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Alterations in serum paraoxonase-1 activity and lipid profile in chronic alcoholic patients infected with *Strongyloides stercoralis*

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ABSTRACT

The objective of this study was to investigate paraoxonase-1 (PON1) activity, cortisol levels, and the lipid profile in the sera of alcoholic and non-alcoholic *Strongyloides stercoralis*-infected and uninfected individuals in a sample of 276 individuals attended at the National Health System in Salvador, Bahia, Brazil. The activity of PON1 was measured by the Beltowski method, serum lipids, and cortisol levels using commercial kits. PON1 activity was low in both alcoholic and non-alcoholic individuals infected with *S. stercoralis*. A positive correlation was observed between PON1 activity and cortisol concentration in alcoholic individuals who were not infected with *S. stercoralis*; whereas a negative correlation occurred in *S. stercoralis*-infected nonalcoholic individuals. The levels of triglycerides, LDL-C, and VLDL-C in *S. stercoralis*-infected alcoholic individuals were significantly lower than in uninfected alcoholic individuals. The high level of HDL-C and the low level of LDL-C, VLDL, triglycerides and PON1 activity in alcoholic patients infected with *S. stercoralis* evidenced an anti-atherogenic pattern.

1. Introduction

The paraoxonase (PON) gene family includes three members, *PON1*, *PON2* and *PON3*, aligned in tandem on the long arms of chromosomes (q21Æ22). Serum PON1 is predominantly synthesized by hepatocytes and released into the circulation associated with high-density lipoproteins (HDL). It is involved in a multitude of biological activities, including the detoxification of organophosphate and the prevention of atherosclerosis. As well, it presumably protects low-density lipoproteins (LDL) against oxidative stress, reducing the formation of macrophage foam cells and inactivate LDL-derived oxidized phospholipids (Costa et al., 2005; Shih et al., 1998).

PON1 activity has been implicated in the pathogenesis of other inflammatory diseases caused by bacteria, viruses, parasites, and excessive alcohol consumption (Akbas et al., 2010; Aviram et al., 1999; Farid et al., 2008; Marsillach et al., 2007). Farid et al. (2009) studying Wistar rats infected with *Nippostrongylus brasiliensis*, observed marked decreases in serum PON1 activity and increased pro-inflammatory cytokines in serum (IL-1, IL-6 and TNF-α). A moderate consumption of alcohol is associated with slight increases in serum PON1 activity and HDL cholesterol (HDL-C) in normal subjects (Hendriks et al., 1998; Rao et al., 2003; Sierksma et al., 2002; van der Gaag et al., 1999). However, in chronic alcoholics with liver cirrhosis, the PON1 activity and HDL-C is very low (Kedage et al., 2010; Marsillach et al., 2007; Rao et al., 2003; Sabesin et al., 1977).

A high parasite load *S. stercoralis* infection has been associated with alcoholism (Silva et al., 2016). Possibly, this is due to the effect of ethanol on the hypothalamic pituitary adrenal axis, increasing the levels of endogenous corticosteroids, leading to larvae differentiation from rhabditoid to filariform infective stage and hyperinfection (Choudhry et al., 2006; László et al., 2001; Ogilvie et al., 1998; Teixeira et al., 2010). Moreover, some studies have demonstrated lipid profile alterations in other parasitic infections (Bansal et al., 2005; Soares et al., 2010). A decrease in total cholesterol, triglycerides, and HDL-C was observed in *Callithrix jacchus* (sagui) infected with *Schistosoma mansoni* (de Ramos et al., 2004) and in guinea pigs infected with *Ascaris suum* (Biaduń, 1989). Others studies with intestinal worms have shown decreased serum lipid levels (in hookworm and *Trichuris* infected patients) with a significant inverse correlation between worm egg excretion and HDL-C levels (Bansal et al., 2005; Wiedermann et al., 1991).

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The purpose of this study was to investigate PON1 activity, cortisol levels, and lipid profile in *Strongyloides stercoralis*-infected and uninfected alcoholic and non-alcoholic individuals.

2. Materials and methods

2.1. Patients and sample details

The present study was performed from September 2012 to March 2014. A total of 276 individuals, who were attended at the National Health System in Salvador, Bahia, Brazil, were included in this study. All of the subjects included in this study lived in emerging neighborhoods, and presented similar demographic and socioeconomic characteristics. Samples of venous blood were collected from patients after fasting for 12 h: a total of 202 alcoholics (71 *S. stercoralis*-infected and 131 uninfected individuals) and from 74 non-alcoholics (13 *S. stercoralis*-infected and 61 uninfected individuals). The chronic alcoholic individuals were voluntarily hospitalized for alcoholism treatment (mean age of 43.9 ± 9.7), diagnosed according to WHO criteria (F10.2, ICD 10, 2002) and the non-alcoholic individuals, apparently healthy, were attended at an outpatient service (mean age of 47.0 ± 13.7). The inclusion criteria were the following: adult males with fecal examination and information on the occurrence, or not, of daily ethanol intake. None of the subjects had used corticosteroids or any other immunosuppressive drugs nor were infected with HIV, HTLV-1 or hepatic virus. The Committee of Ethics in Research of the Nursing School, Federal University of Bahia, Brazil, approved this study and a written informed consent for participation was assigned from each patient, when the clinical specimens were acquired.

2.2. Strongyloidiasis diagnosis

Three fresh fecal samples from each subject were examined on alternate days by three different parasitological methods: spontaneous sedimentation, modified Baermann-Moraes, and agar plate culture (APC). *S. stercoralis* larvae from one gram of feces obtained by the modified Baermann-Moraes method were quantified under a microscope (400× magnification).

2.2.1. Quantification of PON1 activity

All sera samples were stored at −80 °C until measurement of the PON1 activity. Serum PON1 (Sigma Chemical Co., St. Louis, MO) basal activity was assayed according to Beltowski et al. (2005) method with some modifications. Briefly, serum PON1 activity was determined by measuring the initial rate of substrate hydrolysis to *p*-nitrophenol by absorbance at 405 nm. The assay mixture contained 5 mM paraoxon (Sigma Chemical Co., St. Louis, MO), 2 mM $CaCl_2$, and 7 μL of serum in 100 mM Tris–HCl buffer (pH 8.0). The production of *p*-nitrophenol was detected after 5 min in a spectrophotometer (Biotek EL- 800, CA, USA). Enzyme activity was measured at 25 °C in duplicate and all results were presented in nmol per minute per mL, calculated from the E405 of *p*-nitrophenol ($18.050\ L\ M^{-1}\ cm^{-1}$) and expressed in U/mL (1 U of enzyme hydrolysis being equivalent to 1 nmol of paraoxon/min).

2.2.2. Serum cortisol levels

The serum cortisol level was measured by ELISA, in accordance with the manufacturer's instructions (Cortisol AccuBind™ EIA, Monobind Inc., USA). The blood samples were collected in the morning between 7:00 and 9:30 a.m.

2.3. Lipid profile and hepatic biomarkers

The measurement of HDL cholesterol and triglycerides levels was performed by enzymatic methods using commercial kits (Roche Diagnostics; Mannheim, Germany and Pureauto S TG-N; Daiichi Pure Chemicals, Tokyo, Japan, respectively). The serum LDL cholesterol (LDL-C) concentration was estimated using the Friedewald formula (Friedewald et al., 1972). Serum alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, and bilirubin concentrations were measured by standard methods (Beckman-Coulter Fullerton, CA, USA).

2.4. Statistical analysis

Statistical analyses were performed using the statistical software Graph Pad 5.0 (San Diego, USA). The paraoxonase activity cut-off value was established by the ROC curve (receiver operating characteristic) using 29 serum samples from healthy adult individuals and 59 serum samples from *S. stercoralis*-infected patients with normal ALT and AST levels. Fisher's exact test was performed for comparison between cortisol levels and paraoxonase among alcoholic and non-alcoholic individuals, and calculated with 95% confidence intervals. The Spearman correlation test was used to correlate the cortisol levels with PON1 activity in all patient groups. Differences were considered statistically significant when $p < 0.05$. The univariate regression analysis was performed to relate *S. stercoralis* infection and alcoholism. Linear regression analysis was used to verify the correlation between paraoxonase activity and larvae load and paraoxonase activity with *S. stercoralis* and other parasites coinfection.

3. Results

The PON1 activity was significantly lower in *S. stercoralis*-infected patients (both alcoholic and non-alcoholic) than in alcoholic and non-alcoholic uninfected individuals ($p < 0.05$, Table 1). Among the infected patients, the PON1 activity was lower in non-alcoholic than in alcoholic patients ($p < 0.05$).

Parasite load was quantified in 63 infected patients, those in which the Baerman-Moraes was positive. Of these, 82.5%, (52/63) had low parasitic load (range to 1 from 100 larvae/g of feces) and 17.5% (11/63) had high parasite load (range to 101 from 1000 larvae/g of feces). From the patients with low parasitic load, 63.5% (33/52) had paraoxonase activity <100 U/ml and 36.5% (19/52) had paraoxonase activity >100 U/ml. The most of patients with high parasite load, 72.7% (8/11), had paraoxonase activity <100 U/ml. No statically differences were observed between parasite load and PON1 activity ($p > 0.05$; linear regression). From the 71 patients with *S. stercoralis* infection, 23.9% (17/71) were coinfected with other parasites. *S. mansoni* coinfection was observed in 14.1% (10/71), *A. lumbricoides* in 2.8% (2/71), hookworm in 9.9% (7/71), *T. trichiura* in 5.6% (4/71), *Giardia duodenalis* in 2.8% (2/71), *E. nana* in 4.2% (3/71), *E. coli* in 5.6% (4/71) and *E. histolytica/dispar* in 1.4% (1/71) patients. However, analyses by linear regression showed that coinfection had not effect on PON1 activity ($p < 0.05$).

The cortisol level was higher in alcoholic individuals than in non-alcoholic individuals regardless of being infected or not ($p > 0.05$, Table 1). As shown in Fig. 1, serum PON1 activity had a weak positive correlation with cortisol levels in uninfected alcoholic patients (A, $r = 0.248$, $p < 0.05$). However, there was no correlations between serum PON1 activity and cortisol levels in infected alcoholic patients (B, $r = -0.01$, $p > 0.05$). Moreover, serum PON1 activity in non-alcoholic *S. stercoralis* infected patients had negative correlation with cortisol levels (C, $r = -0.602$, $p < 0.05$). A correlation of PON1 activity with sera cortisol levels in uninfected non-alcoholic individuals was not observed (D, $r = -0.11$, $p > 0.05$).

The triglycerides, LDL-C, and VLDL levels were lower in *S. stercoralis*-infected patients than in uninfected alcoholic patients ($p < 0.05$; Table 1). On the other hand, total cholesterol and LDL-C