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Cardiovascular risk and mannose binding lectin in patients with rheumatoid arthritis from southern Brazil

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ABSTRACT

Background: Mannose binding lectin (MBL) appears to be involved in susceptibility to rheumatoid arthritis (RA), in the inflammatory process and in the genesis of atherosclerotic disease.
Objective: To study the association of MBL serum levels and its genotypic variation with carotid arteries intimal thickness (IMT) in RA patients from Southern Brazil.
Methods: MBL serum levels, *MBL2* genotyping and IMT were investigated in 90 RA patients along with their demographic, clinical and laboratory profile. MBL levels and MBL2 genotyping were evaluated in 90 healthy controls.
Results: A significant lower MBL serum concentration was observed in patients with RA in relation to controls (528 ng/mL vs 937.5 ng/mL, $p = 0.05$, respectively). The median IMT in RA patients was 0.59 mm (0.51 to 0.85 mm). There was no correlation between levels of MBL with disease activity, erythrocyte sedimentation rate, autoantibodies presence or IMT (p = NS). A weak and negative correlation was found between MBL and CRP levels (Rho = −0.24; $p = 0.02$;). The *MBL2* variant at codon 54 (variant B) and HYPA haplotype were the most frequently observed in the RA sample (67.5% and 31.7%). *MBL2* wild type (A/A) were associated with lower IMT when compared with heterozygotes (A/O; $p = 0.04$) and low producers (O/O; $p = 0.05$). In addition, high producers genotypes had lower levels of CRP when compared with medium ($p = 0.04$) or with low producers ($p = 0.05$).
Conclusion: RA patients had lower MBL levels than controls. MBL were negatively associated with CRP serum levels; low MBL genotypes producers increased thickness of the IMT than high producers.

1. Introduction

Patients with rheumatoid arthritis (RA) have life expectancy of 5 to 10 times lower than the general population due to increased cardiovascular risk, which is considered to be by 2 to 3 times higher [1]. The reason why these patients have early and accelerated atherosclerosis has been the subject of several studies [1]. Recently, it has been accepted that the chronic inflammatory process seen in RA is related to the genesis and development of atherosclerotic plaques [1, 2].

The mannose binding lectin (MBL) is a serum protein produced in the liver, belonging to the collectin family. Its main function is to activate the complement system via the lectin pathway [3–6]. MBL production is regulated by the MBL gene (*MBL2*), that is located on chromosome 10, and consists of 4 exons and 3 introns [6–8]. Mutations in exon 1 of the *MBL2* gene and variations in the promoter region are known to affect MBL serum levels [6]. The prevalence of mutations in the exon 1 varies according to the population's ethnic background: variations of codon 57 are common in Africans, rare in Europeans and absent in Eskimos. Yet, mutation at codon 54 is a rare finding in Africans but quite common in Europeans and Chineses [3, 9, 10].

The serum concentrations of MBL fluctuate between 0 and 5000 ng/mL in healthy persons [6]. Subjects with levels under 100 ng/mL are considered to have low levels (or MBL deficient); those with levels between 100 and 1000 ng/mL are said have medium levels and those above 1000 ng/mL, to have high levels [8].

The influence of MBL in the development and prognosis of cardiovascular disease is complex and not fully understood. There are paradoxical observations. Some authors have shown that MBL has protective role against the development of atherosclerosis through the clearance of apoptotic cells and cell debris and against infections such as *Chlamydia pneumoniae* and *Helicobacter pylori* [11–13]. However, other studies reported that high levels of MBL may lead to excessive activation of the complement via the lectin pathway, resulting in a pro-atherogenic inflammation and stimulating atherosclerosis or ischemic heart disease [14]. This uncertainty in the understanding of MBL function is directly

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related to the lack of a deeper understanding of the role of this protein in immunity and inflammation [10, 12].

In the context of RA, there are several studies with conflicting results relating serum levels of MBL and its genetic polymorphism with susceptibility, severity, radiographic progression and disease activity [3, 5, 8, 15–18]. Reports on the role of MBL in RA involved in atherogenesis are also diverse. Troelsen et al. reported a dual role for MBL in the cardiovascular risk of RA patients, with high MBL levels having increased risk for myocardial ischemic disease [14]. On other hand in a later study, these same authors, after analysis with linear and quadratic models of the measurement of carotid media intima thickness (IMT) in relation to MBL, found a correlation between low levels of MBL and IMT [19].

In the present study, we aimed to investigate the association between MBL and cardiovascular risk through the carotid artery IMT in patients with RA from a Southern Brazilian population. This association was studied through MBL serum levels and MBL2 genotyping.

2. Materials and methods

2.1. Patients and ethical issues

The study included 90 Southern Brazilians patients with RA, accompanied in a single Rheumatology Clinic from a University Hospital during the period of February 2015 to February 2016. All patients met the classification criteria for RA according to the 1987 American College of Rheumatology (ACR) or ACR/EULAR (European League Against Rheumatism) Classification criteria [20]. This study was approved by the local Ethics in Local Research Committee and all included patients signed consent. For control purposes the MBL in 90 healthy volunteers belonging to the hospital staff, who had no chronic rheumatic disease and no relatives with RA was measured.

2.2. Clinical and laboratory data

Demographic, clinical and laboratory data were obtained through interviews with the patient and/or obtained from the medical records. Collected data included age, gender, ethnicity, smoking, presence of diabetes mellitus (DM), hypertension (HBP), dyslipidemia and statin use and body mass index (BMI). We also analyzed items considered as potential atherosclerotic risk factors associated with AR: presence of anti-citrullinated peptide (anti-CCP), rheumatoid factor (RF), age at disease onset, duration of disease, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), functional index of Steinbrocker [21] and disease activity index calculated by the DAS 28- ESR (Disease Activity Score 28 - ESR) [22].

2.3. Measurement of MBL serum levels and genotyping of MBL2 gene

The serum levels of MBL were determined by the ELISA method (Enzyme Linked Immuno Sorbent Assay) utilizing the anti-MBL monoclonal antibody HYB 131-01 (BioPorto Diagnostics A/S, Copenhagen, Denmark). Individuals with serum MBL < 100 ng/mL were considered low producers or deficient; levels of 100–1000 ng/mL medium producers and levels > 1000 ng/mL high producers [8].

Genotyping was performed by PCR amplification of the promoter region (position −550, −221 and + 4, representing the loci H/L, X/Y and P/Q respectively) and of exon 1 (codons 54, 57 and 52) of the *MBL2* gene and subsequently sequenced using suitable primers, in according to Goeldner et al. [8] Exon 1 mutations comprise exchange of nitrogenous bases in codons 54, 57 and 52, and are respectively referred to as variant B (GGC to GAC, substituting glycine for aspartic acid), variant C (GAA GGA by substituting glycine for glutamic acid) and variant D (CGT by TGT, replacing cysteine by arginine). The wild-type allele is called A [3, 4, 6, 7, 15, 23]. The homozygous for the mutation O/O (where O can be B, C or D) were considered to be low MBL producers; the heterozygotes individuals (A/O) are considered to be intermediary MBL producers and those with homozygous wild allele (A/A) are considered to be high MBL producers.

Polymorphic variations in the promoter region were expressed by the haplotypes HYP, LYQ, LYP, LXP [6]. Low MBL serum concentrations are associated with haplotype LXP [6, 15].

2.4. Measurement of IMT

The measurement of IMT was performed by a single investigator, blind for clinical data, with Esaote® ultrasound apparatus, high resolution, model MyLab40, in B-mode and with a linear transducer of 18 mHz. The patients were studied in a quiet, air-conditioned environment at 22 °C, in the supine position with the neck extended and rotated 45° contralateral to the examined side. The carotid artery was observed in transverse and longitudinal planes, with measurement carried out at a distance of 10 to 20 mm of the carotid bifurcation, in the distal vessel wall [24]. The examination was performed on both sides; for statistical purposes the highest value was considered. The reference values used were 0.4 to 0.7 mm as normal IMT; 0.8 to 1.4 mm as thickened IMT (subclinical atherosclerosis); values greater than or equal to 1.5 mm, as atheroma [25].

2.5. Statistical analysis

Data were collected in frequency and contingency tables. Measures of central tendency were expressed as mean and standard deviation (SD) for parametric samples and median and interquartile range (IQR) for non-parametric samples. Normality was judged by the Kolmogorov Smirnov test.

Comparison between two numerical samples were made using Mann Whitney test when the sample distribution was nonparametric and unpaired *t*-test when parametric. The comparison of three samples was performed by Kruskal Wallis test (nonparametric) and one way Anova (Parametric). Nominal data were compared by Fisher's and chi-square test. Correlation studies were done using Spearman test. When a variable was associated/correlated with several others, independence was tested by multivariate regression. The adopted significance was of 5% and the calculations were made with the aid of the software Medcalc 14.0.

Direct counting was used to estimate the genotypes, haplotypes and allele frequency. Deviations from Hardy-Weinberg equilibrium and the assumption of homogeneity between the distributions of haplotypes were tested using Arlequin 3.1 software.

3. Results

3.1. Description of studied sample

The descriptive data of the RA sample are on Table 1. In the control group there was 69/90 females (76.6%) with age from 20 to 81 years of age (median 52 years; IQR = 45.0–61.0). Pairing date showed $p = 0.33$ for gender and 0.10 for age.

In the RA patients the median carotid artery IMT was 0.59 mm (0.51 to 0.85 mm), with 30% (27/90) of them having IMT higher than 0.8 mm, characterizing subclinical atherosclerosis. MBL serum levels in RA and controls are on Fig. 1.

Concerning MBL2 genetic analysis, the exon 1 variations found in this RA sample were: 67.5% variant B, 10% variant C and 22.2% variant D. The polymorphic variations of promoter regions were: 19.6% LXP; 37.8% HYP; 16.2% LYQ; 26.4% LYP. The haplotype HYPA was the most frequent and found in 31.7% of the patients. We considered A/A as high MBL producers (found in 32.4%); A/O as medium MBL producers (found in 40.25%) and O/O as low MBL producers (found in 27.7%).