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Short communication

The detergent fraction is effective in the detection of IgG anti-*Strongyloides stercoralis* in serum samples from immunocompromised individuals

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ABSTRACT

Human strongyloidiasis is an intestinal helminthiasis that can be fatal particularly in cases of immunosuppression. The aim of this study is to assess the diagnostic accuracy of the detergent fraction (D), purified from total saline extract (SE) of *Strongyloides venezuelensis*, in the detection of anti-*Strongyloides stercoralis* IgG antibodies in serum samples from individuals coming from endemic areas for strongyloidiasis and presenting immunocompromised conditions: human immunodeficiency virus (HIV^+), diabetes mellitus type 2, cancer, tuberculosis and alcoholism. Serum samples from 93 individuals were analyzed by ELISA, as follows: Group 1: 30 immunocompromised individuals with strongyloidiasis; Group 2: 33 immunocompromised individuals without strongyloidiasis and Group 3: 30 healthy individuals. The total saline extract (SE) and detergent fraction (D) showed a sensitivity of 73.33 and 83.33%, and specificity of 82.15 and 86.36%, respectively. The detergent fraction was effective to detect anti-*S. stercoralis* IgG antibodies in immunocompromised individuals with strongyloidiasis and may be applied as an important tool in the immunodiagnosis of human strongyloidiasis related to immunosuppression.

1. Introduction

It is estimated that about 30 to 100 million individuals may be infected by *Strongyloides stercoralis* in the world. Despite being characterized as a self-limited infection, strongyloidiasis can be fatal in immunocompromised individuals [1,2].

Studies have demonstrated the association between hyperinfection by *S. stercoralis* to different immunosuppressive factors such as severe malnutrition, immunosuppressive drug treatments, alcoholism, cancer therapy, chronic renal insufficiency, diabetes mellitus type 2, tuberculosis, autoimmune diseases, human T-lymphotropic virus type 1 (HTLV-1) and HIV infection [1–3].

Definitive diagnosis of strongyloidiasis is accomplished primarily through the identification of larvae in fecal samples using concentration techniques or cultures. However, irregular release of larvae in feces may lead to false-negative parasitological results [3]. Then, serological methods have been routinely used as an alternative to diagnosis because of its accuracy [4].

Some studies demonstrated that the detergent fraction, separated by Triton X-114 and characterized by hydrophobic proteins of infective larvae (L3) of *Strongyloides venezuelensis*, has shown diagnostic efficiency of 85–95% in serologic detection of IgG anti-*S. stercoralis* in immunocompetent individuals [5,6]. In this regard, the aim of this study is to assess the diagnostic accuracy of the detergent fraction of *S. venezuelensis* in the detection of anti-*S. stercoralis* IgG antibodies in serum samples from immunocompromised individuals with strongyloidiasis.

2. Material and methods

Serum samples from 93 individuals being 63 from adult immunocompromised individuals obtained at the Clinics' Hospital of the Federal University of Uberlândia (UFU), State of Minas Gerais, Brazil, and 30 serum samples from adult individual volunteers of the UFU, were analyzed and divided into three groups:

Group 1 (G1): 30 serum samples from immunocompromised individuals with a positive parasitological diagnostic for strongyloidiasis: HIV^+ (n = 7), diabetes mellitus type 2 (n = 2), cancer (n = 6), tuberculosis (n = 5), alcoholics (n = 10);
Group 2 (G2): 33 serum samples from immunocompromised individuals with a negative parasitological diagnostic for strongyloidiasis: HIV^+ (n = 8), diabetes mellitus type 2 (n = 5), cancer (n = 8), tuberculosis (n = 5), alcoholics (n = 7); and
Group 3 (G3): 30 serum samples from healthy individuals.

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All serum samples were obtained from individuals undergoing parasite examination and identification by Baermann (1917) [7] and Lutz (1919) [8], conducted with three samples from each individual, in the three groups. The samples from Groups I and II were obtained from patients affected by single type immunosuppression. Additionally, the samples of Group I were affected just for *S. stercoralis* and the samples of Group II were not affected by any kind of parasitic disease. Group III related healthy individuals based on their clinical observation, without evidence of contact with *S. stercoralis* infection or previous history of strongyloidiasis and three fecal samples tested negative. This study was approved, in 2013, by the Ethics Committee of the Universidade Federal de Uberlândia (UFU), State of Minas Gerais, Brazil, protocol number 307.605.

Parasites were maintained in *Wistar* rats (*Rattus norvegicus*) at the Centro de Bioterismo e Experimentação Animal (CBEA) (UFU), MG, Brazil. The animals were inoculated subcutaneously with *S. venezuelensis* infective larvae (L3). After 7 days of infection, feces were collected for charcoal culture. After 3 days in culture, the infective larvae were collected according to the Rugai method [9], centrifuged at 1500 rpm for 5 min in phosphate buffered saline (PBS) pH 7.2 and stored at −20 °C until use.

The total saline extract was obtained from infective larvae L3 of *S. venezuelensis* according to Gonzaga et al. (2011) [10]. L3 larvae were re-suspended in 0.01 M PBS (pH 7.2) containing protease inhibitors (1 mM ethylene diamine tetracetic acid; 1 mM benzamidine; 1 mM phenyl methyl sulfonyl fluoride; 1 mM aprotinin and 1 mM leupeptin) and subjected to mechanical disruption of 5 cycles of freezing (1 min, −96 °C) and thawing/sonication (5 min, 40 kHz, 4 °C) (Thornton, Electronics Inspec, São Paulo, Brazil). After overnight incubation at 4 °C with constant agitation, the solution was centrifuged at 12,400 ×*g* for 30 min at 4 °C. The protein was quantified according to Lowry et al. (1951) [11] and the solution was stored at −20 °C until use.

To obtain the detergent fraction, the total extract saline (ES) was subjected to protein separation using Triton X-114 according to Feliciano et al. (2010) [5]. To the 1000 μg of ES was added 200 μL of tris buffered saline (TBS), pH 7.4, and 1% Triton X-114. The mixture was incubated at 0 °C for 10 min. Subsequently 6% sucrose (w/v), 0.06% Triton X-114/TBS, pH 7.4, were added and incubated at 37 °C for 10 min. The sample was centrifuged at 3000 ×*g* for 10 min at room temperature. The pellet consisting of the detergent fraction of the total saline extract was precipitated in acetone and then re-suspended in TBS buffer. Protein quantification was done according to Lowry et al. (1951) [11], and the solution was stored at −20 °C until use.

For Enzyme-Linked Immunosorbent Assay (ELISA), preliminary experiments were performed to define the best conditions of antigen, serum, conjugate and plates. High affinity polystyrene microplates (BioAgency, Laboratories, São Paulo, Brazil) were coated separately with 5 μg/mL of total saline extract (ES) or the detergent fraction (D) overnight at 4 °C in 0.06 M carbonate–bicarbonate buffer (pH 9.6). After incubation, the plates were washed three times for 5 min with PBS plus 0.05% Tween20 (PBS-T) and blocked with PBST plus 3% skim milk at 37 °C for 45 min. Serum samples (1/80) were added and incubated for 45 min at 37 °C. After washing with PBST, the anti-human IgG antibody peroxidase conjugated (1/2000) was added and incubated for 45 min at 37 °C. The reaction was revealed by the addition of enzyme substrate (0.03% H_2O_2) and chromogen (o-Phenylenediamine) in 0.1 M phosphate-citrate buffer (pH 5.0). The reaction was incubated for 15 min at room temperature and stopped by adding 2 N H_2SO_4. Optical densities were determined at 492 nm in an ELISA reader (Titertek Plus, Flow Laboratories, McLean, VA). The reactivity index was obtained by the ratio: IR = OD/cut-off. RI values >1 were considered positive.

Statistical analyses were performed with GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The parameters of sensitivity, specificity, area under curve and likelihood ratio for the ELISA were evaluated by receiver operating characteristic (ROC curve) [12]. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

In the present study we used the detergent fraction to detect anti-*S. stercoralis* IgG antibodies in sera from immunocompromised individuals. It was observed that the detergent fraction (D) showed high positivity in the detection of IgG in serum samples from immunocompromised individuals with strongyloidiasis (Table 1 and Fig. 1a,b). Also noteworthy was the absence of false-positive results on serologic detection of IgG in individuals with cancer or affected with tuberculosis and in alcoholics. Using detergent fraction also led to a smaller percentage of false-positive results in healthy individuals. False-positive serological results were observed in HIV^+, diabetic and healthy individuals for both antigens preparations indicating a possible exposure to the parasite in some previous time, maintaining an immunological memory and a possible infection by *S. stercoralis* or cross-reactivity with other parasites, in both cases not detected by the parasitological methods.

The ROC curve data (Fig. 1c,d) confirmed the best diagnostic accuracy of the detergent fraction (D), with 83.33% sensitivity, 86.36% specificity, AUC of 0.91 ($CI_{95\%}$: 0.85–0.97) and LR+ of 6.11, in relation to the total saline extract (SE), that showed 73.33% sensitivity, 82.54% specificity, AUC of 0.86 ($CI_{95\%}$: 0.77–0.95) and LR+ of 4.2. These data corroborate with the results previously obtained for the detection of IgG anti-*S. stercoralis* in serum samples from immunocompetent individuals [5,6]. In this present study we demonstrated that the detergent fraction is also effective in the serological detection of strongyloidiasis in immunocompromised individuals. This aspect is important because immunocompromised individuals may have an ineffective humoral response, with low titers of immunoglobulins in the blood, against parasitic infections that may result in false-negative results.

The transmission of human strongyloidiasis occurs by penetration of infective larvae (L3) in intact skin or mucous membranes. A characteristic of the cycle involves the possibility of autoinfection. This mode of infection increases the parasitic load of infected individuals, being responsible for the long maintenance of the parasite in the host and is characterized as the main factor for the development of severe forms of strongyloidiasis as hyperinfection. Immunocompromised individuals by different causes may develop hyperinfection characterized by pulmonary and gastrointestinal hemorrhage, secondary bacterial and fungal infections due to larvae spreading from the gut to other organs, which may lead to a high mortality rate [4].

Studies conducted in the city of Uberlândia, Minas Gerais State, Brazil, demonstrated that human strongyloidiasis are associated

Table 1
Positivity of different clinical cases against total saline extract (SE) and detergent fraction (D) of *Strongyloides venezuelensis*.

Individuals	Antigens	
	SE	D
	n + (%)	*n* + (%)
Immunocompromised/*S. stercoralis* + (*n* = 30)		
HIV + (*n* = 7)	6 (85.7)	6 (85.7)
Diabetes mellitus 2 (*n* = 2)	2 (100)	2 (100)
Cancer (*n* = 6)	6 (100)	6 (100)
Tuberculosis (*n* = 5)	3 (60)	5 (100)
Alcoholics (*n* = 10)	5 (50)	8 (80)
Immunocompromised/*S. stercoralis* − (*n* = 33)		
HIV + (*n* = 8)	1 (12.5)	2 (25)
Diabetes mellitus 2 (*n* = 5)	2 (40)	3 (60)
Cancer (*n* = 8)	0 (0)	0 (0)
Tuberculosis (*n* = 5)	2 (40)	0 (0)
Alcoholics (*n* = 7)	0 (0)	0 (0)
Healthy (*n* = 30)	6 (20)	1 (3.3)

n number of samples.