Carotid Atherosclerosis in Adolescents and Young Adults With IDDM

Relation to urinary endothelin, albumin, free cortisol, and other factors

MELPOMENI PEPPA-PATRIKIOU, MD
MARIA SCORDILI, MD
ARISTIDIS ANTONIOU, MD
MARIA GIANNAKI, MD
MARIA DRACOPOULOU, MD
CATHERINE DACOU-VOUTETAKIS, MD

OBJECTIVE — To investigate *1*) alterations of carotid intimal-plus-medial thickness (IMT) in subjects with IDDM and 2) the relation of IMT to indexes of diabetic angiopathy and to risk factors of atherosclerosis.

RESEARCH DESIGN AND METHODS — IMT was assessed by ultrasound B-mode imaging in 39 subjects with IDDM (23 male, 16 female young adults aged 17.5 ± 5.2 years, diabetes duration 8.8 ± 5.9) and in 22 control subjects (healthy siblings of the IDDM subjects) of comparable age. Urinary endothelin (UET1) and urinary free cortisol (UFC) were determined by radioammunoassay (RIA), urinary albumin by nephelometry, HbA_{1c} by high-performance liquid chromatography (HPLC), and plasma renin by immunoradiometric assay (IRMA).

RESULTS — The IMT values were greater in IDDM subjects than in control subjects (0.49 ± 0.1 mm, 0.44 ± 0.09 mm, respectively; $P = 0.048$) and greater in IDDM male subjects than in control male subjects (0.52 ± 0.09 and 0.44 ± 0.06 mm, respectively; $P = 0.015$), with no difference between IDDM and control female subjects. The IMT values were greater in diabetic male subjects than in female subjects (0.52 ± 0.09 and 0.45 ± 0.1 mm, respectively; $P = 0.017$). In IDDM subjects, but not in control subjects, there was a positive correlation of IMT to urinary albumin ($P = 0.008$), systolic blood pressure ($P = 0.023$), UET1 ($P = 0.016$), UFC ($P = 0.002$), and BMI ($P = 0.021$). Multiple regression analysis demonstrated that in IDDM subjects the variable that interacts independently with IMT was the BMI ($P = 0.001$).

CONCLUSIONS — IMT, an index of atherosclerosis (macroangiopathy), is increased in IDDM subjects quite early (already in adolescence), and it is positively related to urinary albumin, UET1, blood pressure, and UFC.

Diabetic complications related to micro- and macroangiopathy are the main causes of mortality and morbidity in diabetic patients (1,2). The primary goal of any intervention trial aims at preventing or arresting the progress of diabetic complications. Hence, efforts for obtaining euglycemia should be implemented with efforts for early detection of subclinical angiopathy and with factors that affect their genesis and evolution (3–5).

Studies have shown that carotid intimal-plus-medial thickness (IMT) measured by ultrasound B-mode imaging has a linear relationship to the thickness of the intimal and medial complex detected by pathological and histological criteria (6,7).

Some authors considered the increase of IMT as an index of early atherosclerosis (8,9) and others as an index of advanced atherosclerosis (10). Considering the above data, one expects that IMT measurements by B-mode imaging could be used to detect atherosclerosis of the carotid artery and possibly generalized atherosclerosis. Frost et al. (11), after studying 125 IDDM patients aged ≤40 years, found increased IMT only in patients with diabetic complications.

In the present study, we looked for alterations of IMT in adolescents and young adults with IDDM and in healthy, nondiabetic subjects. Furthermore, the relation of IMT to urinary albumin, UET1, UFC, and risk factors of atherosclerosis was also determined.

RESEARCH DESIGN AND METHODS

The study group comprised 39 subjects with IDDM who were followed in our diabetes center (23 males, 16 females, aged 17.5 ± 5.2 years [7.8–28.4]; diabetes duration 8.8 ± 5.9 years [0.75–22.4]). A total of 22 subjects, who were healthy siblings of the diabetic subjects, were similarly studied and served as control subjects. None of the studied subjects had clinical evidence of angiopathy. Pertinent data of the two groups are depicted in Table 1.

The subjects came to the hospital at 7:30 A.M., after an overnight fast and before the morning insulin injection. They brought in urine collected at home from the previous 24 h. For the urine collection, written instructions were handed out at the previous clinic visit, and explanations were also given by phone the day before urine collection. In addition, at the time of urine delivery they were asked how the urine was collected, and if there was any doubt about the completion of the collection, then the urine was discarded. Blood samples were drawn for the following determinations: blood urea nitrogen, creatinine, total cholesterol, HDL, LDL, triglycerides, renin, and HbA_{1c}. At the same morning, the subjects had a full physical examination, including height and weight measurement.

The blood pressure was measured using a mercury sphygmomanometer in a sitting position after a rest of 5 min. Systolic blood pressure (sBP) was determined by the onset of the "tapping" Korotkoff sounds. The disappearance of Korotkoff

From the Diabetes Center and Endocrine Unit (M.P.-P., C.D.-V.) of First Pediatric Department; the Radiology Department (M.S., A.A.), and the "Aghia Sophia" Children's Hospital (M.G.), Athens University Medical School, Athens, Greece.

Address correspondence and reprint requests to Catherine Dacou-Voutetakis, Associate Professor, First Pediatric Department, Athens University, "Aghia Sophia" Children's Hospital, Goudi, Athens 11527, Greece.

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Abbreviations: dBP, diastolic blood pressure; HPLC, high-performance liquid chromatography; IMT, intimal-plus-medial thickness; IRMA, immunoradiometric assay; RIA, radioammunoassay; sBP, systolic blood pressure; UET1, urinary endothelin; UFC, urinary free cortisol.

Table 1—*Pertinent data of IDDM and control subjects*

	IDDM subjects	Control subjects	*P* value
n	39	22	—
M/F	23/16	11/11	—
Age (years)	17.5 ± 5.2	15.9 ± 7	0.329
Diabetes duration (years)	8.9 ± 5.9	—	—
BMI (kg/m^2)	21 ± 3	18 ± 6.6	0.096
Height (standard deviation score)	0.24 ± 1.1	0.41 ± 1.2	0.738
sBP (mmHg)	116.4 ± 14.6	110 ± 11.7	0.183
dBP (mmHg)	76.8 ± 12	71.7 ± 8	0.157
Mean blood pressure (mmHg)	90 ± 11.9	84.4 ± 9	0.154
Daily insulin (U/kg)	0.83 ± 0.23	—	—

Data are means ± SD.

sounds was characterized as diastolic blood pressure (dBP) (20). Mean blood pressure was estimated as follows:

$$\text{dBP} + \frac{(\text{SBP} - \text{dBP})}{3}$$

For the determination of the IMT, ultrasound high-resolution B-mode imaging, with an echotomographic system (General Electric Logic 500) and an electrical linear transducer (midfrequency of 7.5 MHz), was used. Scanning of the extracranial common carotid arteries in the neck was performed bilaterally according to the anterior-posterior projection. Both measurements were averaged. The greatest value among the four averaged IMT values (two from the left and two from the right) was used as the representative value for each individual. Scanning lasted for an average of 20 min. All the images were conducted by a radiologist and were photographed. The axial resolution of this system was 0.3 mm. The IMT defined by Pignoli et al. (6,7) was measured as the distance from the leading edge of the first echogenic line to the second echogenic line that related to the upper layer of the tunic adventitia.

UET1 was determined by RIA, using commercially available reagents (Nichols Institute, Wijchen, The Netherlands). The interassay and intra-assay variance was 8.2 and 4.5%, respectively. The antibody had the following cross-reactions (endothelin 1, 100%; endothelin 2, 67%; endothelin 3, 84%; big endothelin 1, 2.6%; big endothelin 2, 5.3%; big endothelin 3, 0.2%).

Urinary albumin was determined by nephelometry (nephelometer Turbox [Orion, Espoo, Finland], with commercially available reagents), and was expressed in micrograms per minute.

UFC was determined by RIA using commercially available reagents (clinical assays). The intra-assay and interassay variation was 7.03 and 9.2%, respectively. The antibody had the following cross-reactions: cortisol, 100%; prednisolone, 77%; 6-methylprednisolone, 43%; 11 deoxycortisol, 6.3%; 17-hydroxyprogesterone, 1.2%; and various other corticoids, 0.1%.

Plasma renin (active renin) was determined by IRMA using commercially available reagents (Nichols Institute). The intra-assay and the interassay variance was 2.5 and 7.4%, respectively. The antibody had a high affinity and specificity to active renin and 0.2% cross-reactivity with prorenin.

HbA_{1c} was determined by HPLC. Urea blood nitrogen; total, HDL, and LDL cholesterol; and triglycerides were determined by routine laboratory methods.

Statistical methods

Data are expressed as means ± SD. Differences between the IDDM patients and control subjects were analyzed by the Mann-Whitney *U* test and Student's *t* test as needed. Different correlations were examined by Spearman correlation coefficient. Linear regression analysis and stepwise multiple regression analysis were also performed. A P level < 0.05 was considered statistically significant. The statistical analyses were carried out using the SPSS statistical package on a personal computer.

RESULTS — The results of the different parameters determined are listed in Tables 2 and 3 and Fig. 1. The IMT values (Fig. 1) were greater in IDDM patients than in control subjects (0.49 ± 0.1 and 0.44 ± 0.09 mm, respectively; $P = 0.048$) and greater in IDDM male subjects than in female subjects (0.52 ± 0.09 and 0.45 ± 0.1 mm, respectively; $P = 0.017$), while such a sex difference was not observed in the control subjects (0.44 ± 0.07 and 0.44 ± 0.1 mm, respectively; $P = 0.833$). The IMT values were greater in IDDM male subjects than in control male subjects (0.52 ± 0.09 and 0.44 ± 0.06 mm, respectively; $P = 0.015$) with no difference between IDDM and control female subjects (0.45 ± 0.1 and 0. 44 ± 0.1 mm, respectively; $P = 0.726$). There was no sex difference in the risk factors neither in IDDM subjects nor in control subjects.

In IDDM subjects but not in control subjects there was a positive correlation of IMT with sBP ($r = 0.37$, $P = 0.023$), BMI ($r = 0.44$, $P = 0.021$), urinary albumin ($r = 0.42$, $P = 0.008$), UET1 ($r = 0.40$, $P = 0.016$), and UFC ($r = 0.49$, $P = 0.002$). No correlation of IMT values to age (0.32, $P = 0.067$), plasma renin ($r = -0.20$, $P = 0.476$), total cholesterol ($r = -0.22$, $P = 0.176$),

Table 2—*Results of various parameters studied in IDDM and control subjects*

	IDDM subjects	Control subjects	*P* value
HbA_{1c} (%)	7.2 ± 1.6	3.5 ± 0.4	0.000
Plasma renin (mU/ml)	39 ± 15.9	25.9 ± 11.9	0.05
Urea blood nitrogen (mmol/l)	11.9 ± 2.5	9.7 ± 2.7	0.123
Serum creatinine (mmol/l)	75.1 ± 17.7	68 ± 8.8	0.447
Total cholesterol (mmol/l)	4.65 ± 0.74	4.47 ± 0.62	0.699
HDL (mmol/l)	1.42 ± 0.36	1.19 ± 0.14	0.250
LDL (mmol/l)	2.79 ± 0.62	2.77 ± 0.55	0.882
Triglycerides (mmol/l)	0.9 ± 0.4	1 ± 0.6	0.715
Urinary albumin (μg/min)	25.8 ± 32.6	21.4 ± 25.3	0.04
IMT (mm)	0.49 ± 0.1	0.44 ± 0.09	0.048
Urinary endothelin (pg/24 h)	13129 ± 8019	7994 ± 3302	0.010
Urinary cortisol (μg/24 h)	173 ± 96	99 ± 28	0.001

Data are means ± SD.

Table 3—*Results of the stepwise multiple regression analysis*

Parameter	Univariate correlation coefficient†	*P* value	*B* value	*P* value
BMI	0.500	0.004	0.0161	0.001
Sex*	−0.347	0.015	Not entered	—
HbA_{1c} (%)	0.270	0.056	Not entered	—
Urinary albumin	0.457	0.002	Not entered	—
Diabetes duration	0.281	0.056	Not entered	—
Age	0.421	0.007	Not entered	—
dBP	0.183	0.139	Not entered	—
sBP	0.385	0.009	Not entered	—
Creatinine	−0.252	0.066	Not entered	—
Cholesterol	−0.146	0.191	Not entered	—
Triglycerides	0.282	0.043	Not entered	—
Urinary endothelin	0.400	0.008	Not entered	—
Urinary cortisol	0.361	0.015	Not entered	—
R^2	—	—	—	0.385
Constant	0.149	—	—	0.0004

Analyses were carried out in 39 IDDM patients age 7.8–28.4 years. *Men = 1; women = 2; †Pearson's univariate correlation coefficient.

HDL cholesterol (r = −0.26, P = 0.148), LDL cholesterol (r = −0.16, P = 0.368), triglycerides (r = 0.23, P = 0.162), dBP (r = 0.15, P = 0.35), and mean blood pressure (r = 0.22, P = 0.186) was disclosed. Single linear correlations (Pearson's correlation coefficients) for the above-mentioned pairs showed practically identical results. Multiple regression analysis demonstrated that in IDDM subjects, the variable that interacts independently with IMT was the BMI (P = 0.001) (Table 3).

CONCLUSIONS — The present data indicate that an increase in IMT is evident in IDDM subjects relatively early, namely during adolescence with relatively short diabetes duration (8.8 ± 5.9 years) and in the absence of clinically evident angiopathy. It must be mentioned that the IMT values were greater in diabetic male subjects than in female subjects, while such a sex difference was not found in the control subjects. In reality, the higher IMT values in diabetic subjects were accounted for by IMT values in the male subjects. In this regard, it may be pertinent to note that in another study we have found higher UET1 (12) and UFC (13) values in diabetic male subjects than in female subjects, while no such sex difference was detected in the control subjects.

In this study, the control subjects were first-degree relatives of the diabetic subjects, and the control subjects are expected to share the same genetic background with IDDM subjects, differing only, as far as we can determine, in the presence of IDDM. Furthermore, no clinical evidence of angiopathy was noted. So one expects that IDDM by itself is responsible for the early alterations in IMT.

The correlations of IMT found in this study are thought-provoking. Thus, IMT was positively related to urinary albumin, a sensitive marker of diabetic nephropathy and to UET1, which is possibly linked to diabetic nephropathy as well, or angiopathy in general (12,14,15). Furthermore, the association of IMT to UFC suggests hyperactivity of the adrenals, of whatever mechanism (stress, hypoglycemic episodes, increased endothelin production) might contribute to macroangiopathy. In our study, a positive correlation of IMT to sBP and BMI was also detected.

Pertinent literature data are contradictory. Thus Yamasaki et al. (8) found increased IMT values in subjects with IDDM (age 4–25 years). They further detected that the IMT was positively correlated with diabetes duration and age of the patients, while no other risk factors, such as lipids or blood pressure, were found to be related to IMT. Consequently, Yamasaki et al. (8) considered the carotid artery IMT as an index of early atherosclerosis. Yoneda et al. (9), in studying Japanese patients (age 59 ± 13 years) found that age, male sex, and hyperlipidemia were independent risk factors for carotid atherosclerosis. Kawamori et al. (10) found increased IMT values in IDDM subjects (age 21–66 years) with positive correlation to age, hypertension, and hyperlipidemia. Hence they considered IMT as an index of advanced atherosclerosis (10). Frost et al. (11) found alterations in IMT only in patients with diabetic complications, and they concluded that IDDM alone does not seem to be an index of premature atherosclerosis. Matsushima et al. (16) in another study found increased IMT values in IDDM patients, and they suggested that young

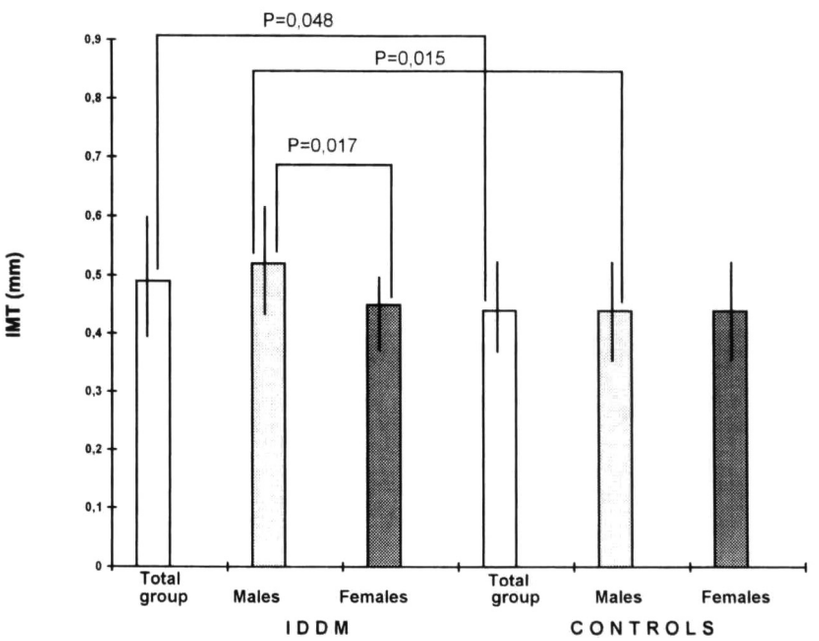

Figure 1—*IMT values in the total group, in male and female diabetic subjects, and in control subjects.*

IDDM patients had advanced atherosclerosis in their carotid arteries, which precedes the onset of microangiopathy. Kanters et al. (17) after studying hyperlipidemic IDDM patients, found that age and blood glucose have an important effect on IMT. Yokohama et al. (18) found increased carotid artery IMT values in IDDM subjects (age 17–39 years) with a positive correlation with dBP and retinopathy. Merrin et al.(19) mentioned that age was the only independent predictive variable of increase in IMT in IDDM subjects.

Our data and those of the literature strongly suggest that IMT determined by high-resolution ultrasonography B-mode imaging can be used as an index of early macroangiopathy and possibly indicate that microangiopathy and macroangiopathy are parallel events. The correlations detected in this study suggest that control of body weight, blood pressure, and better skills for coping with stress may favorably affect the genesis and/or the evolution of diabetic angiopathy.

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