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Pro-inflammatory cytokines correlate with classical risk factors for atherosclerosis in the admixed Brazilian older women

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ABSTRACT

Aim: Measurement of inflammatory markers for risk stratification of vascular disorders has been the focus of numerous investigations worldwide, and usually reveals augmented levels of circulating cytokines/chemokines among carriers of classic risk factors for atherosclerosis. Nonetheless, this low-grade inflammatory milieu detected in aged individuals tends to be influenced by body composition. Moreover, cardiovascular risk factors have a complex genetic etiology, and disregarding the genetic heritage may produce spurious results owing to interethnic differences. In this complex scenario, our study was designed to verify the existence and strength of the association between selected mediators of systemic inflammation and classic risk factors of cardiovascular diseases (CVD).
Methods: In a sample of post-menopausal older women, correlation analyses explored the association of circulating levels of IL1α, IL1β, IL8, IL10 and IL12 with atherosclerosis-related clinical/metabolic parameters, using age, body mass index (BMI), genetic ancestry estimates as standard correction factors. Further adjustment for use of therapeutic agents was applied when appropriate.
Results: Our analyses revealed association of $\log_{10}$-transformed IL-12 titers with VLDL-c levels ($r = .192$; $p = .002$) and with SBP ($r -.185$; $p = .003$), and of $\log_{10}$-transformed IL-8 titers with GLY ($r = .235$; $p < .001$).
Conclusion: Interpretation to the results account to a possible dysregulation of the PPAR signaling pathway to explain the association of IL12 and VLDL-c, and to IL8-driven mechanisms to promote dysglycemia. No previous report sought to investigate the relationship between this set of inflammatory markers and classic risk factors for atherosclerosis correcting for the heterogeneity in genetic admixture and body composition of Brazilian post-menopausal women.

1. Introduction

CVD have been the leading cause of death for the past 50 years, with emphasis to the atherosclerotic disease (Beaglehole & Bonita, 2008). The search of markers for vascular events in populations of different ethnic backgrounds have been carried out worldwide, and now a number of classic cardiovascular risk factors can reliably be identified in middle-aged and older adults, such as diminished levels of high-density lipoprotein cholesterol (HDL-c) and augmented levels of VLDL-c, total cholesterol (TC), SBP/DBP and fasting blood sugar (Casiglia & Palatini, 1998). Nonetheless, a body of evidence remains to be built on what concerns the pathophysiological avenues of dysregulation underlying the onset of these classical risk factors, possibly rendering new markers not devised yet. Consequently, to better identify patients at a higher risk for the atherosclerotic disease, the important players need to be established, especially in aged populations and with widespread applicability.

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Measurement of inflammatory markers for risk stratification and prevention of vascular disorders has been the focus of numerous of these investigations (Ridker, Hennekens, Buring, & Rifai, 2000; Ridker, Rifai, Stampfer, & Hennekens, 2000; Skoog et al., 2002; Vasan et al., 2003; Zhou, Shi, Gao, & Shen, 2001), motivated by observations that inflammation is a key process in the pathophysiology of atherosclerosis and development of acute cardiovascular syndromes. Low-grade, nonspecific inflammatory activity (detected as augmented levels of circulating cytokines and chemokines in the blood) is a highly prevalent finding in the aged population affected by one or more chronic disorders (Dhingra et al., 2007). Thus, it is of much relevance to study the systemic levels of important inflammatory mediators in humans that show high metabolic risk.

Most CVD risk factors have a complex genetic and life style etiology. Disregarding the genetic heritage of individuals for instance may produce non-generalizable findings owing to ethnic differences (genetic, social and cultural) in CVD risk (Chaturvedi, McKeigue, & Marmot, 1993; Cruickshank, Cooper, Burnett, MacDuff, & Drubra, 1991; Deo et al., 2009). The Brazilian population presents great heterogeneity as result of interethnic crosses between Iberian Europeans, Sub-Saharan Africans and the Amerindian, native population. While self-reported race/ethnicity is often applied as a surrogate of an individual's ancestry, the use of explicit genetic information by means of informative molecular markers provide a more accurate estimation of genetic heritage (Lins et al., 2011). Moreover, several studies show that circulating levels of cytokines/chemokines are severely influenced by body composition, especially the fat-mass content (Kim et al., 2006; Suarez-Alvarez et al., 2013), and even implicating adipocyte-released mediators in the development of age-related pathologies (Krabbe, Pedersen, & Bruunsgaard, 2004). In this complex scenario, association studies to isolate the involvement of any particular mediator with the highly intercorrelated variety of age- and obesity-related metabolic disorders should take into account proper adjustment to anthropometry and genetic heritage.

The present study was designed to verify the existence and strength of the association between mediators of systemic inflammatory response and classic risk factors of atherosclerotic disease in a sample of asymptomatic, post-menopausal older women with age as well as genetic ancestry and body composition estimates used as correction factors in our analyses. For this study, we chose to investigate immune mediators whose circulating concentrations have been previously reported as significantly increased in subjects with atherosclerotic lesion or due vascular events (Lee, Yen, Pan, & Chau, 1999; Ridker, Hennekens, et al. 2000; Skoog et al., 2002; Tedgui & Mallat, 2006; Vasan et al., 2003) but with which the literature presented fewer association studies in elderly women.

2. Materials and methods

2.1. Subjects and study design

This report derives from cross-sectional analyses with data obtained from community-dwelling elderly women of the urban outskirts of the Brazilian Federal District, aged 60 or over and inscribed to undergo health screenings and intervention (medical, nutritional and/or pharmacological) to prevent CVD by means of a cohort work know as Prognosis and Therapeutics in Geriatrics (ProTeGer) in Brasília, Brazil. This city (~2.6 million inhabitants) was planned and constructed to bring the administrative capital from the coast to the midwest of Brazil, giving rise to a migration process over the last 50 years. For that reason, the capital's elderly population (~200,000 inhabitants) is considered an expression of the genetic diversity of all Brazilian regions (Nobrega, Faleiros, & Telles, 2009). For the present study, we used data from non-institutionalized consecutive older women aged 60 years or over which have sought the outpatient clinic for preventive care and have never manifested myocardial infarction, stroke or peripheral arterial disease. Additional selection criteria were the absence of autoimmune disease (including rheumatic disorders), chronic or recurrent infections, prior or current neoplastic disease, or use of steroidal or nonsteroidal anti-inflammatory drugs in the past 30 days.

This study was performed in accordance with the Declaration of Helsinki guidelines on good clinical practices and the Institutional ethical committee approved the study. Participation was voluntary and informed written consent was obtained from each subject. Participants were not on nutritional follow-up nor did they practice regular exercises before the biochemical tests and anthropometric/clinical assessments were performed.

2.2. Clinical procedures

Each subject was required to undertake a clinical protocol consisting of biochemistry, anthropometrical and clinical examination for admission in this study. Briefly, blood pressure (mm Hg) was measured after at least 10 min of rest in a sitting position, and was the mean value of a minimum of 2 physician-obtained measurements recorded >3 min apart. BMI (weight (kg)/height (m^2)) was determined with patient in light clothing and without shoes. Waist circumference (WC; cm) was measured midway between the iliac crest and the lower costal margin. Fat mass (FM; kg) and fat-free mass (kg) were measured using dual-energy X-ray absorptiometer (DXA; Lunar DPX-IQ model, software version 4.7e, Lunar Radiation Corp., Madison, WI, USA) according to standard procedures provided by the manufacturer. Venous blood samples were collected into EDTA-containing tube after a 12 h-overnight fasting period. Laboratory tests were performed following routine, colorimetric enzymatic liquid assays for clinical chemistry with reagents from In Vitro Diagnóstica (Brazil) on a Humalyzer 3000 analyzer (Human GmbH, Germany). Serum analyses included fasting serum glucose (mg/dl) and lipid variables [TC, HDL-c and triglycerides (TGLs)] (mg/dl) and Serods® calibration sera. VLDL-c was determined by dividing TGL levels by 5, whereas the Friedewald equation was used to yield LDL-c by subtraction of both VLDL-c and HDL-c from TC. Additionally, current use of any drug for hypertension, diabetes and dyslipidemia was included in each medical record and used in the study to control possible pharmacological effects in the analyses.

2.3. Genetic ancestry estimates

For individual genetic ancestry estimation, we selected 23 ancestry-informative markers (AIMs) that displayed differential allele frequencies among European, African and Amerindian parental populations (Bonilla et al., 2004; Fernandez et al., 2003; Shriver et al., 2005; Smith et al., 2004). The informativeness of these SNPs has been previously evaluated and used to correct the structure of the Brazilian population in different association studies (Benedet et al., 2012; Moraes et al., 2013; Moreno Lima et al., 2007). The list of AIMs genotyped and the laboratory procedures were exactly as described previously (Lins, Vieira, Abreu, Grattapaglia, & Pereira, 2010).

2.4. Cytokine quantification

For the interleukin (IL) analyses, 5 important inflammatory mediators were selected for assessment, as follows: IL1α, IL1β, IL8, IL10 and IL12 (p70). Whole blood was collected into endotoxin-free tubes. Serum was separated from whole blood, stored in aliquots at −80 °C and analyzed in batches using specific enzyme-linked immunosorbent assay kits (BioLegend, Inc., San Diego, CA, USA). Samples quantification was processed in duplicate.