Short Communication

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Evaluation of the cytokine mannose-binding lectin as a mediator of periportal fibrosis progression in patients with schistosomiasis

***Paula Carolina Valença Silva*[1], *Adriana Vieira Gomes*[2],[3], *Georgea Gertrudes de Oliveira Mendes Cahu*[4], *Maria Rosângela Cunha Duarte Coêlho*[4],[5], *Maria Tereza Cartaxo Muniz*[2],[3],[6] *and Ana Lúcia Coutinho Domingues*[7]**

[1]. Centro Acadêmico de Vitória, Universidade Federal de Pernambuco, Vitória de Santo Antão, Pernambuco, Brasil. [2]. Laboratório de Biologia Molecular, Hospital Universitário Oswaldo Cruz, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [3]. Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [4]. Setor de Virologia do Laboratório de Imunopatologia Keizo-Asami, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil. [5]. Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil. [6]. Faculdade de Ciências Médicas, Universidade de Pernambuco, Recife, Pernambuco, Brasil. [7]. Departamento de Medicina Clínica, Universidade Federal de Pernambuco, Recife, Pernambuco, Brasil.

ABSTRACT

Introduction: We hypothesized higher mannose-binding lectin level and classic factors (i.e., age, sex, alcohol consumption, exposure, and specific treatment) are associated with the severity of periportal fibrosis in schistosomiasis. **Methods**: This cross-sectional study involved 79 patients infected with *Schistosoma mansoni* with severe or mild/moderate periportal fibrosis. Serum concentrations of mannose-binding lectin were obtained by enzyme-linked immunosorbent assay (ELISA). **Results:** Higher serum level of mannose-binding lectin was significantly associated with advanced periportal fibrosis. **Conclusions:** Mannose-binding lectin may contribute to liver pathology in schistosomiasis and may represent a risk factor for advanced periportal fibrosis in the Brazilian population studied.

Keywords: *Schistosomiasis mansoni.* Periportal fibrosis. Mannose-binding lectin.

Schistosomiasis is a chronic parasitic infection caused by trematode *Schistosoma* spp. and is a serious public health problem in endemic countries, incurring substantial social and economic burdens. It affects more than 230 million people worldwide and is one of the neglected tropical diseases targeted for elimination by the World Health Organization[1]. Approximately 6 million people are infected with *Schistosoma mansoni* in Brazil, mainly in the northeast region. The disease causes immense morbidity, especially in the State of Pernambuco, where schistosomiasis is continuously expanding to touristic coastal areas of the state[2].

Schistosomiasis causes periportal fibrosis (PPF) and portal hypertension in approximately 6% of infected subjects, usually with the preservation of hepatic function[3]. Some patients with schistosomiasis have a poorly regulated immune response to parasite egg antigens and consequently develop extensive hepatic PPF and subsequent hepatosplenic disease[3]. The evolution of PPF may be influenced by several exposure-related factors such as local environment and behaviors (e.g., alcohol consumption, exposure frequency, specific early treatment, age, and sex) as well as immunogenetic factors that lead to the exacerbation of the host's immune response[4].

Hepatic fibrosis depends on the actions of cytokines including mannose-binding lectin (MBL), which has a central regulatory role[5]. The association between the immune response of MBL and the development of hepatic fibrosis in other liver diseases has been evaluated[5]. The importance of MBL in the innate immune system is related to its multimeric structure as well as its functions as an opsonin and adaptor for the activation of mannose-binding lectin/mannose-binding protein-associated serine protease (MBL/MASPs). Moreover, the MBL serum level and enzyme activity of MBL/MASP-1 complex are elevated in hepatitis C virus (HCV) patients with severe hepatic fibrosis[5]. However, the contribution of MBL in patients with varying severity of *S. mansoni* remains poorly understood[6]. Considering the central role of MBL in the development of severe hepatic fibrosis in other liver diseases, we determined whether serum levels of MBL and classical factors (i.e., age, sex, alcohol, exposure, and treatment) are associated with PPF severity in schistosomiasis in the population of endemic areas of Northeastern Brazil.

This cross-sectional study was conducted from April to December 2013 and evaluated patients infected with *S. mansoni* aged over 18 years who were treated in the Gastroenterology

Corresponding author: Dra. Ana Lúcia Coutinho Domingues. Depto. de Medicina Clínica/Hospital das Clínicas/UFPE. Av. Prof. Moraes Rêgo s/n, Bloco A, Cidade Universitária, 50670-901 Recife, Pernambuco, Brasil.
Phone: 55 81 3271-8534; **Fax:** 55 81 3271-8534
e-mail: alcoutinho@superig.com.br ; paulacvalenca@gmail.com
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Outpatient Clinic of the *Hospital das Clínicas/Universidade Federal de Pernambuco* (CH/UFPE), a reference center for schistosomiasis treatment. All patients lived in endemic areas of schistosomiasis in the State of Pernambuco. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the CH/UFPE (CAAE: 03161512.6.0000.5208). Informed consent was obtained from all patients before blood sampling.

All patients infected with *S. mansoni* examined during the study period were included. Patients with a history of other associated liver diseases including liver cirrhosis, fatty liver disease, or hepatitis B or C, or other clinical forms of diagnosed schistosomiasis were excluded. Hepatitis B and C were excluded on the basis of hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), and hepatitis C virus antibody (anti HCV) measurements[7]. Thus, 79 individuals were included and divided into two groups: the severe group consisted of 39 patients with severe PPF and the hepatosplenic form of the disease, and the mild group consisted of 40 patients with no or mild-to-moderate fibrosis and the hepatointestinal form of the disease. All patients had a history of contact with contaminated water and/or had positive stool test results for *S. mansoni* or prior treatment for schistosomiasis.

The sociodemographic and clinical variables related to the risk factors for developing PPF included sex, age, alcohol consumption, time and site of last contact with contaminated water, and specific treatment. Data were collected by using a precoded structured questionnaire that was applied to individuals by a single investigator.

The diagnosis of the clinical form of the disease was determined on the basis of the patient's medical history and clinical examination. In addition, upper-abdominal ultrasonography was performed by a single experienced operator using an Acuson X 150® (Siemens, Munich, Germany) with a 3.5-mHz convex transducer in order to confirm the diagnosis and exclude other liver diseases. PPF pattern was evaluated according to Niamey's classification[8] as follows: A, absence of fibrosis; B, dubious; C, peripheral; D, central; E, advanced; and F, very advanced fibrosis.

Serum MBL levels were measured using the commercial Human MBL Quantikine® ELISA Kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. The results are expressed in pg/mL based on standard curves (limit of detection: 0.029ng/mL). The Cutoff of 881ng/mL was derived from the average serum levels of MBL in the severe group.

The Kruskal-Wallis test was used to compare the serum levels of MBL between groups. Variables showing $p < 0.20$ in the univariate analysis were entered into non-conditional logistic regression analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the fibrosis pattern as the dependent variable and the selected factors as independent variables. The level of significance was set at $p < 0.05$. Epi Info version 3.5.5. (CDC, Atlanta, GA, USA) was used for all statistical analyses.

The associations of sociodemographic and clinical variables with PPF severity are shown in **Table 1**. The mean ± SD age of the patients was 54 ± 13 years. The patients were predominantly women. Male sex tended to be associated with severe PPF (OR = 2.85; 95% CI: 1.80-8.18; p = 0.050) with borderline significance. Moreover, higher serum MBL level was significantly associated with severe PPF (OR = 2.97; 95% CI: 1.08-8.29; p = 0.032). Median serum MBL level differed significantly between the mild and severe PPF groups (867.62 vs. 874.04ng/mL, respectively p = 0.003).

The final logistic regression model included age and alcohol consumption along with serum MBL level. Only serum MBL level was significantly associated with severe PPF after adjusting for confounding factors (p = 0.012, **Table 2**). As this association was inverse, serum MBL level may be a protective factor against severe PPF.

The relationship between MBL and hepatic fibrosis in other hepatic diseases remains controversial[5]. The present study evaluated the influences of serum MBL level, age, sex, alcohol, exposure, and treatment on PPF severity in schistosomiasis. However, the classic factors studied were not associated with PPF severity. Thus, immunogenetic factors appear to be more important when predicting PPF severity.

PPF is a well-known multifactorial manifestation caused by complex interactions between genetic and environmental factors[9]. However, the classic factors should be considered when evaluating the role of MBL in PPF.

Serum MBL levels were significantly higher in the severe PPF group than the mild PPF group; this result is concordant with recent data indicating increased complement activation and activity of the MBL/MASP-1 complex in HCV-induced hepatic fibrosis[5] [10] [11], thus corroborating the proinflammatory role of MBL in chronic disease.

Hepatitis C virus patients have higher MBL levels than controls and are associated with more severe hepatitis[5] [10] [12] [13]. Brown et al.[5] investigated the influence of MBL level in patients with chronic HCV infection, non-HCV liver disease, and healthy controls from the Trent HCV cohort study in the UK[14] [15]; they analyzed 147 infected patients and 111 healthy controls, and found MBL levels were significantly higher in HCV patients with severe fibrosis than that in patients with mild fibrosis and controls. However, MBL levels did not differ significantly between HCV and non-HCV liver disease patients.

Most studies that compared the distribution of MBL levels with respect to the severity of fibrosis report no significant association[11]. Pedroso et al[12] compared 102 Euro–Brazilian patients with moderate and severe chronic hepatitis C with sex- and age-matched HCV-seronegative healthy controls and found overall circulating levels of MBL did not differ significantly between groups. However, it is important to note that the exclusion of patients with no or little fibrosis from that study may have weakened the association between disease progression and MBL concentration.

El Saadany et al.[13] studied 80 Egyptian patients with chronic HCV infection with different patterns of hepatic fibrosis and 20 control subjects; they found MBL levels tended to be higher in the mild liver fibrosis group than the controls, while MBL levels were significantly higher in the severe fibrosis group than the mild fibrosis and control groups.

TABLE 1 - Associations of sociodemographic and clinical variables with periportal fibrosis severity in patients with schistosomiasis in Pernambuco, Brazil.

	Severe n = 39		Mild n = 40				
Characteristics	n	%	n	%	OR	95% CI	ρ
Sex							
male	19	48.7	10	25.0	2.85	1–8.28	0.050
female	20	51.3	30	75.0	1		
total	39	100.0	40	100.0			
Age (years)							
18–40	4	10.3	10	25.0	1		
41–60	19	48.7	17	42.5	2.79	0.63–13.15	0.220
≥ 61	16	41.0	13	32.5	3.08	0.66–15.31	0.189
total	39	100.0		100.0			
Alcohol consumption							
yes	5	12.8	2	5.0	2.79	0.44–22.44	0.205
no	34	87.2	38	95.0	1		
total	39	100.0					
Last exposure with contaminated water (years)							
<1	3	7.7	6	15.0	1		
1–25	20	51.3	19	48.7	2.11	0.38–12.64	0.465
≥ 25	16	41.0	15	48.4	2.13	0.37–13.41	0.457
total	39	100.0	40	100.0			
Specific treatment							
treated	36	92.3	33	82.5	2.55	0.53–13.71	0.310
untreated	3	7.7	7	17.5	1		
total	39	100.0	40	100.0			
Serum MBL level (ng/mL)							
≥ 881	25	64.1	15	37.5	2.97	1.08–8.29	0.032
< 881	14	35.9	25	62.5	1		

OR: odds ratio; **CI:** confidence interval; **MBL:** mannose-binding lectin.

TABLE 2 - Risk factors for severe periportal fibrosis.

Variable	Adjusted OR	95% CI	p[a]
Age (years)			
18-40	1		
41-60	2.35	0.57–9.54	0.231
≥ 61	2.67	0.63–11.30	0.181
Alcohol consumption			
yes	1		
no	0.20	0.03–1.28	0.091
Serum MBL level concentration (ng/mL)			
≥ 881	1		
< 881	0.280	0.10–0.75	0.012

OR: odds ratio; **CI:** confidence interval; **MBL:** mannose-binding lectin. [a] $p_{model} = 0.024$.

Thus, the present finding suggesting high serum MBL levels play an important role in the severity of PPF corroborates other studies reporting similar findings[5] [12]. Therefore, further larger studies analyzing the association between MBL expression and disease outcomes are required to corroborate the present findings and confirm the profibrotic activity of MBL. Furthermore, the influences of MBL and other classic factors on the severity of immune fibrosis in schistosomiasis warrant a large cohort study with PPF monitoring in order to confirm this association in different ethnicities as well as clarify the roles of other environmental risk factors and immunogenetic factors in the evolution of this disease.

In conclusion, MBL may contribute to liver pathology in schistosomiasis and may be a risk factor for PPF severity in the Brazilian population. Moreover, it could be used to predict the severity of advanced PPF in schistosomiasis and thus the severity of liver pathology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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