Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Plasma markers of inflammation and prediction of cardiovascular disease and mortality in African Americans with type 1 diabetes

Monique S. Roy [a,*], *Malvin N. Janal* [b], *Juan Crosby* [c], *Robert Donnelly* [c]

[a] *Institute of Ophthalmology and Visual Science, Rutgers University, Newark, NJ, United States*
[b] *Department of Epidemiology and Health Promotion, NYU College of Dentistry, New York, NY, United States*
[c] *Department of Pathology and Laboratory Medicine and NJMS Molecular Resource Facility, Rutgers University, Newark, NJ, United States*

ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form
29 October 2015
Accepted 28 December 2015
Available online 12 January 2016

Keywords:
Biomarkers
Inflammation
Diabetes
Cardiovascular disease
Mortality

ABSTRACT

Background: To determine whether plasma levels of markers of inflammation are predictive of the incidence of cardiovascular disease (CVD), hypertension, or mortality in African Americans with type 1 diabetes mellitus.
Methods: A total of 484 African Americans with type 1 diabetes were included. At baseline and 6-year follow-up, a clinical interview and examination were conducted to document CVD and systemic hypertension. Venous blood for glycated hemoglobin and cholesterol was obtained and albumin excretion rate measured. Mortality was assessed annually between baseline and 6-year follow-up by review of the social security death index. Baseline plasma levels of 28 inflammatory biomarkers were measured using multiplex bead analysis system.
Results: After adjusting for baseline age and other confounders, African Americans with type 1 diabetes in the highest quartile of plasma interferon-inducible protein 10 (IP-10) were three times more likely to develop CVD than those in the lowest quartile. African Americans with type 1 diabetes in the lowest quartiles of plasma stromal derived factor-1 (SDF-1) had a 75% higher risk of death than patients in the highest quartile, independently of age, low density lipoprotein cholesterol, body mass index, hypertension, and albuminuria.
Conclusion: In African Americans with type 1 diabetes, high plasma IP-10 is an independent predictor for incident CVD and low SDF-1 an independent predictor for mortality.

1. Introduction

Macroangiopathy, hypertension, and associated morbidity are common complications of type 1 diabetes, particularly in African Americans [1–5]. Older age, body mass index, systemic hypertension, proteinuria, and hypercholesterolemia are clinical predictors of CVD [3]. Pathological findings associated with large vessel disease also suggest that inflammation may participate in the initiation and progression of the atheroma process [6,7]. Among markers of inflammation and endothelial dysfunction, the acute phase protein C-reactive protein has been the most consistently associated with either presence or incidence of CVD [8,9]. Circulating CRP is elevated in patients

* Corresponding author. Tel.: +1 973 972 2029; fax: +1 973 972 2068.
E-mail address: monique.roy@rutgers.edu (M.S. Roy).
http://dx.doi.org/10.1016/j.diabres.2015.12.014

with atherosclerosis and predicts CVD and hypertension in selected at risk populations [10,11].

In type 1 diabetes, blood levels of various inflammatory markers have been shown to be elevated [12–14]. However, the few studies evaluating inflammation as a potential marker for macroangiopathy in mostly Caucasian persons with type 1 diabetes have yielded conflicting results because of cross-sectional designs, a restricted range of inflammatory markers being measured, and/or methodological problems associated with low plasma (or serum) levels of the markers [12,15].

We previously assembled, examined, and subsequently re-examined (6 years later) a large cohort of African Americans with type 1 diabetes (the New Jersey 725) [1,16]. In those patients, prevalence of CVD including hypertension and mortality are particularly high [2–5]. The purpose of the present study was to examine whether specific plasma markers of inflammation or endothelial dysfunction predict the incidence of CVD (i.e., coronary disease, stroke, or lower extremity amputation), hypertension, and/or mortality in this cohort independently of the known clinical predictors.

2. Methods

2.1. Study population

The original cohort consisted of 725 African Americans with type 1 diabetes who participated in the New Jersey 725 study between 1993 and 1998 [1]. Patients were identified from among 68455 African Americans listed in the New Jersey Department of Health computerized Hospital Discharge Data as having a diagnosis of DM. Of those, a review of randomly chosen 13615 patient charts was conducted in 31 New Jersey participating hospitals. Patients with a discharge diagnosis of type 1 diabetes, acute onset of DM, treated with insulin before 30 years of age, and currently on insulin were included [17]. The use of insulin was confirmed at the time of first contact with the patient. Of the 875 eligible patients, 725 were enrolled. Of the 725, 508 (70.1%) underwent a 6-year follow-up examination, 44 (6.1%) could not be located, 34 (4.7%) refused examination, and 139 (19.2%) had died [16].

At the 6-year follow-up, 25 (4.9%) participants who were no longer on insulin were excluded for any further analyses. Also excluded from the analyses were seven patients with systemic conditions which could be confounders in the relationship between the inflammatory markers and incidence of CVD: two patients with systemic lupus, one with Buerger's disease, and four with congenital heart disease. This report concerns patients who had baseline plasma samples available for measurement of the inflammatory markers, had a 6-year follow-up examination (or died before the 6-year follow-up), did not have any infection, tumor, or renal failure, and were at risk for incident CVD ($N = 320$), hypertension ($N = 218$), or death ($N = 484$). The mean (±standard deviation) follow-up was 6.0 (±0.3) years.

2.2. Procedures

Patients were examined in University Hospital's Eye Clinic, Newark, NJ. The same procedures were followed at baseline and follow-up visits. Upon arrival, informed written consent was obtained. A structured clinical interview was conducted to document medical [past history of coronary disease, stroke, or lower extremity arterial disease (LEAD)], and socio-demographic and life-style variables (smoking, alcohol consumption). Height and weight were obtained. Blood pressure was measured twice in sitting and standing positions, and the average was used. Beck Depression Inventory (BDI), the Hostility and Direction of Hostility Questionnaire (HDHQ) were obtained.

A 4-h timed urine collection was obtained for measurement of albumin excretion rate (AER) and creatinuria, using spectrophotometry (Quest Diagnostics, Madison, NJ). Venous blood was drawn for measurement of creatinine by spectrophotometry, high (HDL) and low (LDL) lipoprotein cholesterol and total cholesterol using an enzymatic assay and separation spectrophotometry (Quest Diagnostics), and of total glycated hemoglobin using high-pressure liquid chromatography (LabCorp, Burlington, NC). A 2 mL venous blood sample was collected by venipuncture in an EDTA-coated vacutainer tube, the content was thoroughly mixed and plasma separated by centrifugation and stored frozen at −70 °C for future assay.

The research followed the tenets of the Declaration of Helsinki. The Institutional Review Board of the New Jersey Medical School, Newark, NJ approved the study.

2.3. Measurements of the inflammatory biomarkers

Juan Crosby, who measured the markers, was masked to the clinical data. Baseline plasma samples were analyzed for 28 inflammatory biomarkers: 9 cytokines and soluble receptors [interleukin-1α (IL-1α), IL-2 receptor (IL-2R), IL-6, IL-10, IL-12p40, IL-12p70, soluble CD40 ligand (sCD40), tumor necrosis factor (TNF-α), interferon-γ (IFN-γ)]; three growth factors, granulocyte macrophage colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF); 12 chemokines [eotaxin, fractalkine, growth-related oncogene-α (GRO-α), IL-8, monocyte chemoattractant protein-1(MCP-1), MCP-3, regulated on activation normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1ß (MIP-1β), IP-10, SDF-1, neutrophil-activating peptide (ENA-78)]; 4 soluble adhesion molecules, E-selectin, soluble intercellular adhesion molecule (sICAM-1), soluble vascular cellular adhesion molecule-1 (sVCAM-1), and C-reactive protein (CRP).

Biomarker concentrations were measured using a multiplex bead analysis system (Milliplex X-MAP, EMD Millipore Corp, Billerica, MA). Intra-assay and inter-assay variations were <15% and 18%, respectively. Measurements were done using 25 μL samples. After overnight incubation in 96-well plates, the specific fluorescence corresponding to each biomarker was measured on the Luminex 100 instrument (Luminex, Austin, TX). Quantification was done against 4-parameter logistic regression-generated standard curves using the reference cytokine standards supplied by the kit manufacturer. For statistical analysis, the biomarker concentrations below the lowest concentration point in the standard curves were given a value of zero.