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Carotid Intima-Media Thickness, Ankle-Arm Index, and Inflammation Profile in Mexican Patients with Early and Late Onset Type 2 Diabetes

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ABSTRACT

Background: Type 2 diabetes is strongly linked to an increased incidence of cardiovascular outcomes. Carotid artery intima-media thickness and ankle-arm index are non-invasive complementary measures as subclinical markers of atherosclerosis. **Objective:** To evaluate the association of carotid intima-media thickness, ankle-arm index, and inflammation profile in Mexican patients with early- and late-onset type 2 diabetes mellitus. **Material and Methods:** We included 145 subjects at an academic medical center: 77 patients with early-onset (< 40 years of age) and 33 patients with late-onset (≥ 40 years) type 2 diabetes mellitus, and 35 healthy volunteers. Clinical history, anthropometrics, blood chemistry, lipids profile, glycosylated hemoglobin A1c, cytokines, and high-sensitivity C-reactive protein were determined; carotid and lower limb ultrasound were taken. Groups were compared with ANOVA or Kruskal-Wallis, Student's *t* or Mann-Whitney U. Spearman or Pearson correlation and logistic regression analysis were used. **Results:** There were anthropometric and biochemical differences between the three groups. Concentrations of interleukin-1β, -4 and -6 were significantly higher in patients with late versus early onset diabetes. There were differences in carotid intima-media thickness and ankle-arm index between early and late onset. Age, body mass index, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein, waist circumference, and glycosylated hemoglobin A1c showed direct correlation with carotid intima-media thickness, while ankle-arm index showed inverse correlation with blood pressure, glycosylated hemoglobin A1c, time with disease, age at onset, triglycerides, and fibrinogen. Multivariate analysis showed an association between carotid intima-media thickness and disease duration; ankle-arm index with disease duration and high-sensitivity C-reactive protein; while only body mass index associated with end diastolic flow velocity. **Conclusions:** Our findings suggest that carotid intima-media thickness and ankle-arm index are associated with inflammation markers and could be included in the evaluation of type 2 diabetes mellitus patients, according to disease onset and duration. There are important differences in interleukin concentrations between early- and late-onset type 2 diabetes mellitus. Additionally, measurement of high-sensitivity C-reactive protein is suggested in patients with abnormal ankle-arm index. (REV INVES CLIN. 2015;67:240-9)

Key words: Type 2 diabetes. cIMT. Ankle-arm index. Inflammation marker. Early onset.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic disease with long-term morbidity and high mortality as a result of atherosclerosis[1]. In the last few years, the clinical spectrum of diabetes in Mexico has expanded to affect people under 40 years of age. Between 1993 and 2006, the overall prevalence of T2DM doubled, associated with obesity and a clinical profile of metabolic syndrome. It appears that early-onset T2DM is becoming more common and may increase future hospitalizations due to diabetes with a corresponding increment in associated healthcare costs[2]. In fact, the 2012 National Health and Nutrition Survey in Mexico (Encuesta Nacional de Salud y Nutrición, ENSANUT) estimated that 9.17% of Mexicans have been diagnosed with diabetes[3].

The decrease in the age of onset of T2DM is related with an increase in obesity in the young, including children and adolescents[4]. There is an inverse relationship between the degree of obesity and the age of T2DM onset, even in young adults[5].

Early-onset T2DM is a complex metabolic disorder with multifactorial and polygenic pathology that is characterized by hyperglycemia secondary to an important gradual deficit in the secretion of insulin by pancreatic β-cells in a shorter time, compared with late-onset T2DM, and which also includes the presence of insulin resistance[6].

Cardiovascular disorders in the population with diabetes are 2-3 times more frequent than in the non-diabetic population[7]. The inflammatory process in diabetic patients is essential for the development and progress of atherogenesis, ending in the formation of atherosclerotic plaques, which, during their development, may fissure or erode, causing the formation of thrombi. Various systemic inflammation markers have been studied to evaluate this process, including ultrasensitive C-reactive protein (hs-CRP), interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 8 (IL-8), and tissue necrosis factor alpha (TNF-α)[8,9]. Today, the rise in these markers is thought to be related to a greater expression of IL-6 or TNF-α in intraabdominal adipose tissue, with increase in leptin concentrations and decrease of adiponectin (an anti-inflammatory and anti-atherogenic protein)[10].

To evaluate anomalies in carotid intima-media thickness (cIMT), readings should be compared with the population of reference. The cIMT measured by B-mode echography in real time is directly related with different phases of atherosclerosis development[11]. At the same time, it has been reported that flow velocity relates with vessel distensibility and diameter, constituting a homodynamic measure of vessel function[12]. Although in general a cIMT is considered abnormal when it is > 0.908 mm, any point in the cohort above the 80th percentile of the study population is considered pathological[13]. The presence of plaque during the study is considered an equivalent of high cardiovascular risk, and allows the reclassification of an individual from the intermediate-risk category to a high-risk, thus supporting the need for intervention. It has been demonstrated that an increase in cIMT in patients with T2DM (≥ 60 years old) without a history of coronary or cerebrovascular disease progresses to disease 10-times faster than in matched subjects without diabetes. Independent predictors of progression were: baseline cIMT ($p < 0.001$), glycosylated hemoglobin A1c (HbA1c) ($p < 0.001$), and age ($p = 0.001$)[14].

The Bogulasa Heart Study correlated cIMT with classic cardiovascular risk factors in young populations where systolic blood pressure, race, age, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) explained 17% of the variability of cIMT[15]. In Mexico, there are few studies that evaluate cIMT in patients with type 2 diabetes. Cantú-Brito, et al. studied the prevalence of atherosclerotic lesions in 145 Mexicans over 55 years of age; in 44.1%, the cIMT was > 1 mm (normal value < 1 mm), with a sensitivity of 91.3% and a specificity of 92.7%[16].

Twenty-percent of patients with diabetes have asymptomatic peripheral arterial illness (PAI), which may be sub-clinical and only detected through vascular imaging techniques[17]. A low ankle-arm index (AAI) has indicated a risk of mortality from cerebrovascular and coronary disease of 5.9-fold and 6.6-fold, respectively[18]. In Mexico, PAI affects 19.8% of the population. Its prevalence increases with age and it is more frequent in men than in women before the age of 40. An AAI ≤ 0.90 defines PAI, with a sensitivity of 95% and a specificity of 99%, compared with arteriography (current gold standard), and is associated with an increased risk of cardiovascular morbidity and mortality[19]. The increase in the presence of an AAI ≤ 0.9 and its correlation with a greater number of atheromatous lesions in carotid echography and greater cIMT has been analyzed[20].

Guidelines for initiating cardio-protective treatment in patients with early-onset diabetes have not been established, unlike in late-onset diabetes patients. Therefore, this study aimed to evaluate the association of cIMT, AAI, and inflammation profile in Mexican patients with early- and late-onset type 2 diabetes. This study may serve as a basis for generating new evidence concerning the atherogenicity of early-onset diabetes in the Mexican population. The usefulness of noninvasive complementary measurements, such as cIMT and AAI, as subclinical markers of atherosclerosis should be explored further.

MATERIAL AND METHODS

An analytical, cross-sectional study was designed. The study was approved by the Ethics Committee of the Mexican Social Security Institute (IMSS). From the patients with type 2 diabetes who attended scheduled visits at the Unidad de Investigación en Epidemiología Clínica, UMAE Hospital de Especialidades Centro Médico Nacional Siglo XXI of IMSS, 353 were invited to participate. Inclusion criteria were: ≥ 20 years of age, either sex, with type 2 diabetes, and free of chronic or acute inflammatory disease. Exclusion criteria were: diabetic ketoacidosis, hyperosmolar coma, secondary cause of diabetes, alcoholism or tobacco use, primary hyperlipidemia (homozygote or heterozygote hypercholesterolemia), chronic renal failure, acquired or non-acquired immunodeficiency, rheumatic or immunological disease, under topical or systemic steroids or immunoregulators, arrhythmia or conduction disorders (second or third degree blockage, auricular fibrillation), presence of known valvulopathy or myocardiopathy, diagnosed ischemic cardiopathy, any kind of neoplasia, and failure to agree to participate or to sign an informed consent.

Patients who fulfilled the inclusion criteria were contacted by telephone, and those who accepted participation were evaluated for inclusion in the study. A total of 110 patients and 35 healthy IMSS workers, who volunteered to participate as controls, signed the informed consent.

Inclusion criteria for healthy controls were: fasting plasma glucose < 100 mg/dl, systolic blood pressure < 130 mmHg, diastolic blood pressure < 85 mmHg, total cholesterol < 200 mg/dl and triglycerides < 150 mg/dl. Group I included the 35 healthy volunteers (21 women and 14 men). Group II included 77 patients that had early-onset T2DM (< 40 years of age). Group III patients had late-onset T2DM (≥ 40 years of age).

In all subjects, a medical history was recorded and anthropometric measurements were taken according to the Lohman technique[21]; blood pressure was recorded and laboratory studies performed. A carotid ultrasound (US) was performed, along with US of the lower limbs. Laboratory tests included blood chemistry, lipids profile, HbA1c, cytokines (IL-1β, IL-4, IL-6), and hs-CRP. Blood chemistry and the lipids profile were determined by commercial methods (Beckman); HbA1c was estimated using high-resolution liquid chromatography (Bio-Rad, Hercules, CA); cytokines (IL-1β, IL-4, and IL-6) were determined by the ELISA method with PeproTech (Rock Hill, NJ). The hs-CRP was measured using high-sensitivity human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX).

Ultrasound was used for automatic measurements of cIMT and was performed by a single experienced radiologist, who was blinded to subject group assignment, in the Radiodiagnostic Service of Hospital de Especialidades CMN Siglo XXI.

Both flow velocities (peak systolic velocity and end diastolic velocity) were measured. The segment of the common carotid artery, the bifurcation (carotid bulb), and internal carotid artery were the same in each survey. All exams were performed according to a predetermined and standardized protocol with right and left sides averaged for the three angles[22]. High-resolution US equipment (Philips HDI 5000) was used with specific software for carotid arteries and a wide-band lineal transducer (7-12-MHz). All images were photographed and stored. Images were analyzed by the same radiologist with the Philips's QLAB 4.2.1 Advanced Ultrasound Quantification Program. The peripheral vascular US was performed using a wide-band linear transducer of 8 MHz and calibrated sphygmomanometer. The patient was placed in dorsal decubitus with at least five minutes of repose. Systolic blood pressure (SBP) was measured in the posterior tibia artery of both lower extremities and the higher value was used as the numerator for blood pressure. Likewise, SBP was taken at the brachial artery of both upper extremities and the higher value used as denominator. The blood pressure value for each of the lower extremities is the result of dividing SBP of each

Table 1. Baseline clinical and anthropometric parameters of study subjects according to age at onset of type 2 diabetes

	Group I Control group (n = 35)	Group II Early-onset (< 40 years) (n = 77)	Group III Late-onset (≥ 40 years) (n = 33)	*Group I vs. Group II vs. Group III p value	†Group II vs. Group III p value
Age (years)	42.6 ± 7.6	39.2 ± 6.2	52.3 ± 4.2	< 0.001	< 0.001
Sex (M/F)	14/21	34/43	17/16	0.567	0.478
Duration of disease (years)	–	4.5 ± 3.0	3.8 ± 3.3	< 0.001	< 0.001
Weight (kg)	61.9 ± 9.0	79.6 ± 17.9	74.9 ± 13.9	< 0.001	0.187
Height (cm)	1.61 ± 0.09	1.60 ± 0.09	1.57 ± 0.07	0.123	0.114
BMI (kg/m^2)	23.7 ± 2.10	30.6 ± 5.63	30.1 ± 5.38	< 0.001	0.642
Waist circumference	82.0 ± 12.2	98.5 ± 14.2	100.4 ± 11.7	< 0.001	0.497
SBP (mmHg)*	100 (100-110)	110 (100-120)	110 (100-120)	0.005	0.869
DBP (mmHg)*	70 (60-77)	70 (70-80)	70 (70-80)	0.456	0.564
Medication use: n (%)					
– Oral hypoglucemiants	–	54 (70.1)	24 (72.7)	–	0.081
– Insulin (SC)	–	23 (29.9)	9 (27.3)	–	0.783
– Statins and Fibrates	–	13 (16.9)	6 (18.2)	–	0.869
– Antihypertensives	–	55 (71.4)	32 (96.9)	–	0.216
– Antiaggregants	–	15 (19.5)	8 (24.2)	–	0.574
History of dyslipidemia n (%)	0	13 (16.8)	6 (18.2)	–	0.649
History of hypertension n (%)	0	10 (12.9)	3 (9.1)	–	0.641
Physical activity n (%)	1 (2.9)	5 (6.5)	2 (6.1)	0.901	0.846
Adherence to diet n (%)	–	13 (16.9)	4 (12.1)	–	0.902

Data represent the n (%) or mean ± SD.
*Median (interquartile range, Q25-Q75); *Kruskal-Wallis or ANOVA was used.
†Student *t* or Mann-Whitney U was used, each according to data distribution.
BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; SC: subcutaneous.

lower extremity by the higher SBP of the upper extremities, using the lower number for analysis. All the information was recorded in a database specially designed for the study.

Obesity was defined as body mass index (BMI) ≥ 30. Arterial hypertension was diagnosed according to recommendations[23] or if the subject was using antihypertensive medication. Dyslipidemia was defined according to the third panel of the National Program of Cholesterol Education[24].

Statistical Analysis

ANOVA or Kruskal-Wallis was used to compare the variables among the three groups. Student *t* or Mann-Whitney *U* compared between early- and late-onset diabetes. Spearman or Pearson correlation, as appropriate, correlated cIMT, AAI, and flow velocities for the variables studied, and logistic regression analysis was used to identify predictors of risk. Statistical significance of $p < 0.05$ was determined. These analyses were performed with the statistical package SPSS (v15, Chicago, IL).

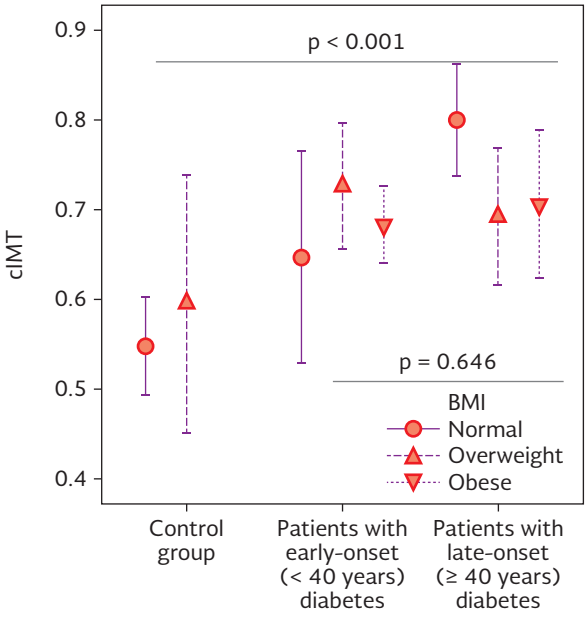
RESULTS

A total of 353 subjects were evaluated: 198 women (56%) and 155 men (44%); the average age of participants was 46.1 ± 9.6 years and the BMI was 29.0 ± 5.7 kg/m^2. Group II included 77 patients with early-onset T2DM (before the age of 40): 43 women and 34 men 20-50 years old with disease duration of 4.5 ± 3.0 years. Group III included 33 patients with late-onset T2DM (≥ 40 years of age): 16 women and 17 men 43-60 years old with disease duration of 3.8 ± 3.3 years.

Clinical and anthropometric characteristics of all the subjects are shown in table 1.

As expected, there were differences in BMI and waist circumference, with the control subjects being slimmer and with less abdominal fat (Table 1). When comparing

Figure. 1. Comparison of carotid intima-media thickness with body mass index in the sample population. Comparison of carotid intima-media thickness with body mass index (ANOVA was used to compare the three groups and Student *t* for patients with early (< 40 years) vs. late (≥ 40 years) onset of type 2 diabetes).
cIMT: carotid intima-media thickness; BMI: body mass index



the increase in cIMT with BMI, we found that being overweight led to greater cIMT in the control group and patients with early-onset diabetes, while in the late-onset group a normal weight was associated with a greater cIMT. The presence of diabetes appeared to lead to a greater cIMT ($p < 0.001$), although the differences between early- and late-onset were not significant ($p = 0.464$) (Fig. 1).

On the other hand, in the biochemical parameters, levels of glucose and HbA1c showed significant differences between the three groups ($p < 0.001$); they were higher in the early-onset group, although the difference between early- and late-onset diabetes was not significant. There was also a significant difference in the lipid profile between the three groups, as expected, except for LDL-C ($p = 0.292$). However, when compared between groups II and III, these differences lost their significance. Serum markers of inflammation showed significant differences when compared with the healthy control group ($p < 0.001$), and IL-1β, IL-4 and IL-6 maintained significance between the two groups with diabetes, showing higher concentrations in those with late-onset diabetes, although fibrinogen ($p = 0.677$) and hs-CRP ($p = 0.233$) lost significance and were higher in the early-onset group (Table 2). Upon analyzing cIMT with the degree of metabolic control in diabetic patients according to HbA1c values, a slight correlation is seen ($R^2 = 0.216$) (Fig. 2).

When analyzing the groups according to thickness of the common carotid-intima media, we found a significant difference when comparing Groups II (0.692 ± 0.154 mm) and III (0.715 ± 0.130 mm) against Group I (0.566 ± 0.172 mm) ($p < 0.001$), but not when comparing early- and late-onset groups ($p = 0.464$) (Table 3).

The AAI was greater in Group I (1.19 ± 0.08) compared with Group II (1.08 ± 0.11) and Group III (1.10 ± 0.11) ($p < 0.001$), but not when comparing early- and late-onset diabetes ($p = 0.589$). There were no significant differences in flow velocities between groups. The presence of atheroma plaque was documented in the carotid arteries of 10.4% of patients from Group II and 6.1% of Group III ($p = 0.469$) (Table 3).

Several variables correlated with cIMT and AAI, but not with flow velocities, including systolic and diastolic blood pressure, HbA1c, diabetes duration, age at onset, and triglycerides. Age, BMI, HDL-C, CRP, and waist circumference significantly correlated with cIMT but not AAI, while the reverse was true for fibrinogen. The only variable that correlated with flow velocity was BMI with end diastolic velocity ($p = 0.041$) (Table 4).

Logistic regression analysis was performed using the upper 75th percentile of cIMT (> 0.600 mm) and AAI (< 1.19) found in the study group. After adjusting for age, BMI, blood pressure, waist circumference, HDL-C, LDL-C, triglycerides, IL-1β, IL-4, IL-6, HbA1c, fibrinogen, high-density C-reactive protein, with cIMT, age at onset and duration remained independently significant (beta 0.048 with β exponent of 1.050 [$p = 0.052$; 95% CI: 1.000-1.102] and beta 0.251 with β exponent of 1.286 [$p = 0.007$; 95% CI: 1.072-1.542]), respectively. However, for AAI, disease duration and C-reactive protein were the variables that remained significant (beta –0.271 with β exponent of 0.763 [$p = 0.029$; 95% CI: 0.598-0.972] and beta 1.753 with β exponent of 5.771 [$p = 0.026$; 95% CI: 1.236-26.954]), respectively. Similarly, BMI proved to be a risk predictor for end diastolic velocity (beta 1.640 with β exponent of 5.158; $p = 0.041$; 95% CI: 1.072-24.818) (Table 5).

Table 2. Biochemical characteristics and inflammation markers of the study population

	Group I Control group (n = 35)	Group II Early-onset (< 40 years) (n = 77)	Group III Late-onset (≥ 40 years) (n = 33)	Group I vs. Group II vs. Group III p value	Group II vs. Group III p value
Glucose (mg/dl)†	88.0 (81-95)	152 (113.5-269)	134 (106.5-220)	**< 0.001**	0.147
Total cholesterol (mg/dl)*	183.0 ± 18.2	203.4 ± 53.6	206.3 ± 39.5	**0.048**	0.780
Triglycerides (mg/dl)†	99 (74-125)	189 (142-338)	214 (155-292.5)	**< 0.001**	0.914
HDL-C (mg/dl)*	60.6 ± 14.02	38.7 ± 10.81	41.7 ± 12.03	**< 0.001**	0.206
LDL-C (mg/dl)*	102.1 ± 21.85	108.3 ± 41.94	115.8 ± 32.79	0.292	0.363
HbA1c (%)†	5.7 (5.4-5.9)	7.4 (6.1-10.4)	7.0 (6.3-9.0)	**< 0.001**	0.667
Interleukin-6 (pg/ml)†	172.3 (31.2-550.7)	549.7 (132.6-612.5)	568.5 (549.7-625.1)	**< 0.001**	**0.030**
Interleukin-4 (pg/ml)†	7.9 (6.8-146.2)	159.3 (1.9-321.6)	212.0 (159.3-318.6)	**< 0.001**	**0.026**
Interleukin-1β (pg/ml)†	46.4 (28.0-117.3)	265.0 (15.7-502.8)	451.4 (393.5-509.3)	**< 0.001**	**0.010**
Fibrinogen (mg/dl)*	309.5 ± 60.6	373.2 ± 75.0	366.5 ± 78.2	**< 0.001**	0.677
Ultrasensitive C-reactive protein (mg/dl)†	0.50 (0.30-1.97)	3.03 (1.51-6.53)	2.33 (0.73-5.37)	**< 0.001**	0.233

Data represent n (%), or means ± SD; Median (interquartile range, Q25-Q75) according to data distribution. ANOVA or Kruskal-Wallis was used to compare the three groups.
*Student *t*.
†Mann-Whitney U to compare groups II and III.
HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; HbA1c: glycosylated hemoglobin A1c.

Figure. 2. Correlation of carotid intima-media thickness with level of glycosylated hemoglobin A1c using Spearman Rho correlation. cIMT: carotid intima-media thickness; HbA1c: glycosylated hemoglobin A1c.

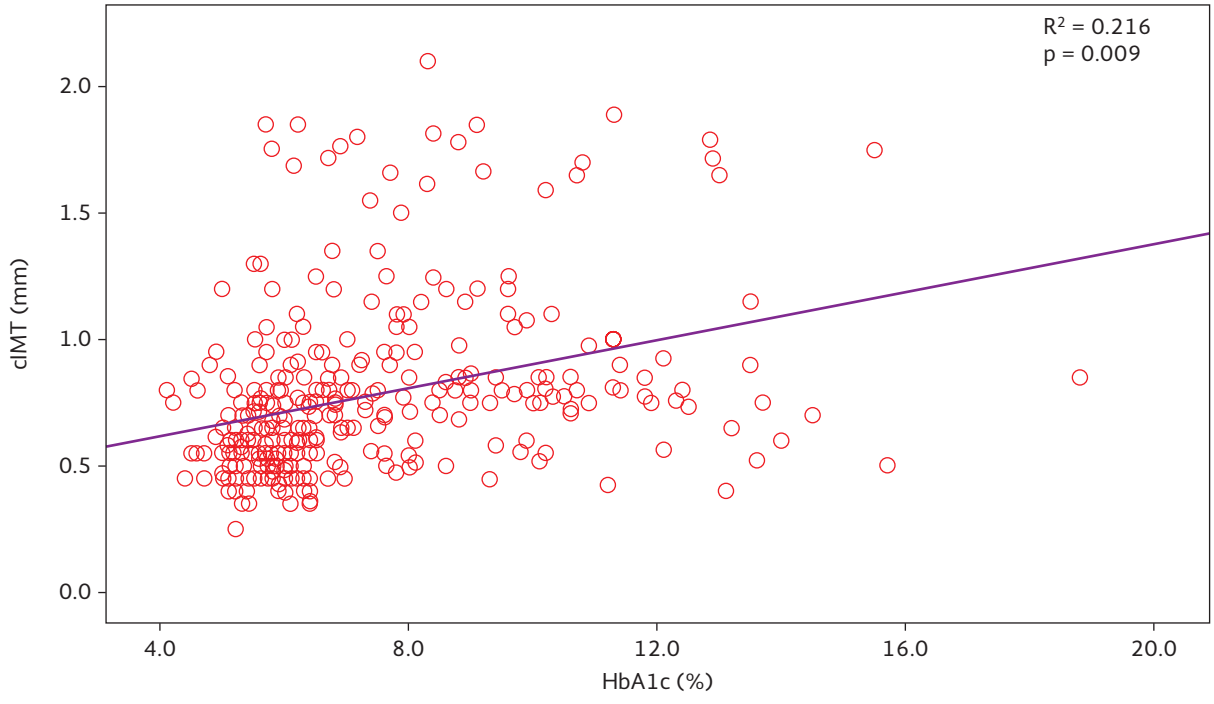


Table 3. Parameters of vascular ultrasound in subjects according to age at onset of type 2 diabetes mellitus

	Group I Control group (n = 35)	Group II Early-onset (< 40 years) (n = 77)	Group III Late-onset (≥ 40 years) (n = 33)	Group I vs. Group II vs. Group III p value	Group II vs. Group III p value
cIMT (mm)	0.566 ± 0.172	0.692 ± 0.154	0.715 ± 0.130	< 0.001*	0.464‡
Mean AAI	1.19 ± 0.08	1.08 ± 0.11	1.10 ± 0.11	< 0.001*	0.589‡
Peak systolic velocity	74.8 ± 17.4 (61.6-84.5)	74.2 ± 12.3 (66.5-82.1)	75.4 ± 11.1 (65.8-83.3)	0.907†	0.625‡
Atheroma plaque n (%)	–	8 (10.4%)	2 (6.1%)	–	0.469§

Data represent the mean ± SD or number (%).
*ANOVA.
†Kruskal-Wallis was used to compare the three groups.
‡Student *t*.
§Xi^2.
Xi^2 o Student *t* were used to compare 2 groups, according to distribution.
cIMT: carotid intima-media thickness; AAI: ankle-arm index.

Table 4. Correlation coefficient between carotid intima-media thickness, peak systolic and end diastolic velocities and related variables

	cIMT		End diastolic velocity		Peak systolic velocity		Ankle-arm index	
	r	p	r	p	r	p	r	p
Age (years)	0.202*	**0.015**	–0.054	0.523	0.082	0.328	–0.132	0.122
BMI (kg/m²)	0.216†	**0.009**	–0.175*	**0.041**	–0.054	0.518	–0.043	0.606
SBP (mmHg)	0.293†	**0.001**	–0.103	0.216	–0.119	0.153	–0.334†	**0.001**
DBP (mmHg)	0.275†	**0.001**	–0.008	0.926	–0.120	0.150	–0.285†	**0.001**
HbA1c (%)	0.216†	**0.009**	0.017	0.837	0.081	0.331	–0.238†	**0.005**
Duration of disease (years)	0.483†	**0.001**	–0.024	0.772	0.087	0.298	-0.472†	**0.001**
Age at onset (years)	0.332†	**0.001**	0.007	0.931	0.086	0.306	–0.236†	**0.006**
Total cholesterol (mg/dll)	0.055	0.514	–0.092	0.270	–0.007	0.938	–0.028	0.749
Triglycerides (mg/dl)	0.270†	**0.001**	–0.030	0.719	–0.033	0.695	–0.249†	**0.003**
LDL-cholesterol (mg/dl)	0.122	0.143	–0.100	0.231	0.021	0.799	0.036	0.678
HDL-cholesterol (mg/dl)	–0.268†	**0.001**	–0.011	0.896	–0.086	0.301	0.155	0.070
C-reactive protein (mg/dl)	0.279†	**0.004**	0.029	0.764	0.179	0.065	–0.010	0.918
Fibrinogen (mg/dl)	0.159	0.064	–0.122	0.158	0.136	0.115	–0.348†	**0.001**
Waist circumference (cm)	0.342†	**0.001**	–0.155	0.070	–0.025	0.768	–0.060	0.475

Spearman or Pearson (as appropriate) correlation coefficient:
*Significant correlation: < 0.05 (bilateral).
†Significant correlation: < 0.01 (bilateral).
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low density lipids; HDL: high density lipids; HbA1c: glycosylated hemoglobin A1c.

DISCUSSION

This study confirms that there are important differences between early- and late-onset type 2 diabetes, and between cIMT and AAI. The AAI findings highlight the importance of measuring hs-CRP. The most recent evidence suggests that early-onset T2DM is a phenotype of a more aggressive disease than late onset, with greater development of cardiovascular complications[5,25].

When analyzing the metabolic state in the groups, we found lower levels of total cholesterol, triglycerides, and HDL-C and LDL-C to be more prevalent in patients

Table 5. Logistic regression analysis for the association between carotid intima-media thickness, flow velocity, ankle-arm index and related variables

Carotid intima-media thickness			
	*β coefficient	β exp (95% CI)	p value
Age at onset	0.048	1.050 (1.000 – 1.102)	0.052
Duration of disease	0.251	1.286 (1.072 – 1.542)	**0.007**
End diastolic velocity			
– BMI	1.640	5.158(1.072-24.818)	**0.041**
Ankle-arm index			
– Duration of disease	-0.271	0.763(0.598 – 0.972)	**0.029**
– C-reactive protein	1.753	5.771(1.236 – 26.954)	**0.026**

*The β coefficient of carotid intima-media thickness (> 0.600 mm) and ankle-arm index (< 1.19) was calculated for the highest quartile and adjusted for each variable: age, body mass index, systolic blood pressure, diastolic blood pressure, waist circumference, high-density lipid cholesterol, low-density lipid cholesterol, triglycerides, interleukin 1-β, interleukin-4, and interleukin-6, glycosylated hemoglobin A1c, fibrinogen, high-density C-reactive protein.

with early-onset T2DM compared with the late-onset group, all of which were abnormal but not significant. This confirms the observations of Haffner, et al. and stresses the presence of slightly elevated concentrations of LDL-C, or even normal levels[26].

In theory, the presence of a higher pro-atherogenic lipid profile in patients with early-onset T2DM should be accompanied by a rise in serum inflammation markers such as hs-CRP, VCAM-1, ICAM-1, SP-selectin and SE-selectin, as referred to by Tousoulis, et al.[27]. However, in our study, while it is true that we found significant differences in interleukins, hs-CRP, and fibrinogen when comparing the three groups, we were not able to document differences between early-onset and late-onset T2DM, probably because group III included patients with less time with T2DM.

The American Heart Association (AHA) has recommended the use of carotid echocardiography in selected patients with intermediate global risk[28]. Large prospective studies in individuals have demonstrated conclusively that cIMT is a risk factor independent of cardiac coronary disease or stroke[1,29,30]. Our work is one of the first to use the quantification of cIMT and AAI in patients with T2DM under 40 years of age, considering that by age alone they would be considered at low risk.

The study determined the mean cIMT of the posterior wall of the common carotid in healthy subjects in our population (0.566 mm), using a value within the range of 0.25-1.5 mm referred to in the Cardiovascular Health Study, as a group at low risk of developing infarction or ictus (1.1%/year)[31,32]. In our study, patients with T2DM showed higher mean cIMT values compared with the control group, but without differences between Groups II and III (p = 0.464).

When we analyzed atherosclerosis plaque at the carotid level, almost twice the number of the patients with early-onset T2DM had atheroma plaque compared with the late-onset group (10.4 vs. 6.1%, respectively). These percentages are lower than that found by other authors, including Salonen and Salonen, who found 23.3% in asymptomatic individuals with various cardiovascular risk factors[33].

Carotid intima-media thickness has been associated with age, SBP, presence of diabetes, and LDL-C concentration, among other factors. In individuals < 45 years of age, cIMT has been related with six risk factors for cardiovascular disease[30]. The importance of the independent risk factor for developing atherosclerosis as manifested by increased cIMT has been confirmed[34]. But the increase in cIMT in our study was only statistically associated with the increase in SBP, independent of lipid levels, which suggests that in addition to endothelial damage, there is another mechanism involved in the accumulation of lipids on the arterial wall[35,36].

On the other hand, atherosclerosis is an inflammatory disease. Numerous epidemiological studies have demonstrated that hs-CRP is an inflammatory marker and is a predictor of future cardiovascular events in both

men and women[8,9]. Our results agree with the literature, documenting a linear correlation between the number of risk factors and an increase in cIMT in the three groups, although without statistical significance between groups II and III. In this study, cIMT was also associated with the direct increase of hs-CRP (subclinical inflammation), suggesting an increase in atherosclerosis risk. The group of patients with early-onset T2DM had concentrations of 3.03 mg/dl compared with the late-onset group with 2.33 mg/dl, which confirms the usefulness of this marker to better categorize the risk of cardiovascular events in each individual, mainly in subjects with intermediate risk, as suggested by the AHA[28]. No significant differences were found between the two groups, probably due to the duration of the disease.

Regarding the AAI, its usefulness is accepted for evaluating subclinical peripheral arterial disease. It has been shown to be an important predictor of vascular disease in other areas, so that an AAI ≤ 0.9 or > 1.4 is associated with greater mortality from cardiopathic ischemia, and, to a lesser degree, from cerebrovascular disease[20,37].

In regards to hs-CRP, a relationship was seen with the values obtained for AAI in logistic regression analysis, showing more than five-times the risk of AAI ≤ 0.9. The importance of duration of disease in our study is observed both for cIMT and AAI, showing an inverse association with AAI and a positive one with the increase in cIMT. These results are similar to those stated by other authors[20,37]. It is also important to note that obesity reduces vasodilation reserve and vascular wall compliance[38].

There are several limitations to this study. One is the small sample size. As noted, more than half of the patients considered did not meet the inclusion criteria. Another is the lack of follow-up, which did not allow us to see future development of cardiovascular complications as indicated by abnormal cIMT or AAI values. The same is true of atheromatous lesions. In addition, the short time with diabetes of the sample did not allow us to observe progression of these complications. The designation of early- and late-onset was based on the time of detection of the disease and could not consider the time with diabetes prior to that point. This may explain the negative association of disease duration with abnormal AAI.

There are differences between early- and late-onset type 2 diabetes in both cIMT and AAI. The IL-1β, IL-4 and IL-6 concentrations were significant between the two groups with diabetes, showing higher concentrations in those with late-onset diabetes. A cIMT above the 75th percentile significantly associated with age at diagnosis and duration of the disease. An increase in cIMT and AAI depended in great measure on the duration of T2DM. The cIMT was also affected by the age at onset of the disease, while AAI associated with hs-CRP, indicating a fivefold risk of abnormal AAI with elevated CRP, a finding which appears to agree with O'Hare, et al.[37].

In conclusion, our findings suggest that cIMT and AAI associate with inflammation markers and could be included in the evaluation of all patients, according to disease onset and duration. There are important differences in interleukin concentrations between early- and late-onset T2DM. Additionally, measurement of hs-CRP is suggested in patients with abnormal AAI.

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