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Original Research

Influence of diabetes on ambulation and inflammation in men and women with symptomatic peripheral artery disease

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ABSTRACT

Objective: To determine whether diabetes and sex were factors associated with ambulatory function, endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity in patients with peripheral artery disease (PAD) and claudication.
Materials/Methods: Ambulatory function of 180 symptomatic men and women with PAD was assessed during a graded maximal treadmill test, 6-minute walk test, and 4-meter walk test. Patients were further characterized on endothelial effects of circulating factors present in the sera using a cell culture-based bioassay on primary human arterial endothelial cells, and on circulating inflammatory and vascular biomarkers.
Results: Men and women with diabetes had greater prevalence ($p = 0.007$ and $p = 0.015$, respectively) of coronary artery disease (CAD) than patients without diabetes. To assure that this difference did not influence planned comparisons, the data set was stratified on CAD. Diabetic men with CAD had a lower peak walking time (PWT) during the treadmill test and a slower 4-meter gait speed compared to non-diabetic men with CAD ($p < 0.05$). Diabetic women with CAD had a lower PWT compared to their non-diabetic counterparts ($p < 0.01$). Additionally, diabetic men with CAD had higher pigment epithelium-derived factor ($p < 0.05$) than their non-diabetic counterparts, and diabetic women with CAD had higher leptin ($p < 0.01$) and interleukin-8 levels ($p < 0.05$).
Conclusions: In patients with PAD, diabetic men and women with CAD had more severe claudication than their non-diabetic counterparts, as measured by shorter PWT, and the men had further ambulatory impairment manifested by slower 4-meter gait speed. Furthermore, the diabetic patients with CAD had elevations in interleukin-8, leptin, and PEDF.

Introduction

PAD is a significant medical concern, as it is a highly prevalent [1], costly [2], disabling [3,4], and deadly condition [5]. Exercise therapy is a primary treatment for patients with PAD, as the COT, PWT, and 6-minute walk distance increase following a program of exercise rehabilitation [6–9]. However, the response to a program of exercise rehabilitation is variable, as we recently found that diabetic women responded poorly to a program of exercise compared to other subgroups of patients despite no difference in exercise adherence [10].

The relatively poor exercise response in diabetic women with PAD may be due to several possible factors. We have previously found that women have greater impairment in ambulation [11] and vascular function [12] compared to men. Furthermore, we recently found that women have greater inflammation than men [13], and that inflammation and anti-oxidant capacity were predictors of COT, PWT, and calf muscle hemoglobin oxygen saturation during exercise [14].

Abbreviations: ABI, ankle/brachial index; COT, claudication onset time; HsCRP, high sensitivity C-reactive protein; NF-κB, nuclear factor K-light-chain-enhancer of activated B cells; PAD, peripheral artery disease; PEDF, pigment epithelium-derived factor; PWT, peak walking time; ROS, reactive oxygen species

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In addition to sex differences in ambulation and inflammation, diabetes has been found to impair ambulation in several [15,16], but not in all studies [17]. Diabetes may differentially impact women and men with PAD, but little is known about the sex-specific effect of diabetes on ambulatory and inflammatory profiles in patients with symptomatic PAD.

The primary aim of the current study was to determine whether diabetes and sex were factors associated with ambulatory function, endothelial cell inflammation, oxidative stress, and apoptosis, and with circulating biomarkers of inflammation and antioxidant capacity in patients with PAD and claudication. We hypothesized that patients with diabetes have impaired ambulation, greater endothelial inflammation, cellular ROS production, and apoptosis, and worse circulating inflammatory biomarkers and antioxidant capacity than those without diabetes. Furthermore, we hypothesized that the negative impact of diabetes on these outcome measures are more prominent in women than in men.

Methods

Patients

Approval and informed consent

The institutional review board at the University of Oklahoma Health Sciences Center approved the procedures of this study. Written informed consent was obtained from each patient at the beginning of investigation.

Recruitment

Patients who were not currently exercising were recruited from vascular laboratories and vascular clinics from the University of Oklahoma Health Science Center for possible enrollment into an exercise rehabilitation program to treat leg pain secondary to PAD.

Baseline clinical characteristics obtained from a medical history and physical examination

Patients were evaluated in the morning at the Clinical Research Center, at the University of Oklahoma Health Science Center. Patients arrived fasted, but were permitted to take their usual medications. To begin the study visit, patients were evaluated with a medical history and physical examination in which demographic information, height, weight, waist circumference**,** cardiovascular risk factors, co-morbid conditions, claudication history, ABI, and a list of current medications were obtained. Following the medical history and physical examination, nursing personnel obtained blood samples, and exercise personnel performed the exercise tests. The nursing and exercise personnel were blinded to the results from the medical history and physical examination, including the diabetes status of the patients.

Inclusion and exclusion criteria

Patients with symptomatic PAD were included in this study if they met the following criteria: (a) a history of ambulatory leg pain, (b) ambulatory leg pain confirmed by treadmill exercise [3], and (c) an ABI ≤ 0.90 [18] at rest or ≤0.73 after exercise [19]. Patients were excluded for the following conditions: (a) absence of PAD (ABI > 0.90 at rest and ABI > 0.73 after exercise), (b) non-compressible vessels (ABI ≥ 1.40), (c) asymptomatic PAD, (d) use of medications indicated for the treatment of claudication (cilostazol or pentoxifylline) initiated within three months prior to investigation, (e) exercise limited by other diseases or conditions, (f) active cancer, (g) end stage renal disease defined as stage 5 chronic kidney disease, and (h) abnormal liver function. A consecutive series of 268 individuals were evaluated for eligibility, and 180 patients were deemed eligible for inclusion into the study.

Patients were grouped according to their diabetes status and sex. Diabetes was confirmed through medical history and list of medications, or by a glucose value of greater than or equal to 126 mg/dl in those patients without history or medication for diabetes. All patients with diabetes had Type 2 diabetes.

Measurements

COT and PWT obtained from a graded maximal treadmill test

Patients performed a graded treadmill test to determine study eligibility, and then repeated the test on a following visit within one week to obtain the primary outcome measures of COT and PWT as previously described [3,6]. Using our procedures, the test-retest intraclass reliability coefficient is $R = 0.89$ for COT [3] and $R = 0.93$ for PWT [3].

Total walk distance obtained from a 6-minute walk test

Patients performed an over-ground, 6-minute walk test supervised by trained exercise technicians, as previously described from our laboratory [20]. The total distance walked during the test was recorded. The test-retest intraclass reliability coefficient is $R = 0.94$ for total 6-minute walking distance [20].

Gait speed obtained from a 4-meter walk test

Gait speed was measured from a 4-meter walk test in a hallway [21]. Patients performed this test twice at their usual walking pace, and the faster of the two walks was used in the analyses. The test-retest intraclass reliability coefficient is $R = 0.96$ for the velocity to walk four meters [22].

Blood sampling

Blood was drawn by venipuncture from an antecubital vein, collected in vacutainers, and distributed in 0.5 ml aliquots. The samples were stored at –80 °C, and were subsequently batched for analysis.

Endothelial cell cultures

A cell culture-based bioassay approach utilizing cultured primary human arterial endothelial cells was used to characterize the endothelial effects of circulating factors present in the sera of patients. In brief, endothelial cells (purchased from Cell Applications, Inc., San Diego, CA, after passage 4; age of the donors is unknown) were initially cultured in MesoEndo Endothelial Cell Growth Medium (Cell Applications, Inc.) followed by Endothelial Basal Medium supplemented with 10% fetal calf serum until the time of serum treatment, as described [23]. Inter-individual variance is unlikely to contribute to observed differences because detector cells used for each in vitro study were from the same donor. For treatment, fetal calf serum was replaced with serum (10%; for 24–48 h) collected from our patients [23]. Cells cultured in Endothelial Basal Medium supplemented with 10% fetal calf serum served as an additional control.

Apoptosis assay

Cultured endothelial cells were treated with sera from patients for 24 hours. Caspase activities using Caspase-Glo 3/7 assay kit (Promega, Madison, WI) were measured to assess apoptotic cell death, as previously reported [23].

Cellular ROS production

Hydrogen peroxide production in detector endothelial cells was measured fluorometrically using the Amplex Red/horseradish peroxidase assay to determine cellular oxidative stress induced by factors present in the sera [23].