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Mannose-binding lectin gene polymorphism and risk factors for cardiovascular disease in postmenopausal women

Claudio Lera Orsatti[a,*], Eliana Aguiar Petri Nahás[a], Jorge Nahas-Neto[a], Fabio Lera Orsatti[b], Iara Moreno Linhares[c], Steven Sol Witkin[d]

[a] Department of Gynecology and Obstetrics, Botucatu Medical School, Sao Paulo State University– UNESP, Botucatu, São Paulo, Brazil
[b] Department of Sports Science, Institute of Health Sciences – UFTM, Uberaba, Minas Gerais, Brazil
[c] Department of Gynecology and Obstetrics, University of Sao Paulo Medical School, Sao Paulo, Brazil
[d] Department of Obstetrics and Gynecology, Division of Immunology and Infectious Diseases, Weill Cornell Medical College, New York, NY, USA

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ABSTRACT

Background: Inflammatory responses may be altered in postmenopausal women and predispose to cardiovascular disease (CVD). Genetic factors can also influence susceptibility to CVD. Mannose-binding lectin (MBL) is a component of the innate immune system and an activator of the complement cascade. We evaluated the association of genetic polymorphism of MBL (*MBL2*) on risk factors for CVD in postmenopausal women.
Methods: In this cross-sectional study, 311 Brazilian women (age ≥45 years and amenorrhea ≥12 months) were included. Exclusion criteria: presence of previous or current CVD, insulin dependent diabetes, chronic kidney disease, autoimmune diseases and cancer. Clinical, anthropometric and biochemical assessments were performed to evaluate the cardiovascular risk factors. DNA was extracted from buccal cell and polymorphisms at codons 54 and 57 in the *MBL2* were determined by polymerase chain reaction (PCR). For statistical analysis, the chi-square and logistic regression (odds ratio, OR) were used.
Results: The presence of the polymorphic allele for codon 54 was found in 25.8% of women (A/B = 22.6%, B/B = 3.2%) and for codon 57 in 12.2% (A/C = 10.8%, C/C = 1.4%). The polymorphism at codon 54 was significantly associated with the presence of hypertension (OR 0.55, 95% CI 0.31–0.99, $p = 0.044$) and insulin resistance assessed by HOMA-IR (OR 0.46, 95% CI 0.24–0.91, $p = 0.025$). No significant associations were observed between the polymorphism at codon 57 with risk factors for CVD.
Conclusion: In postmenopausal women, the polymorphism at codon 54 of the *MBL2* was associated with lower risk for hypertension and insulin resistance that are important risk factors for CVD.

1. Introduction

With the increase in a woman's life expectancy, greater attention is being given to menopause and its consequences. The risk of cardiovascular disease (CVD), especially coronary heart disease (CHD), increases throughout life, and is highest in women after menopause (Mosca et al., 2011). CHD results from a multifactorial process of progressive development, which involves various factors such as hypercholesterolemia, smoking, hypertension, diabetes, stress, sedentary lifestyle and obesity (Kannel et al., 2004; Yusuf et al., 2004). CHD is frequently fatal but more than half of women with CHD do not present with early symptoms (Mosca et al., 2011). Hence, the identification of asymptomatic individuals who are more predisposed to develop CVD is crucial forthe early initiation of effective preventive strategies.

Individual differences in the inflammatory profile may modulate the severity and timetable to develop CVD (Olivieri et al., 2006). Inflammation plays a key role in the initiation and progression of atherosclerosis in the CVD (Libby, 2002). Atherosclerosis is defined as a chronic, progressive and systemic process resulting from the inflammatory and fibroproliferative response of endothelial surface of arteries (Stoll and Bendszus, 2006). The activity of several genes that code for pro-inflammatory mediators, have been

Abbreviations: CVD, cardiovascular disease; MBL, mannose-binding lectin; HT, hormone therapy; BMI, body mass index; WC, waist circumference; TC, total cholesterol; TG, triglycerides; CRP, C-reactive protein; PCR, polymerase chain reaction; IR, insulin resistance; HOMA-IR, homeostasis model assessment-insulin resistant.

* Corresponding author at: Department of Gynecology and Obstetrics, Botucatu Medical School, Sao Paulo State University – UNESP, Distrito de RubiãoJúnior s/n, 18618-970 Botucatu, São Paulo, Brazil. Tel.: +55 14 38116227; fax: +55 14 38821933.
E-mail address: claudiorsatti@gmail.com (C.L. Orsatti).

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shown to influence development of atherosclerosis (Alipour et al., 2011).Mannose-binding lectin (MBL) is a protein component of the innate immune system (Dommett et al., 2006). By binding to carbohydrate moieties and inducing activation of the complement cascade MBL is involved in the clearance of microorganisms (Babovic-Vuksanovic et al., 1999), circulating antigen–antibody complexes (Roos et al., 2001), and cells that have undergone programmed cell death (apoptosis) (Ogden et al., 2001). MBL also can bind directly to macrophages (Ghiran et al., 2000). Each of these activities results in pro-inflammatory immune system activation. Single nucleotide polymorphisms have been identified in exon 1 of the gene coding for MBL (*MBL2*) that result in formation of an altered MBL protein that is rapidly degraded (Garred et al., 2006). Thus, individuals positive for these polymorphisms have reduced levels of MBL in circulation. MBL genetic variants are relatively common, with an incidence of 12%–25% in Caucasian populations (Garred et al., 2006).

In recent years, there has been an emerging interest in MBL. This is partly due to its central role as a recognition molecule in the complement system, but also because of the potential clinical implications of the genetically determined differences in *MBL2* and serum levels between individuals (Garred, 2008). Epidemiological studies have indicated that genetically determined changes in serum MBL levels may influence development of infectious, autoimmune, metabolic and CVD, the polymorphisms in the *MBL2* have been related to CVD in different populations (Garred, 2000; Garred et al., 2003; Munthe-Fog et al., 2014; Schoos et al., 2013). However, published studies on the influence of MBL levels and/or *MBL2* polymorphisms on CVD have been inconsistent. A small study in Norwegian patients with severe atherosclerosis suggested that*MBL2* polymorphisms may have earlier onset of CVD and a more rapid progression (Madsen et al., 1998). In a large prospective case-control study of healthy men and women in the United Kingdom elevated circulating levels of MBL were associated with an increased risk of subsequently developing CVD in men but not in women (Keller et al., 2006). Most studies involving the *MBL2* polymorphism in relation to CVD have been performed in predominantly male populations. There are no reports involving only women, especially postmenopausal women who are at high risk for CVD. Based on the above-described findings, we evaluated the association of genetic polymorphism of MBL (*MBL2*) on risk factors for CVD in postmenopausal women.

2. Methods

2.1. Study design and sample selection

This is a clinical, analytical, cross-sectional and comparative study. The study population was postmenopausal women, aged 45–70 years, attending a public outpatient center in a University Hospital in Southeastern Brazil from February 2011 to June 2012. The sample size estimation was based on the study of Garred et al. (2006) who demonstrated a maximum frequency of the *MBL2* polymorphisms to be present in 25% of the population. Considering this frequency, with a 5% level of significance and a 10% type-II error (90% test power), the need to evaluate at least 292 participants was estimated. Women whose last menstruation was at least 12 months prior to study initiation and age ≥45 years old were included. The exclusion criteria were: (1) known high cardiovascular risk due to existing or preexisting CHD, cerebrovascular arterial disease, abdominal aortic stenosis or aneurysm, peripheral artery disease, chronic kidney disease; (2) history of: hepatitis B and C, acute infection, lower genital tract infection, chronic inflammatory or autoimmune diseases (ulcerative colitis, Crohn's disease, rheumatoid arthritis, lupus, etc.), cancer, and addiction to either alcohol or illicit drugs. Fig. 1 describes the 311 women that were included. Informed consent was obtained from all participants and the study was approved by the Research Ethics Committee of Botucatu Medical School, Sao Paulo State University/UNESP.

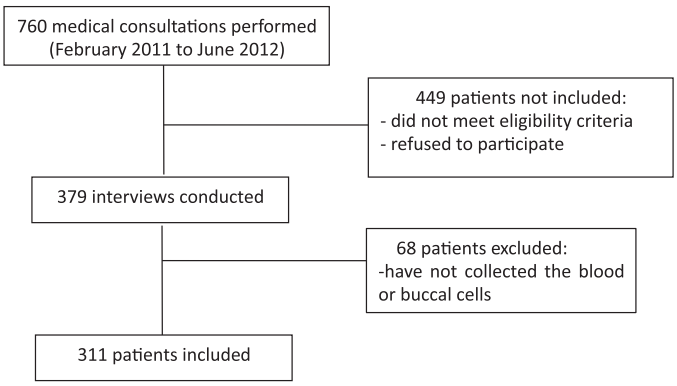

Fig. 1. Flow chart of women included in the study.

2.2. Measurement of variables

During the consultation, all subjects underwent individual interviews in which the following data were collected: age, time since menopause, current smoking, use of hormone therapy (HT), personal history of hypertension, diabetes and physical activity, as well as family history of CHD (acute myocardial infarction in 1st degree relative male aged <55 years and female aged <65 years). Blood pressure was measured using a standard aneroid sphygmomanometer on the right arm with patients in the sitting position, forearm resting at the level of the precordium and the palm of the hand facing upwards, after a 5-min rest. Smokers were defined as persons who reported smoking regardless of the number of cigarettes smoked. Women who practiced aerobic physical exercise of moderate intensity for at least 30 min, five times a week (150/min/week) or resistance exercise three times a week were considered to be active (Garber et al., 2011). Women showing three or more of the following diagnostic criteria proposed by the US National Cholesterol Education Program/Adult Treatment Panel III (NCEP-ATP III) (Expert Panel on Detection and Treatment of High Blood Cholesterol, 2001) were diagnosed as positive for MetS: waist circumference > 88 cm; triglycerides ≥ 150 mg/dL; HDL cholesterol < 50 mg/dL; blood pressure ≥ 130/85 mmHg or under therapy; fasting glucose ≥ 100 mg/dL or under therapy.

The following data for anthropometric measurements were obtained: weight, height, body mass index (BMI = weight/height^2) and waist circumference (WC). Weight and height were determined with a standard balance beam scale (max. 150 kg, 0.1 kg accuracy) and portable wall anthropometer (0.1 cm accuracy), respectively, with patients wearing lightweight clothes and no shoes. BMI was classified according to the system: lower than 25 kg/m^2 was defined as normal, from 25 to 29.9 kg/m^2 as overweight, above 30 kg/m^2 as obesity. Waist circumference was measured at the midpoint between the lowest rib and the top of the iliac crest. The patients were advised to remain in the orthostatic position and the reading was performed at the moment of exhalation. This measurement was performed by a single evaluator. Any WC exceeding 88 cm was considered elevated (Expert Panel on Detection and Treatment of High Blood Cholesterol, 2001).

Blood samples were collected from each subject after a 12-hour fast. Triglycerides, total cholesterol, HDL, glucose, and C-protein reactive (CRP) were measured by a colorimetric dry-chemistry method (Johnson & Johnson®, Rochester, NY, USA) in an automated