Original Research

Plasma Glucose Levels and White Blood Cell Count Are Related with Ankle Brachial Index in Type 2 Diabetic Subjects

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Key words: **Type 2 diabetes mellitus, ankle brachial index, fasting plasma glucose, white blood cell count.**

Introduction: Type 2 diabetes mellitus (T2DM) is a major risk factor for peripheral arterial disease (PAD). A simple, noninvasive method for the estimation of PAD is ankle brachial index (ABI). The aim of the present study was to determine whether there is an association between ABI and cardiovascular risk factors in T2DM subjects without apparent macrovascular disease.

Methods: A total of 125 T2DM subjects (84 males, 41 females, mean age ± standard deviation 62.2 ± 9.1 years) who had no apparent macrovascular disease and who attended the Health Center of Erymantheia between January 2008 and June 2009 were recruited to the study.

Results: Of the study subjects 20% (n=25) had ABI <0.90. Univariate linear regression analysis showed that ABI was significantly associated with history of hypertension (p=0.02), fasting serum glucose levels (p=0.02), serum urea levels (p=0.005), serum uric acid levels (p=0.007) and white blood cell (WBC) count (p=0.04). Multivariate linear regression analysis demonstrated significant independent associations between ABI and fasting serum glucose levels (p=0.03) as well as WBC count (p=0.03).

Conclusions: Elevated plasma glucose and WBC count increase the risk of PAD in asymptomatic diabetics.

Manuscript received: October 4, 2009;
Accepted: April 16, 2010.

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It is well known that subjects with type 2 diabetes mellitus (T2DM) often suffer from macrovascular complications and T2DM is a well established risk factor for peripheral arterial disease (PAD).[1,2] In addition, higher rates of subclinical cardiovascular disease among diabetic patients have been reported in comparison with healthy counterparts.[3]

Ankle brachial index (ABI) is a simple, easy to perform, noninvasive method for the estimation of PAD.[4,5] ABI is the ratio of the ankle to brachial systolic blood pressure, and a value of <0.90 indicates the presence of PAD.[4,5] However, relatively few data exist regarding the relationship between ABI and cardiovascular risk factors in T2DM subjects. A recent study using ABI in T2DM subjects showed that PAD risk factors differ significantly between the sexes.[6] Another study showed that elevated uric acid (UA) levels were a significant risk factor for PAD in subjects with T2DM.[7]

The objective of the present study was to determine whether there is an association between ABI and the established as well as the novel cardiovascular risk factors in subjects with T2DM who have no apparent macrovascular disease.

Methods

Subjects and procedures

A total of 125 subjects (84 males / 41 females, mean age ± standard deviation:

62.2 ± 9.1 years) with T2DM who had no apparent macrovascular disease and who attended the Health Center of Erymantheia between January 2008 and June 2009 were recruited to the study. The diagnosis of diabetes was based on the American Diabetes Association criteria.[8] Macrovascular disease was defined as a history of previous myocardial infarction, presence of angina, revascularization procedures or stenosis >50% of the coronary arteries, a history of cerebrovascular disease, or PAD based on a previous lower limb Doppler ultrasound examination. According to their medical records, subjects without any of the above criteria for the presence of macrovascular disease were included in the study.

A detailed medical history, including current medication and smoking habits, was obtained, and a thorough physical examination was performed. The purpose of the study was clearly explained to all subjects, who then volunteered to participate. The study was approved by the local ethics committee.

All measurements were performed in the morning, after a 10-12 hour fast. The subjects were advised not to eat, smoke, or drink coffee before examination. Blood samples were drawn for measurement of fasting serum glucose, HbA1c, urea, creatinine, lipid profile, white blood cell (WBC) count, and UA and high sensitivity C-reactive protein (hsCRP) levels. The antidiabetic medications were given to the patients at the end of the examination.

Blood pressure was measured three consecutive times, one minute apart, in the sitting position using an appropriate cuff size. The mean value of the last two measurements was calculated and used in the analysis. Arterial hypertension was defined according to the current guidelines, i.e. systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg, or when the patients were on antihypertensive treatment.[9] Body weight (with subjects in light clothing without shoes) and height were measured, and body mass index (BMI) was calculated. Waist circumference was measured with a soft tape while the subject was standing, midway between the lowest rib and the iliac crest.

Analytical methods

Fasting serum glucose, lipids and creatinine concentrations were measured using a Technicon analyzer RA-XT. Low density lipoprotein (LDL) cholesterol concentrations were calculated using the Friedewald formula.[10] HbA1c was measured by high-performance liquid chromatography (HPLC) (Roche Diagnostics, Mannheim, Germany) with a non-diabetic reference range of 4.0-6.0%; hsCRP was determined using ADVIA 1650 (Bayer, Elkhart, IN, USA). Estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft-Gault formula.[11]

Measurement of ABI

Measurement of ABI was based on the method described in previous studies.[4,5] Doppler ultrasound (8 MHz probe; Medacord PVL, MedaSonic Inc, Mountain View, CA, USA) was used to measure the systolic blood pressure in the bilateral brachial, posterior tibial and dorsal pedal arteries while the patient was supine after a 20 min rest. The occluding cuffs (55×12.5 cm) were applied just above the malleoli for the measurement of ankle pressures. The right and left ABI were calculated automatically by the device, by dividing the highest pressure in the dorsal pedal or posterior tibial arteries on the right and left sides, respectively, by the highest brachial pressure on either side.[4,5,7] The minimum value of ABI was used for the analysis.

Statistical analysis

Statistical analysis was preformed using programs available in the SPSS statistical package (SPSS 15.0, Chicago, USA). All variables were tested for normal distribution of the data. Data are shown as mean ± SD, unless stated otherwise. Univariate linear regression analysis was performed to look for a relationship between ABI and the variables of interest in the study population. Multivariate linear regression analyses were then performed to look for independent associations between ABI and the variables of interest. All independent variables in the multivariate analyses models were tested for multicolinearity. A p-value <0.05 (two-tailed) was considered statistically significant.

Results

Twenty-five (20%) of the study subjects had values of ABI <0.90. The demographic and clinical characteristics of the study population are shown in Table 1.

In the total study population, univariate linear regression analysis showed that ABI was significantly associated with presence of hypertension (p=0.02), fasting serum glucose levels (p=0.02), serum urea

Table 1. Demographic and clinical characteristics of the study population.

Males/females n (%)	84 (67.2) / 41 (32.8)
Age (years)	62.2 ± 9.1
Waist (cm)	101.2 ± 10.8
BMI (kg/m^2)	28.7 ± 4.3
Duration of diabetes (years)	8.9 ± 7.0
HbA1c (%)	7.5 ± 1.6
Systolic blood pressure (mmHg)	138.1 ± 21.2
Diastolic blood pressure (mmHg)	76.9 ± 10.6
Serum glucose (mg/dL)	162.9 ± 58.7
Total cholesterol (mg/dL)	205.8 ± 42.9
HDL cholesterol (mg/dL)	43.1 ± 10.3
LDL cholesterol (mg/dL)	137.5 ± 39.0
Triglycerides (mg/dL)	130.9 ± 67.7
Serum urea (mg/dL)	41.7 ± 12.9
Serum creatinine (mg/dL)	0.9 ± 0.3
eGFR (ml/min/1.73m^2)	99.3 ± 27.8
Serum uric acid (mg/dL)	4.5 ± 1.3
WBC (μL^{-1})	6547 ± 1562
hsCRP (mg/dL)	6.3 ± 1.4
Hypertensives, n (%)	41 (35.2)
Dyslipidemics, n (%)	37 (29.6)
Current smokers, n (%)	17 (13.6)
Diabetes treatment:	
Antidiabetic tablets n (%)	98 (78.4)
Insulin n (%)	26 (20.4)

Data are given as mean value ± standard deviation or as n (%). BMI – body mass index; HDL – high density lipoprotein; LDL – low density lipoprotein; eGFR – estimated glomerular filtration rate; WBC – white blood cell count; hsCRP – high-sensitivity C-reactive protein.

levels (p=0.005), serum UA levels (p=0.007) and WBC count (p= 0.04).

There was a suggestive association with the duration of diabetes (p=0.09) and insulin therapy (p=0.09). No significant associations were found between ABI and sex, age, waist circumference, presence of dyslipidemia, smoking status, BMI, HbA1c, systolic and diastolic blood pressure, oral antidiabetic treatment, serum creatinine, total cholesterol levels, high density lipoprotein cholesterol levels, LDL-cholesterol levels, triglyceride levels, hsCRP, or eGFR.

Multivariate linear regression analysis demonstrated, after controlling for the presence of hypertension, serum urea and UA levels, significant independent associations between ABI and fasting serum glucose levels (p=0.03) as well as WBC count (p=0.03) (Table 2).

Discussion

The results of the present study showed that ABI was related with fasting serum glucose levels as well as WBC count in T2DM subjects. A secondary, but noteworthy, result was the high proportion of diabetic subjects without apparent macrovascular disease who had ABI values lower than 0.90.

Studies in populations with different cardiovascular risk factors have showed a relationship between ABI and glycemic control.[12-16] Two studies in hypertensive subjects showed that high fasting glucose levels and a history of diabetes were associated with low ABI.[12,13] Another study in a high risk population showed that ABI was related with diabetic status, independently of other cardiovascular risk factors.[14] Also, a study in subjects with newly diagnosed PAD showed an association between ABI and the presence of diabetes.[15] A large scale study showed that an association existed between higher levels of HbA1c and PAD, even among subjects without diabetes.[16]

However, the data in the literature regarding the effect of glycemic control on PAD in T2DM subjects are conflicting. A study in T2DM subjects showed that glycemic control in terms of fasting serum glucose and HbA1c was not associated with PAD.[6] The above findings are in agreement with the results of the Hoorn study, showing that ABI was not associated with serum glucose levels in T2DM subjects.[17]

WBC count, a common marker of systemic inflammation, is known to be associated with atherosclerotic cardiovascular disease[18] and the progression of subclinical atherosclerosis.[19] A large epidemiological study showed that blood rheological factors and leukocyte activation are associated with lower limb ischemia in the general population.[20] In a representative sample of the United States population, after adjustment for other inflammatory markers, monocytes were significantly and independently associated with PAD.[21] A recent study in Greece showed an association between aortic distensibility, an index of arterial stiffness, and WBC count in healthy subjects, showing the important role of underlying inflammation.[22] It is well known that inflammation initiates PAD through the adherence of leukocytes to the vessel wall, the increased membrane permeability and the production of cytokines, leading to the recruitment of macrophages and the proliferation of macrophages and smooth muscle cells within the vessel wall.[23,24]

Finally, in the present study a high proportion of diabetic subjects had values of ABI lower than 0.90, implying subclinical atherosclerosis. Higher rates of subclinical cardiovascular disease among diabetic subjects have also been reported in different studies.[3,25,26] Additionally, a Spanish study showed that the preva-

Table 2. Univariate and multivariate regression analysis: the association between various parameters and ankle brachial index in the study population.

	Univariate analysis		Multivariate analysis	
	Correlation coefficient	p	Standardized coefficients beta	p
Age	-0.10	0.26	-	-
Sex	-0.09	0.31	-	-
Waist	-0.12	0.15	-	-
BMI	-0.01	0.92	-	-
Duration of diabetes	-0.15	0.09	-	-
Systolic blood pressure	-0.14	0.12	-	-
Diastolic blood pressure	-0.01	0.96	-	-
HbA1c	-0.07	0.45	-	-
Serum glucose	-0.21	**0.02**	-0.18	**0.03**
Total cholesterol	0.07	0.41	-	-
HDL- cholesterol	-0.01	0.87	-	-
LDL- cholesterol	0.12	0.19	-	-
Triglycerides	-0.09	0.34	-	-
Serum urea	-0.25	**0.005**	-0.15	0.08
Serum creatinine	-0.12	0.19	-	-
eGFR	0.06	0.48	-	-
hsCRP	-0.07	0.45	-	-
Serum uric acid	-0.24	**0.007**	-0.14	0.14
White blood cell count	-0.18	**0.04**	-0.18	**0.03**
Hypertensives	-0.21	**0.02**	-0.13	0.14
Dyslipidemics	-0.07	0.46	-	-
Current smokers	-0.10	0.26	-	-
Insulin	-0.15	0.09	-	-
Antidiabetic tablets	0.13	0.14	-	-

Significant associations are highlighted. Abbreviations as in Table 1.

lence of a low ABI was elevated in asymptomatic subjects with coronary or cerebrovascular disease.[14]

Study limitations

A major limitation of the present study was the small number of diabetic subjects with ABI values lower than 0.90. Therefore, further analysis of the study variables between diabetic subjects with ABI <0.90 versus diabetic subjects with ABI >0.90 could not be done in order to find possible determinants of PAD. Another major limitation was that we used the patients' medical history in order to exclude subjects with apparent macrovascular disease, since we were not able to perform either noninvasive or invasive tests for the diagnosis of macrovascular disease. Finally, the diabetic subjects were recruited from the population of a rural area attending a referral Health Center; therefore, our results can not be extrapolated to the total diabetic population.

Conclusion

The results of the present study showed that elevated plasma glucose levels and WBC count increase the risk of PAD in asymptomatic diabetics. Therefore, interventions aiming at better glycemic control might help diabetic subjects who are at increased risk of subsequent cardiovascular events.

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