Peripheral artery disease and oxidative stress in patients in a program for preventing complications of diabetes mellitus and dyslipidemia

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Abstract

Introduction: Peripheral artery disease (PAD) is mainly caused by atherosclerosis but also involves hyperglycemia and dyslipidemia, which trigger oxidative stress and lead to vascular damage.

Objectives: To determine the prevalence of PAD in patients with type 2 diabetes mellitus (DM2) and/or prediabetes and/or dyslipidemia, to identify some risk factors and to establish whether urinary levels of 8-isoprostane-f2α (an oxidative stress marker) are elevated in patients with PAD.

Design: A cross-sectional, nonprobabilistic, convenience sampling study.

Materials and methods: The sample included 146 patients with DM2 and/or prediabetes and/or dyslipidemias from the Universidad Nacional de Colombia.

Risk factors, symptoms related to PAD, ankle-brachial index measurement and biochemical variables (HbA1c%, fasting blood glucose, lipid profile, creatinine and albuminuria) were recorded. Urine levels of 8-isoprostane-f2α were determined by ELISA. The 8-iso-PGF2α/creatine concentration were analyzed using the statistical package R. Risk factors were compared using ANOVA/Kruskal-Wallis. ROC curves were generated to analyze the discriminant power of the biomarkers. The joint analysis of laboratory results and risk factors was performed using multivariate logistic regressions.

Results: PAD was identified in 10 diabetic patients. Risk factors were smoking, dyslipidemia, poor metabolic control, overweight or obesity. There was no evidence of increased urinary 8-isoprostane-f2α in these subjects.

Conclusions: A low prevalence of PAD was found in subjects with DM2. There was no evidence of increased 8-isoprostane-f2α measured by ELISA in patients with PAD. The extension of the study with different markers of oxidative stress and the use of other techniques is recommended (**Acta Med Colomb 2019; 44. DOI: https://doi.org/ 10.36104/amc.2019.1257).**

Key words: d*iabetes, dyslipidemia, peripheral artery disease, oxidative stress, 8-isoprostane-f2α.*

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Background

Peripheral artery disease (PAD) affects approximately 12 million people in the United States (1). The most important risk factors for developing the disease are type 2 diabetes mellitus (T2D) and smoking (2-4). The clinical picture of PAD varies from the absence of symptoms to classic claudication (5-7). The diagnosis can be made by multiple methods; the most cost-effective index is the ankle-brachial index (ABI) (8, 9). PAD is mainly caused by atherosclerosis, the pathogenesis of which is mediated by multiple molecules and signaling pathways (10-12). Hyperglycemia causes an overproduction of free radicals and reactive oxygen species (ROS) and the formation of advanced end-glycation products that cause endothelial damage (13-15). ROS also interact with cell membrane lipids, leading to lipid peroxidation and increasing the formation of 8-isoprostane-f2α (8-iso-PGF2α). Elevated concentrations of LDL cholesterol (LDL-c) favor the development of atherosclerotic plaques via the oxidation of LDL (LDLox), which is involved in processes related to the production of ROS and the expression of pro-inflammatory genes (16). Efforts have been made to measure oxidative stress using biomarkers;

8-isoprostane-f2α has the advantage of being quantifiable in casual urine (17).

Justification

Patients with DM2 have a two to three times greater risk of suffering from PAD (2), and this risk can lead to severe complications such as pressure ulcers and amputation (18), which is why early diagnosis is important. In Colombia, to our knowledge, there is no study on the prevalence of PAD in the diabetic and/or dyslipidemic populations. Multiple studies have associated DM2 and dyslipidemia with increased oxidative stress (19) and PAD (20, 21). Several biomarkers have been used to measure oxidative stress, one of which is quantifiable 8-iso-PGF2α (17) in urine. It is of great interest to establish the prevalence of PAD and its relationship with metabolic control, body mass index (BMI) and oxidative stress, determined by urinary measurement of 8-iso-PGF2α, in patients in a program for the prevention of complications of diabetes mellitus and dyslipidemia offered by the Faculty of Medicine of the National University of Colombia (interdisciplinary team consisting of a physician, nutritionist and physiotherapist). Efforts to identify possible risk factors and events associated with the presence of PAD are also being made. The understanding of these relationships could advance the prevention and treatment of complications in diabetic patients and/or patients with dyslipidemia.

Materials and Methods

After the patients signed the informed consent form, the following measurements were recorded: body mass index (BMI kg/m^2), waist circumference (cm) and blood pressure (mmHg). An OMRON® scale, Health o Meter® height rod, 3M Littman® stethoscope, Welchallyn® tensiometer and Huntleigh® Doppler system were used. An instrument designed to detect PAD risk factors was administered; the instrument included items pertaining to pathology and family history, habits (consumption of alcohol and cigarettes and physical activity levels), symptoms related to PAD (pain in the lower limbs while walking, standing or sitting; pain or the absence of it when stopping walking), atypical symptoms (pain in the thigh or buttock, erectile dysfunction, paresthesia in the extremities), physical examination (identification of ulcers and neurological examination with a tuning fork, monofilament and reflex hammer) and the measurement of ABI, for which systolic blood pressure was determined with a Doppler system and tensiometry in the brachial artery and the dorsalis pedis and posterior tibial arteries (22). Glycated hemoglobin (HbA1c), fasting blood glucose, triglycerides, total cholesterol, high-density cholesterol (HDL-c), LDL-c (calculated according to the Friedewald formula), creatinine and albuminuria were evaluated at every follow-up in the laboratory of the Health Services Unit of the National University of Colombia (Unisalud). Before the ABI determination, urine was collected and separated into 2 aliquots that were stored at -80 °C until analysis for subsequent determination of creatinuria by the colorimetric method and of levels of 8-iso-PGF2α by ELISA according to the manufacturers (Cell Biolabs).

Type of study. The study had a cross-sectional, nonprobabilistic, convenience sampling design and used descriptive tools to explore the behavior of the phenomenon, such as the correlation of variables with various demographic and thematic domains.

Sample size. The sample studied included 146 patients with DM2 and/or prediabetes and/or dyslipidemias who were treated at the Health Services Unit of the National University of Colombia (Unisalud) and participated in a program for the prevention of complications of diabetes mellitus and dyslipidemia offered by the Faculty of Medicine of the National University of Colombia. Patients attend periodically (every three months) under medical supervision at the School of Medicine.

Inclusion criteria. Users of Unisalud who were men or women older than 18 years with DM2 and/or prediabetes diagnosed according to the criteria of the *American Diabetes Association* (ADA) and/or dyslipidemia according to the criteria of the *American Association of Clinical Endocrinologists* (AACE).

Exclusion criteria. Active autoimmune disease, active neoplasia, psychiatric disorders being treated with medication, pregnancy.

Statistical analysis. Data and concentration results of the biomarker (8-iso-PGF2α/creatinuria) were analyzed using the statistical package R. The comparison and study of the different risk factors were analyzed by ANOVA/Kruskal-Wallis tests. ROC curves were generated to analyze the discriminant power of the biomarker. Multivariate logistic regressions were performed for the joint analysis of the different laboratory results and risk factors. When the differences between variables met the 95% confidence criterion ($p<0.05$), they were considered statistically significant.

Ethical considerations. The present project complied with the ethical principles for medical research on humans established by the World Medical Association in its Declaration of Helsinki. Furthermore, it was evaluated and approved by the Ethics Committee of the School of Medicine of the National University of Colombia.

Results. The general characteristics of the 146 subjects are shown in Table 1.

After the ABI analysis, 10 subjects with ABI less than 0.9 were found. These patients had DM2; therefore, the prevalence of PAD in subjects with DM2 was 8.7%. Thirty-three percent of the 115 subjects with DM2 had poor metabolic control, with an HbA1c≥7%; regarding LDL-c, 46% of the diabetic subjects had good control of dyslipidemia. Of the subjects with coronary artery disease, 21% had poor metabolic control (HbA1c≥7%), and 17% had LDL-c within the recommended target (<70 mg/dL). Of the 146 subjects, 47% were overweight, and 21% were obese. The 10 patients with PAD, three women and seven men aged between 69 and 85

years, presented with T2DM and dyslipidemia. One of the 10 subjects smoked, three showed intermittent claudication, and three showed poor metabolic control. Nine of the subjects with PAD had a BMI≥25 kg/m^2. Risk factors for PAD were smoking, OR 1.51 (CI 0.18, 13); dyslipidemia, OR 1.62 (CI 0.2, 13.42), and poor metabolic control, OR 1.04 (CI 0.26, 4.22); having a BMI≥25 kg/m^2 increased the risk of PAD by four times. Finally, physical activity was found to be a protective factor against PAD, with an OR of 0.41 (CI 0.1, 1.73).

8-iso-PGF2α was measured in the 10 patients with PAD (diagnosed according to an ABI<0.9) and in 30 selected patients from the initial sample who were classified into four groups according to metabolic control, age and history of coronary artery disease (23; 29). The results are shown in Table 2.

None of the subjects showed an abnormal 8-iso-PGF2α/creatinine ratio, i.e., greater than 0.86 pg/mg. When the different groups were compared, no statistically significant difference was found (p=0.759).

The results for group 4 (subjects with good metabolic control, without coronary heart disease and without PAD) were compared with those of the other groups, and no significant differences were found (Table 3).

Table 1. *General characteristics of the study population.*

General characteristics	Number of patients (%)
Total	146 (100)
Females	72 (49)
Males	74 (50)
Age 40-60 years	15 (10)
Age 60-70 years	51 (35)
Age 70-90 years	79 (54)
Age 90-100 years	1 (0.6)
Type 2 diabetes mellitus	115 (78)
Prediabetes	29 (19)
Dyslipidemia	127 (86)
Systemic hypertension	85 (60)
Heart disease	23 (15)
BMI 20-24.9 kg/m^2	38 (26)
BMI 25-29.9 kg/m^2	69 (47)
BMI 30-34.9 kg/m^2	31 (21)
BMI 35-39.9 kg/m^2	7 (4)
LDL-c less than 100 mg/dL	81 (55)
LDL-c 100 -159 mg/dL	53 (36)
LDL-c 160 -189 mg/dL	10 (6)

BMI: body mass index (kg/m^2); LDL-c (low-density cholesterol)

Discussion

The prevalence of peripheral artery disease found in the present study was lower than that described in the North American, Australian, Belgian, Costa Rican and Mexican populations (1, 25-28). There are no prevalence data in Colombia; however, this can be considered a pilot study (29). The patients in the study received regular medical check-ups, which facilitated the identification of limitations that hinder adherence to management and metabolic control; therefore, the prevalence of PAD in this group could be lower than that of other groups without regular follow-up. In this study, 67% of the diabetic patients were within HbA1c targets. This is in agreement with the UKPDS study (30), in which good glycemic control was associated with a decreased risk of macrovascular complications. Likewise, a systematic review and meta-analysis of the University of Bern found that better glycemic control in patients with DM1 and DM2 is associated with a decrease in the incidence of macrovascular events, especially PAD and stroke (31). Smoking habits were noted in the patients with PAD; although the intensity and duration of smoking were not investigated, it has been

Table 2. *F2-ISoPs/creatinine ratios (pg/mg) by group.*

		Group					
		Group 1 with PAD	Group 2 Poor metabolic control, no heart disease, >65 years	Group 3 Poor metabolic control, no heart disease, <65 years	Group 4 Good metabolic control, no heart disease, >65 years	Group 5 Good metabolic control, heart disease, >65 years	Subtotal
F2-ISoPs/creatinine ratio (pg/mg)	Mean	0.0102	0.0241	0.0159	0.0209	0.0029	0.0148
	Standard deviation	0.0273	0.0579	0.0351	0.0497	0.0033	0.0383
	Maximum	0.088	0.1671	0.0953	0.1434	0.0098	0.1671
	95th percentile	0.088	0.1671	0.0953	0.1434	0.0098	0.1193
	Median	0.0013	0.0023	0.0022	0.0017	0.0013	0.0018
	5th percentile	0.0005	0.0009	0.001	0.0009	0.0007	0.0007
	Minimum	0.0005	0.0009	0.001	0.0009	0.0007	0.0005

Table 3. *Statistical significance of the comparison of the F2-ISoPs/creatinine ratio (pg/mg) between the groups.*

Comparison groups		P value
Group 4	Group 1	0.3
Group 4	Group 2	0.453
Group 4	Group 3	0.412
Group 4	Group 5	0.171

found that the prevalence and incidence of PAD is higher in patients with high tobacco consumption and prolonged exposure time (32-35). The majority of the patients with PAD had a BMI≥25. A study with a Chinese population observed that the increase in BMI was directly related to the presence of PAD (35). Obese patients show elevated levels of triglycerides and circulating free fatty acids, which have been associated with increased production of free radicals (37). In a group of obese diabetic patients from Iran, an increase in the plasma concentration of 8-iso-PGF2α measured by ELISA was observed (38). All patients with PAD in this study had DM2 and dyslipidemia. T2DM and dyslipidemia increase oxidative stress and increase the risk of PAD (11, 12, 39). 8-iso-PGF2α, the most abundant marker of its class and a good biological marker of oxidative stress, also has the advantage of being quantifiable in a urine sample. No increase in 8-iso-PGF2α was found in the patients with PAD, and no statistically significant differences between the patients with PAD and the other groups. Several factors could explain this result: ELISA was used for its quantification, while other studies used liquid or gas chromatography coupled with mass spectrometry (40, 41). In addition, urinary concentrations may be different than plasma concentrations because in urine, only free forms are measured (42).

Conclusions

A low prevalence of PAD was found in patients with DM2 from a program for the prevention of complications of DM2 and dyslipidemia offered by the School of Medicine of a Colombian university. This low prevalence may be due to the periodic monitoring of patients. Regular monitoring by an interdisciplinary group composed of a doctor, nutritionist and physiotherapist seems to have a favorable effect on modifiable risk factors. There was no evidence of increased urinary levels of an oxidative stress marker, 8-iso-PGF2α, as measured by ELISA in patients with PAD. Extension of the study with other markers of oxidative stress and the use of techniques such as liquid or gas chromatography coupled with mass spectrometry is recommended.

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