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Archives of Medical Research ■ (2018) ■

Archives of Medical Research

PRELIMINARY REPORT

Mannose Binding Lectin and Pentraxin 3 in Patients with Diabetic Retinopathy

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Received for publication November 7, 2017; accepted June 22, 2018 (ARCMED-D-17-00635).

Background. Mannose binding lectin (MBL) is a protein of the complement system and pentraxin-3 (PTX3) is an acute phase protein both with an important role in inflammatory diseases, such as diabetic retinopathy (DR).

Aim of the Study. To evaluate whether plasma MBL and PTX3 levels are associated with the development of DR and if patients with and without DR can be distinguished.

Methods. The patients were divided into three groups: diabetic without DR; with mild/moderate DR, and with severe/proliferative DR. PTX3 and MBL levels were measured with enzyme-linked immunosorbent assay kits.

Results. A total of 74 patients were included. A significant association was observed between high levels of MBL and severe DR; 47% of patients with severe/proliferative DR had high levels of MBL, whereas 12% of the patients with diabetes but no DR had high levels of MBL ($p = 0.008$; odds ratio [OR]: 6.06; 95% confidence interval [CI]: 1.4−25.0). High levels of MBL were more frequent in patients with severe/proliferative disease (47%) when compared to those with mild/moderate DR (20%), $p = 0.04$ (OR: 3.46; 95% CI: 1.0−11.8). PTX3 levels were similar among the groups and were not related to the development or severity of DR.

Conclusion. We found a significant association between high plasma MBL levels and DR development as well as with severe/proliferative DR. We observed no relationship between plasma PTX3 levels and the development or severity of DR.

Key Words: MBL, Pentraxin 3, Diabetic retinopathy, Biomarkers.

Introduction

Diabetes mellitus (DM) is a chronic systemic disease that causes several micro and macrovascular complications. The prevalence of DM is increasing due to a sedentary lifestyle, obesity, aging and the growing population; about 366 million people will have DM by 2030 (1). One of the most devastating microvascular complications of DM is diabetic retinopathy (DR), which can lead to irreversible blindness. The major complications of DR include intraocular neovascularization, inter-retinal edema, hemorrhage, exudates, and microaneurysms (2,3) Studies have demonstrated that the duration of diabetes (4) and rigorous control of hyperglycemia are the main risk factors associated with the progression of DR (2,3) In addition, inflammation plays an important role in the development of diabetic vascular complications leading to DR (5). DR is characterized by abnormal growth and leakage of small blood vessels, mainly due to excess production of vascular endothelial growth factor (6), resulting in local edema and tissue damage. The mechanisms involved in DR pathogenesis are multiple and unclear; however, factors, such as dysregulated

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0188-4409/$ - see front matter.
https://doi.org/10.1016/j.arcmed.2018.06.003

vascular regeneration, oxidative and hyperosmolar stress, and inflammation, play a key role in the development of the disease (7).

Some cells function to detect tissue stress, among which tissue resident macrophages, promote host defense and release inflammatory mediators, including complement components. DR pathology may occur as a result of tissue injury or immune dysregulation. In addition to retinal innate immune cells, soluble pattern recognition molecules (PRM) such as mannose binding lectin (MBL) and pentraxin-3 (PTX3) play a critical role protecting the retina from exogenous and endogenous insults. In contrast, both proteins may lead to dysregulated activation of complement and an inflammatory response leading to retinal disease (8) MBL and PTX3 are important components of the innate immune response, as they are part of the first line of defense against infection (9). MBL is synthesized in the liver, circulates in the blood, and can leave the circulation due to vascular leakage under inflammatory conditions. In these cases, MBL can be detected on the mucosal surface (10). Human MBL is encoded by the *MBL2* gene, which largely defines plasma MBL concentrations even considering that MBL levels may vary ten-fold between individuals with an identical MBL genotype (9−11). Plasma MBL levels corresponding to particular polymorphic *MBL2* genotypes vary widely, making it impracticable to deduce the plasma MBL level from a given *MBL2* genotype, or predict the *MBL2* genotype from a given plasma level (10). MBL deficiency affects 5−30% of the general population according to ethnicity (10) and low levels of this protein have been related to vulnerability to infections caused by a range of microorganisms (9,10) Conversely, higher levels of MBL may enhance tissue damage due to unwarranted activation of complement (9,12).

PTX3 is an acute-phase reactant protein and a member of the long pentraxin family which is produced and released locally by cells types and originally identified as an early induced protein in response to proinflammatory stimuli (13). High levels of PTX3 have been reported in cardiovascular disease, such as ischemic heart disease (14), unstable angina (15) and heart failure (16). Plasma PTX3 levels predict 3 month mortality in patients with myocardial infarction after adjusting for major risk factors (16) In addition, PTX3 levels are associated with endothelial dysfunction and with the severity of renal disease, suggesting a role in peripheral vascular damage (17).

PTX3 modulates all three complement pathways (e.g., classical, alternative, and lectin pathways) and interacts together with components, such as ficolin 1, ficolin 2, MBL, C1q, Factor H, and C4b (18). Thus, the interactions between PTX3 and the complement proteins may have extensive implications in host defense and regulation of inflammation (19). In fact, the interaction between PTX3 and MBL promotes recruitment of molecules onto the surface of recognized microorganisms and amplifies the complement-mediated innate response (19) because the formation of PTX3/MBL complexes induces C1q recruitment and promotes C4 and C3 deposition on the surface of the microorganism, thereby increasing phagocytosis of the pathogen (18). On the other hand, both proteins promote opsonisation of apoptotic cells to regulate injury to normal cells mediated by the complement membrane attack complex (18).

High plasma and vitreous levels of PTX3 have been reported in other retinopathies, such as age-related macular disease (20) and retinal vein occlusion (7). However the relationship between PTX3 level and DR has barely been explored. As PTX3 level reflects chronic inflammatory disease, it could be a biomarker for DR. Only two studies have addressed the role of MBL in DR and both were in a Chinese population. Those studies described high levels of MBL in patients with DR and suggested that this protein is a strong biomarker for this disease (21,22). Because there is wide variability in MBL levels of those who are genetically determinate, it is extremely important explore the levels in another population. So, far, no study has evaluated MBL levels and DR in other ethnicities.

In this study, we evaluated whether plasma MBL and PTX3 levels are associated with the development of DR and whether they can be used to differentiate mild from severe DR in a Brazilian population.

Materials and Methods

This was an observational, cross-sectional study. Participants were recruited from the retina and vitreous service of the Federal University of Paraná, Brazil. Approval from the Institutional Review Board Ethics Committee was obtained for this study, which followed the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Assessment of Diabetic Retinopathy

Subjects underwent a complete ophthalmic examination, including best corrected visual acuity, tonometry, biomicroscopy, and a fundus evaluation using both a 78 diopter lens and indirect ophthalmoscopy following pupil dilation.

Retinopathy was considered present if any characteristic lesions were detected, according to the Early Treatment Diabetic Retinopathy Study standard set for microaneurysm, hemorrhage, cotton wool spots, intraretinal microvascular abnormalities, hard exudate, venous beading, and new vessel images (23) Retinopathy severity was classified as without DR, mild non-proliferative diabetic retinopathy (NPDR), moderate NPDR, severe NPDR, and proliferative retinopathy.

Patients were excluded if they presented with a history of cardiac disease, kidney disease, cerebral disease, cancer,