Clinical, Laboratory and Ultrasonographic Evaluation of Patients with Acute Schistosomiasis Mansoni

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1. Introduction

Schistosomiasis is a parasite chronic disease caused by the helminth of genus *Schistosoma*. The infection is common in parts of Africa, South America, Middle East, Caribbean and Asia where it is a leading cause of morbidity and mortality [1]. Recent estimates suggest there are 700 million people at risk worldwide with almost 200 million infected in Africa alone [2]. In Brazil, the schistosomiasis infection is caused by *Schistosoma mansoni*, where estimates suggest a range from 2.5 million [3, 4] to 12 million people infected [5]. In the state of Minas Gerais, schistosomiasis mansoni is prevalent in 519 out of 853 municipalities, with an estimated number of one million infected people in an area of 300.000 km² [6].

One of the greatest public health problems in countries where the disease is highly endemic, including Brazil, is schistosomiasis control for the following reasons: (a) large intermediary hosts dissemination and their escape mechanisms from molluscicides and from biological

control due to high costs and low efficacy; (b) high charges associated with implementation of sanitary conditions and water supply and the intense contact of rural population with polluted water, as well as engagement in agricultural and fishing activities; (c) the long time needed for sanitary education and for the community to adhere to controlling programs; (d) individual or massive treatment has been shown efficient for controlling the morbidity, but not for reducing prevalence due to reinfection; (e) individual protection is unlikely, except for specific groups of exposed people; (f) until the current days, there is no effective vaccine for preventing schistosomiasis [7, 8].

According to previous data published by our group [9, 10], the proliferation of rural tourism in endemic areas may be an important contributing factor to the outbreak of schistosomiasis cases. The preservation of natural environment on the site, in order to attract visitors from urban centers, unintentionally contributes to create an ideal habitat for the intermediate host. Additionally, most areas chosen for leisure activities accommodate rural communities without any type of sanitation and, thus, represent a permanent risk of contamination of the flowing water.

In this context, visitors from urban areas, who never had previous contact with the parasite, contract the infection, and develop acute schistosomiasis [11]. Acute schistosomiasis is associated with a primary exposure and is more commonly seen in non-immune individuals traveling through endemic regions [12]. The clinical symptoms most commonly observed in such patients include fever, general weakness, headache, nausea, vomiting, diarrhea, anorexia, colic, weight loss, dry cough and hepatosplenomegaly accompanied by marked eosinophilia and leucocytosis [13-16]. However, these clinical symptoms may be confused with a number of infections such as visceral leishmaniasis, typhoid fever, malaria, tuberculosis, viral hepatitis, mononucleosis and bacterial infections [17]. Hence, the diagnosis of acute schistosomiasis becomes a challenge for the assistant doctor due to the wide diversity of non-specific symptoms presented by the patients; in addition, the presence of eggs in stool may not to be easily detected by parasitological examination in this phase of the infection.

Abdominal ultrasound is a complementary tool often used to assist the diagnoses of the *S. mansoni* infection, mainly on the study of the liver damage caused by the chronic infection. It is an inexpensive method, not radioactive or invasive, which provides immediate results and can be used for epidemiological fieldwork in endemic areas. The hepatic ultrasound pattern in patients with severe hepatosplenic schistosomiasis is characterized by pronounced periportal thickening. However, reports of ultrasound studies in patients with acute schistosomiasis are still scarce [18], and additional trials are necessary to evaluate the benefits and limitations of the ultrasound as a tool of clinical evaluation at the acute phase of the *S. mansoni* infection [19].

From the immunological point of view, the acute phase of the infection is characterized by a series of humoral and cellular immunological events. Hiatt et al. [20] showed elevations of IgG, IgM, IgE, and high titers of total antibodies in serum of acute patients that indicated the illness is associated with intense immune activity, while the magnitude of the IgE responses was related to the intensity of the infection. De Jesus et al. [16] showed that there was no significant difference in total IgE level between patients with

acute and chronic schistosomiasis. In addition, Caldas et al [21] showed that specific IgG, IgM and IgE titers against egg and worm antigens in acute patients do not differ from those presented by chronic patients. Regarding cellular immunological events, nitric oxide (NO) represents an important and versatile messenger in biological systems, and it has been identified as a cytotoxic factor in the immune system, presenting anti- or pro-inflammatory properties under different circumstances [22]. Oliveira et al. [23] demonstrated that human peripheral blood mononuclear cells (PBMC) are capable of *in vitro* NO production and the inhibition of its production through the addition of N omega-nitro-L-arginine methyl ester (L-NAME) is responsible for an exacerbated granulomatous reaction.

In this report, we evaluated the clinical/laboratorial parameters and ultrasonographic features of patients who suffered acute schistosomiasis resulting from a simultaneous exposure in a country house in the metropolitan area of Belo Horizonte, Minas Gerais State, Brazil.

2. Population, materials and methods

2.1. Study population

The patients evaluated in this study acquired the acute phase of *S. mansoni* infection in a country house in the rural district of the municipality of Igarapé in the metropolitan area of Belo Horizonte, Brazil. The country house is frequently leased during the weekends and long holidays. It is a large house with a pool of water supplied by a stream. Beside the pool, the water from the stream was channeled into open channels to form a shower. During the inspection of the country house, researchers at the Research Center Rene Rachou (CPqRR) – Oswaldo Cruz Foundation, at the Brazilian Ministry of Health detected that the place did not have basic sanitation. All the water used was damped, discarded in a dam located near the house. Out of forty-two individuals who were in the country house, thirty-eight participated in the study and were previously evaluated. After a preliminary assessment, we found that nineteen infected patients had been treated previous to our evaluation and five patients did not fulfill the inclusion criteria described below. Therefore, these patients were excluded from the study apart from other four subjects who had negative stool examination for *S. mansoni*. Thus, the group of patients evaluated was composed by ten individuals, five women and five men, aging 14 to 31 years, with parasite load ranging from 8 to 768 eggs per gram of feces (epg).

To participate in the study, patients in the acute phase of schistosomiasis have fulfilled the following criteria for inclusion: age between 10 and 65 years; did not report having received any treatment with anti-helminthic drugs in the last 24 months; diagnosis of acute schistosomiasis mansoni based on epidemiological data (recent contact with *S. mansoni* cercariae-contaminated water), clinical symptoms (acute enterocolitis, nausea, vomiting, abdominal pain, fever, headache, weight loss, cough, cercarial dermatitis, hepatomegaly and splenomegaly) and laboratory data (eosinophilia and viable *S. mansoni*

eggs in stool). Each volunteer or their legal guardian signed their informed consent. In addition, we also excluded from this study all patients who had one of the following conditions: unable to hold examinations proposed; alcoholism, defined as above average weekly consumption of 420 grams of ethanol (daily average over 60 g of ethanol) [24]; pregnancy, defined by laboratory criteria; significant anemia defined as hemoglobin less than 10 g/dl [25] and any other significant systemic disease, acute or chronic, that could interfere with the results of the proposed methods.

After blood collection, all patients who had positive stool examination for *S. mansoni*, regardless of participation in the study, were submitted to treatment with the standard Brazilian dose of Praziquantel (50-60 mg/kg).

A group of healthy volunteers formed by nine individuals, one woman and eight men aged 25 to 42 years, blood donors of the blood bank of Hospital Felicio Rocho, Belo Horizonte, Minas Gerais, Brazil constituted a control group (CT). It is important to mention that these individuals were screened and selected after serological tests for negativity for Chagas disease, leishmaniasis, human immunodeficiency virus (HIV), hepatitis, and did not report previous infection with *S. mansoni*.

The study was carried out according to the National Health Council resolution 196/96, which regulates the research involving human beings, and was approved by the Ethics Committees of the Faculty of Medicine, Federal University of Minas Gerais, Oswaldo Cruz Foundation, and the Brazilian National Committee on Ethics in Research.

2.2. Parasitological examination

Parasitological examination was performed using the Kato-Katz method [26]. The presence and the number of *S. mansoni* eggs per gram of feces were determined through examination of six blades per one stool sample for each patient. The results are presented as the arithmetic mean of number of eggs. The Kato-Katz method is the method of choice to measure infection level and has been used extensively in epidemiological studies.

2.3. Evaluation of clinical parameters

All the individuals who came into contact with water contaminated by cercariae were subjected to a detailed history, performed by a physician of our team. The survey used during this study contained data referring to number of the protocol, name, gender, age, education and place of birth and questions about the clinical symptoms/signs such as fever, diarrhea, nausea, vomiting, abdominal pain, cough, weight loss, headache, asthenia, facial edema and cercarial dermatitis.

2.4. Evaluation of hematological parameters

Peripheral blood was collected in 5 mL vacuum tubes containing ethylenediamine tetraacetic acid (EDTA) as anticoagulant (Vacutainer, Beckton Dickinson, CA, USA), approximately 40 days after contact with contaminated water by cercariae and only once prior to treatment with

praziquantel. The hemograms were performed in automated hematological electronic counter (Coulter MD18, E.U.A). The parameters measured were hemoglobin, number of erythrocytes and hematocrit values as well as total and differential counts of leukocytes including absolute counts of eosinophils, neutrophils, lymphocytes and monocytes.

2.5. Total IgE measurement

The plasma levels of total IgE antibodies were quantified using *Colorimetric Immunoenzymatic kit* (SYM Total IgE, Symbiosis Diagnostic, Brazil). Aliquots of 25 μL of plasma and six standards (Reagent kit SYM Total IgE Symbiosis Diagnostic, Brazil) at concentrations of 0, 5, 25, 50, 150 and 400UI/mL were dispensed in duplicates in wells of flat-bottomed plates, previously sensitized with streptavidin, together with 100 μL of monoclonal anti-human IgE conjugated to biotin (Reagent kit SYM Total IgE Symbiosis Diagnostic, Brazil). The plates were incubated for 30 minutes at room temperature in the dark and washed five times with 300 μL/well of wash solution (Reagent kit SYM Total IgE Symbiosis Diagnostic, Brazil). Subsequently were added 100 μL of a second monoclonal anti-human IgE conjugated to peroxidase. The plates were incubated for 30 minutes at room temperature, in the dark, and washed five times with 300 μL/well of wash solution. Then, 100 μL of chromogen-substrate solution (tetramethylbenzidine-TMB) (Reagent kit SYM Total IgE Symbiosis Diagnostic, Brazil) were added by well, and the plate was incubated for 15 minutes in the dark. The reaction was stopped by adding 100 μL of 1N sulfuric acid (H_2SO_4) per well (Reagent kit SYM Total IgE Symbiosis Diagnostic, Brazil). The optical density of samples and standards was measured in an automatic reader (Molecular Devices Versa Max, California, USA) using a 450 nm filter.

2.6. Nitric oxide plasma levels

The plasma levels of nitric oxide were quantified using *Immunoenzymatic kit QuantiChromTM Nitric Oxide* (Quantitative Colorimetric Determination of Nitric Oxide, BioAssay Systems, USA). The assays were performed according to the manufacturer's recommendations. The deproteination of samples was made by adding 80 mL of zinc sulfate ($ZnSO_4$) 75 mM final concentration and 120 mL of sodium hydroxide (NaOH) 55 mM final concentration. Samples were centrifuged at 400 g for 5 minutes at 4°C. Then 70 mL of glycine buffer was added in each sample. Afterwards, aliquots of 100 μL of plasma and eight standards at concentrations of 0, 5, 10, 15, 20, 30, 40 and 50 μM mL were dispensed in duplicates in 1.5 mL tubes. Then, samples were placed in the presence of cadmium activated with 200 mL of Buffer Activation and incubated for 15 minutes. The samples were transferred in duplicates to their respective wells in flat-bottomed plates and added to 50 mL of reagents A and B. The optical density of samples and standards was measured in an automatic reader (Molecular Devices Reader-Precision, USA) using a 540 nm filter.

2.7. Evaluation of hepatic enzymes

For the analysis of hepatic functions for the study group, plasma concentration of the enzymes ALT (alaline amino transferase), AST (aspartate amino transferase) and γ-GT

(gamma-glutamyl transferase) were quantified by kinetic method using *ALT/GPT Liqui-form, AST/GOT Liquiform and GAMA GT Liquiform kits* (Labtest Diagnostic, Brazil) in the device Wiener Lab Metrolab 2300 plus (Model CM-200). The results were expressed in UI/mL.

2.8. Ultrasonographic analysis

Ultrasonographic evaluation was performed using a Nemio SSA/550ª machine (Toshiba, Town, Japan) with a 3-MHz sector probe for patients ≥ 10 years old and a 5-MHz probe for patients under 10 years old. The group of healthy individuals non-infected with *S. mansoni* (CT group) consisted of twelve volunteers, paired by sex and age with the in-fected patients. Seven acute patients, three women and four men aged 14 to 31 years old have had ultrasonographic evaluation. All study population was examined by the same physician (MCBA). Liver size, portal-vein diameter, thickness of the central walls and pe-ripheral portal branches, spleen size and splenic vein diameters were assessed as descri-bed [27, 28]. The liver was also examined for surface smoothness. Portal vein diameter was measured at its entrance into the liver and its bifurcation inside the liver. The spleen was evaluated by using oblique and longitudinal scanning of the left upper quad-rant. The gallbladder was examined for wall thickness and stones. The periportal thick-ness was evaluated according to previous established criteria by [27-29].

2.9. Statistical analysis

The statistical analysis of the data was made through GraphPad PRISM® 5.00 software release for Windows (La Jolla, CA, USA). Student's t test was used for parametric data comparison between two groups, while for non-parametric data we used the Mann-Whitney test. In all cases, the data were considered significant at P < 0.05.

3. Results

3.1. Demographic data and intensity of S. mansoni infection

Out of forty-two people who were present in the country house, thirty-eight (90.5%) partici-pated in this study and reported contact with water contaminated with cercariae. Out of these, thirty-four (89.5%) had eggs of *S. mansoni* in their feces and four (10.5%) were repeatedly negative. According to criteria previously mentioned, twenty-four patients were excluded from the study and ten patients were considered to be on an ongoing acute phase of schisto-somiasis. In the acute group (ACT), there were no significant differences in age between females and males (23.0 ± 7.8 and 19.8 ± 4.5 years old, respectively, p value = 0.45) and the intensity of infection did not differ regarding gender, (205.6 ± 318.5 and 98.4 ± 70.7 eggs/g of feces, respectively, P value = 0.50). The ACT group presented parasite load ranging from 8 to 768 eggs per gram of feces (152.0 ± 224.7).

Parameter	Subjects		
	Total individuals	Evaluated individuals	Acute patients
Number of subjects (%)	42 (100%)	38 (90.5%)	10 (23.8%)
Age			
Range	01 - 65	01 - 65	14 - 31
Mean ± SD	22.4 ± 15.2	22.5 ± 15.5	21.4 ± 6.2
Median	21.5	20.5	22.5
Gender			
Female	25 (59.5%)	22 (57.9%)	05 (50.0%)
Male	17 (40.5%)	16 (42.1%)	05 (50.0%)
Parasitological Examination			
Negative	----	04 (10.5%)	0
Positive	----	34 (89.5%)	10 (100%)
Egg counts (eggs/g of feces)			
Range	----	0 - 768	8 - 768
Mean ± SD	----	50.8 ± 132.2	152.0 ± 224.7
Median	----	10.0	86.0

Table 1. Characterization of the study population

3.2. Clinical manifestations

The magnitude of clinical manifestations of schistosomiasis varies from light to severe intensity. Two patients showed serious clinical symptoms and required hospitalization due to the severity of the disease. The patients reported the onset of symptoms 20-30 days after water exposure. Headache and fever (60.0% and 50.0%, respectively) were the most common symptoms among patients, followed by diarrhea and weight loss (both 40.0%). Other clinical symptoms/signals were also recorded such as nausea/vomiting, abdominal pain, cough, asthenia, facial edema (each one with frequency of 30.0%) as well as urticaria and cercarial dermatitis (both 10.0%).

Table 2 highlights the main features observed in different human studies evaluating acute phase of schistosomiasis mansoni performed in distinct brazilian states such as Pernambuco,

Sergipe and Minas Gerais. Although in these studies the frequency of exposed/infected individuals was similar ranging 90-100% and the incubation period has also occurred in comparable times such 20-30 days, we have observed strongly heterogeneous symptoms between them. Nonetheless, our study demonstrated some similar clinical symptoms in comparison to those found by Barbosa et al. 2001b [30] like the frequency of fever (50.0% and 54.0%, respectively), cough (30.0% and 33.0%, respectively) and urticaria (10.0% and 8.0%, respectively). However, other parameters presented distinct results like the frequency of headache (60.0% and 33.0%, respectively) and diarrhea (40.0% and 25.0%, respectively).

Parameters	Barbosa Et Al. 2001a [15]	Barbosa Et Al. 2001b [30]	De Jesus Et Al. 2002 [16]	This Study
Number of patients	11	12	31	10
Site/State	Ilha de Itamaracá Pernambuco	Escada Pernambuco	Aracaju Sergipe	Igarapé Minas Gerais
Exposed/infected	92%	100%	92%	90%
Incubation period (days)	15-30	20	20-30	20-30
Headache	36%	33%	87%	60%
Fever	100%	54%	90%	50%
Diarrhea	64%	25%	81%	40%
Cough	91%	33%	91%	30%
Abdominal pain	64%	N.E.	93%	30%
Urticaria	18%	08%	N.E.	10%
Cercarial dermatitis	100%	N.E.	N.E.	10%
Hepatomegaly	75%	N.E.	35%	86%
Transaminases (increase)	36%	N.E.	38%	N.L.

N.E. - not evaluated

N.L. - normal levels

Table 2. Main features observed in different human studies evaluating the acute phase of schistosomiasis mansoni

3.3. Plasma levels of total IgE and nitric oxide

The plasma levels of total IgE and nitric oxide from the ACT group are illustrated in Figure 1. The analysis of the results showed that the ACT group presented a significant ($P < 0.05$) increase of these parameters as compared with the CT group.

3.4. Hematological profile

The main findings related to the hematological profile from the ACT group are illustrated in Figures 2 and 3. The analysis showed a significant reduction in the concentration of hemoglobin in the ACT group as well as an increase in the total leukocytes count as compared with the CT group. Increased total leukocytes were reflected in the absolute values of lymphocytes and eosinophils also increased in the ACT group as compared with the CT group. No significant difference was found between these two groups regarding other analyzed parameters such as number of erythrocytes, percentage of hematocrit, and absolute values of neutrophils and monocytes.
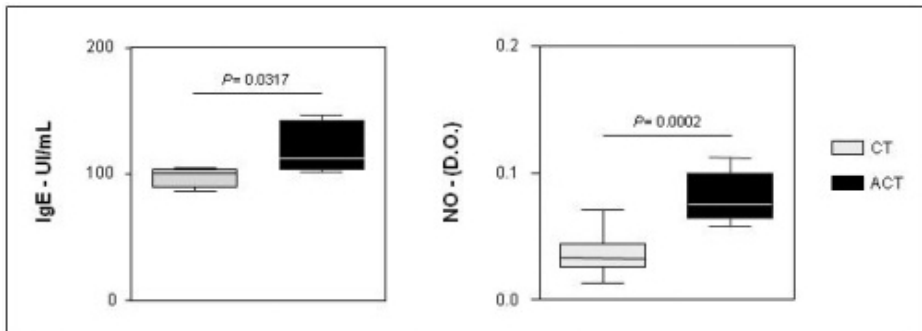

Figure 1. Total plasma IgE and nitric oxide (NO) levels in acute schistosomiasis patients (ACT=10) and non-infected individuals (CT=09). The results are expressed in box-plot format. The box stretches from the lower hinge (defined as the 25th percentile) to the upper hinge (the 75th percentile) and therefore contains the middle half of the scores in the distribution. The median is shown as a line across the box. Therefore 1/4 of the distribution is between this line and the top of the box and 1/4 of the distribution is between this line and the bottom of the box. Significant differences (connecting lines) were considered at $P < 0.05$.
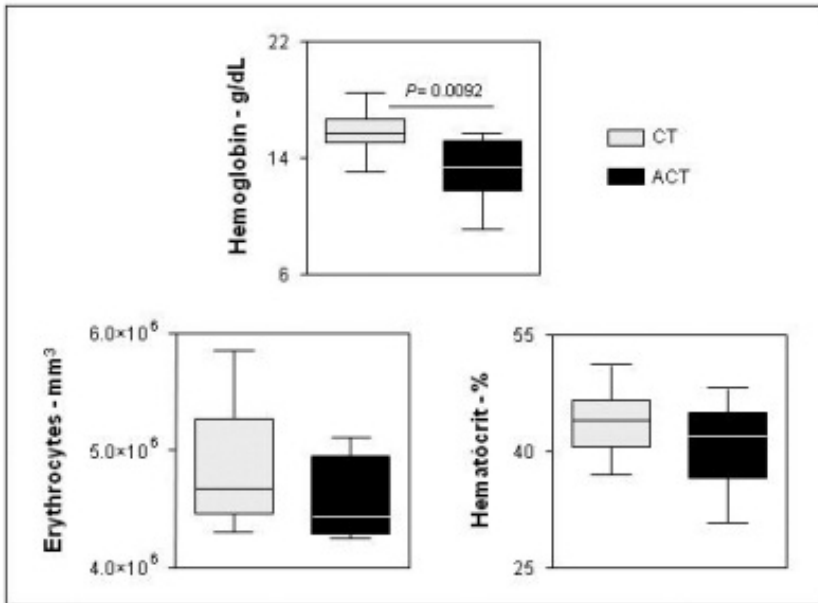
Figure 2. Hematological profile (hemoglobin-g/dL, erythrocytes/mm³ and percentage of hematocrit) from acute schistosomiasis patients (ACT=10) and non-infected individuals (CT=09). The results are expressed in box-plot format. The box stretches from the lower hinge (defined as the 25th percentile) to the upper hinge (the 75th percentile) and therefore contains the middle half of the scores in the distribution. The median is shown as a line across the box. Therefore 1/4 of the distribution is between this line and the top of the box and 1/4 of the distribution is between this line and the bottom of the box. Significant differences (connecting lines) were considered at $P < 0.05$.
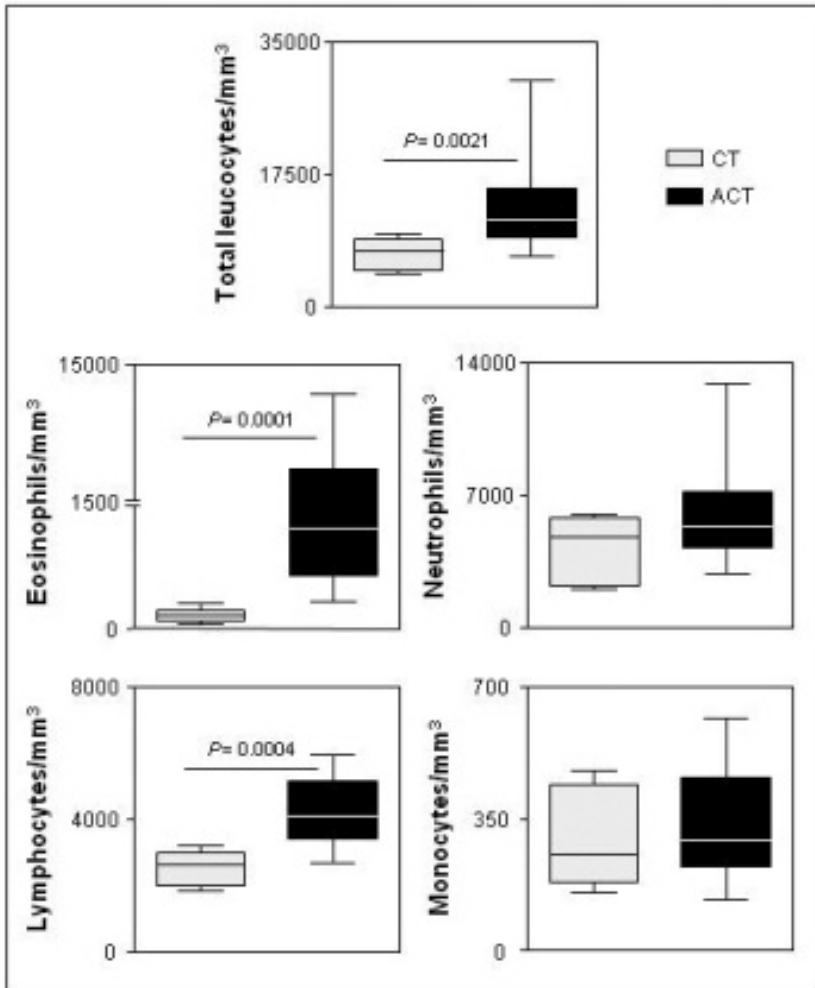
Figure 3. Total and differential leukocyte counts from acute schistosomiasis patients (ACT=10) and non-infected individuals (CT=09). The results are expressed in box-plot format highlighting the gap of 50% of data set measurement for absolute values in mm³ of each leukocyte subset analyzed. Significant differences (connecting lines) were considered at $P < 0.05$.

3.5. Profile of hepatic enzymes

Acute patients presented levels of ALT, AST and γ-GT enzymes similar to reference values and there were no significant differences between these levels and those presented by CT group. (Table 3).

Hepatic Enzymes	Control Group (N = 9)	Acute Patients (N = 8)
ALT (alaline amino transferase) (3-50 U/mL)*	12.5 ± 11.2	8.6 ± 5.5
AST (aspartate amino transferase) (12-46 U/mL)*	18.7 ± 5.9	23.0 ± 6.7
γ-GT (gamma-glutamyl transferase) (9-61 U/L)*	23.4 ± 9.9	33.9 ± 16.6

Values are represented as mean ± standard deviation

* Reference values

Table 3. Hepatic serological tests in the study group

3.6. Ultrasonographic analysis

The results demonstrated that the ACT group presented some significant differences as compared with the CT group: medium increase in the measurement (in mm) of longitudinal left/right lobe of liver (117.4 ± 19.7 and 99.0 ± 12.3 or 150.1 ± 18.2 and 102.2 ± 16.0, respectively), size of longitudinal spleen (110.6 ± 16.3 and 91.2 ± 12.7, respectively) as well as dimension of Hilar portal vein wall (3.4 ± 1.0 and 1.5 ± 0.3, respectively) (Table 4). Periportal lymph node enlargement was observed in six of seven patients (85.7%). The periportal lymph nodes were larger, round or ovoid in shape and sharply defined with thin halos surrounding hypoechoic areas (Figure 4). An additional ultrasonographic feature observed was an incipient periportal echogenic thickening named as grade I fibrosis (Figure 5).

Site Of Measurement	Control Group (N = 12)	Acute Patients (N = 7)
Longitudinal left lobe of liver	99.0 ± 12.3	117.4 ± 19.7*
Anteroposterior left lobe of liver	46.7 ± 10.0	53.3 ± 10.9
Longitudinal right lobe of liver	102.2 ± 16.0	150.1 ± 18.2*
Anteroposterior right lobe of liver	68.6 ± 11.9	63.4 ± 9.6
Longitudinal spleen	91.2 ± 12.7	110.6 ± 16.3*
Anteroposterior spleen	35.1 ± 6.3	43.5 ± 11.1
Portal vein	10.0 ± 1.2	9.9 ± 1.9
Hilar portal vein wall	1.5 ± 0.3	3.4 ± 1.0*
Splenic vein	6.4 ± 1.3	7.1 ± 1.4
Superior mesenteric vein	6.7 ± 1.1	7.1 ± 1.3
Periportal lymph node	N.V.	1.8 ± 1.0*

Values are represented in mm as mean ± standard deviation

* Statistical difference ($p < 0.05$)

N.V. - not visualized (normal abdominal lymph nodes are usually not visualized by sonography)

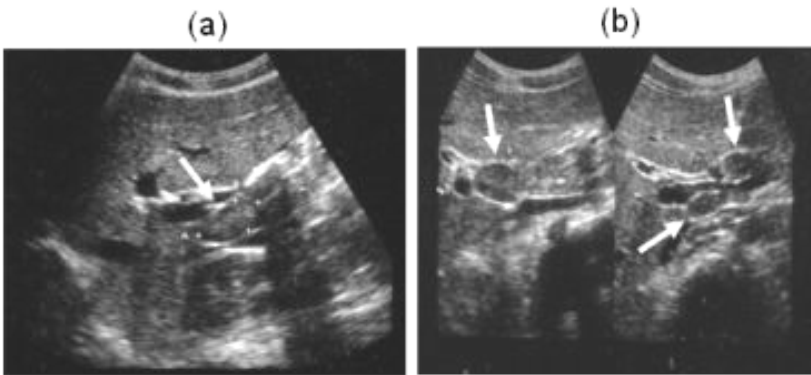**Table 4.** Ultrasonographic features of the study group

Figure 4. Representative ultrasonographic images of periportal lymph nodes from acute schistosomiasis patients. The arrows indicate larger lymph nodes, ovoid in shape (a) and rounded (b) with thin halos surrounding hypoechoic areas. Ultrasonographic analysis was performed with a Nemio SSA/550ª machine (Toshiba).
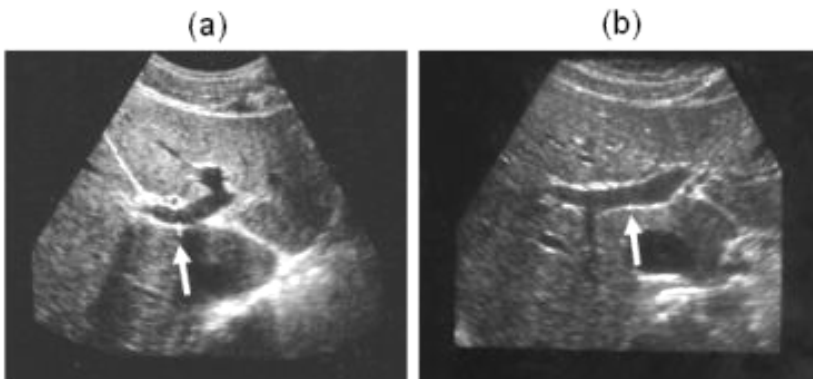

Figure 5. Representative ultrasonographic images of liver from acute schistosomiasis patient (a) and healthy volunteer (b). The arrows indicate incipient periportal echogenic thickening (a) and no periportal echogenic thickening (b). Ultrasonographic analysis was performed with a Nemio SSA/550ª machine (Toshiba).

4. Discussion

The transmission of *S. mansoni* may vary depending on the ecology of the disease and the social standing of the population in which it occurs. The prevalence and intensity of the infection is subject to cultural practices, specific for each locality or situation and usually associated to economic, domestic or leisure activities [15, 31].

During the last decades, the traditional epidemiological pattern showed a tendency to change. Accelerating migration from the countryside to cities threatened to overwhelm

existing water and sanitation systems, and to increase urban schistosomiasis [9, 32, 33]. In Brazil, especially in urban areas, acute schistosomiasis outbreaks were observed and documented with a certain frequency [9, 14, 15, 17, 34-36]. The increase of acute schistosomiasis cases also indicates that a non-immune fraction of the population became exposed to the disease [9, 10, 36].

The incidence of the acute form of schistosomiasis mansoni is certainly underestimated. This illness has been mainly described as a disease of travelers. Many scientific publications concerning this acute disease refer to groups of tourists, fishermen or sailors originally from a non-endemic country who have visited a tropical zone [17, 37, 38]. However, as schistosomiasis is a focally distributed infection [17, 39, 40], the acute form is also diagnosed in inhabitants from endemic countries who do not live in endemic areas. Nevertheless, acute disease is seldom recognized in infected patients from endemic areas. According to Lambertucci [41], acute schistosomiasis is most evident in primary infection in non-immune individuals. Among people living in endemic areas, the acute phase may pass undiagnosed. Because in endemic areas exposure to infection occurs early in life, symptoms would be inconspicuous and diagnosis not even suspected.

The clinical features of the acute phase of schistosomiasis mansoni present a wide spectrum [42, 43] and the relative contribution of host and parasite factors in the pathogenesis of the disease is not completely elucidated [20, 43-45]. According to Neves [46], the clinical forms of the disease will depend on the interaction of at least three sequential events: (a) the evolution phase of the worms, whether before or after oviposition and the deposition of eggs in the tissues; (b) the organ predominantly involved by young or mature worms and by their eggs; and (c) the type and the qualitative/quantitative deviation of the total and local host response to antigenic products derived from the disintegration of schistosomula, adult worms and their eggs.

The main clinical findings presented in our study such as headache, fever, diarrhea and weight loss were consistent with those found by other authors [9, 15-17]. Different intensities of clinical manifestation are observed in patients with acute schistosomiasis, some of them evolved with a relatively severe picture while others with mild symptoms. In our study, we have also observed a similar profile.

The development of non-apparent clinical form characterized by blood eosinophilia and a positive immediate cutaneous reaction in the initial phase of the *S. mansoni* infection was described by Rocha et al. [47]. According to previous data in the literature [13, 15, 16, 43], patients with acute phase of schistosomiasis in our study had an increased absolute number of eosinophils in comparison to reference data ranging between 400 to 600 cells/mm^3 [48]. Eosinophils are thought to play a major role in a variety of human diseases, including allergic inflammation, malignancy, and host defense against helminth infections. However, the exact role(s) of eosinophils in schistosomiasis regarding immunopathology remains unclear. Eosinophils may participate in Ab-dependent protective immune responses [49]. Human eosinophils express IgE receptors that participate in an IL-5-dependent Ab-dependent cell-mediated cytotoxicity reaction against schistosomula in vitro. Eosinophils also mediate the destruction of miracidia and schistosome eggs

[50]. Experimental studies have shown that the eosinophilia would be induced by type 2-cytokines as interleukin (IL)-4, IL-9, IL-13 and mainly by IL-5 [51]. According to Rocha et al. [43], the eosinophilia may be considered a possible pathogenic factor in acute schistosomiasis. The specific granules of the eosinophil contain lysosomal hydrolases as well as several cationic proteins like the major basic protein (MBP) [43, 52], the eosinophil cationic protein (ECP) and a neurotoxin, the eosinophil-derived neurotoxin (EDN), besides a peroxidase [43, 53]. The MBP in low concentrations is toxic to schistosomula but this action is non-selective since mammalian cells can also be damaged [43, 54, 55]. Both peroxidase and ECP are also toxic for helminths and mammal cells [43, 56, 57]. The cytolysis or eosinophil degranulation with the release of granule proteins can contribute to the genesis of local pathological alterations causing dysfunction or damage to the cells, especially when a great number of eosinophils are involved [43, 53, 58].

According to this report, acute patients presented increased plasma levels of IgE and nitric oxide as compared with the CT group. Our result is similar to the data published by De Souza et al. [59], which evaluated the humoral immune response through quantifying of the total IgE levels from patients with acute clinical form of schistosomiasis. Thus, we suggest that total IgE may be related to disease morbidity, as established by Pereira et al. [60]. Nitric oxide (NO) has been identified as an important cytotoxic factor and versatile messenger in the immune system being responsible to induce both anti- and/or proinflammatory effects [61]. There are broad direct and indirect evidences that NO can act as an anti-schistosomal and antiparasitic molecule [62-64]. Moreover, NO produced by human leukocytes has been shown to kill larval schistosome parasites [64, 65]. Studies in experimental models of acute schistosomiasis showed that the inhibition of iNOS resulted in cachexia and exacerbated hepatic pathology, suggesting that in schistosomiasis, NO limits hepatocyte damage [66-68].

From two to eight weeks after a first contact with natural water infested by *Schistosoma* cercariae, susceptible infected patients may present a syndrome comprising a period of 2 to 30 days of fever, diarrhea, toxemia and weakness, weight loss, abdominal pain, cough, mialgya, and arthralgia, edema, urticaria, nausea/vomiting and hepatosplenomegaly [7]. Until recently, acute schistosomiasis diagnosis was only based on epidemiological and clinical features, presence of *S. mansoni* eggs in stools and eosinophilia. However, the diagnosis becomes a challenge to the physicians due to the non-specificity symptoms as well as the lack of positivity of the subjects for *S. mansoni* eggs in feces in earlier stage of the infection. In this context, abdominal ultrasound can be a complementary tool to assist the diagnoses of the *S. mansoni* infection. The ultrasonographic findings presented in this study were consistent with those described by Barata et al. [11]. According to this report, ultrasonographic evaluation showed non-specific increase in the size of liver, incipient periportal echogenic thickening, in addition to lymph nodes easily identified in the periportal region. The authors have also performed a histological liver analysis from hepatic biopsies of three patients, and the data demonstrated that the periportal thickening seen on ultrasound was probably produced by inflammatory infiltration of the portal tracts caused by schistosomiasis, which disappeared after treatment with oxamniquine.

However, liver biopsy is an invasive procedure that is seldom justified. It is important to mention that the ultrasonographic features of the lymph nodes, liver and spleen in acute schistosomiasis, although highly sensitive, are not pathognomonic of acute schistosomiasis, and are also seen in other infectious diseases such as acute hepatitis and other viral diseases [19, 69, 70].

Normal abdominal lymph nodes are usually not visualized by sonography. Neoplastic lymph nodes are generally rounded and hypoechoic, sometimes with an irregular shape and borders [19, 71, 72]. Lymphomas can also affect periportal and peripancreatic lymph nodes, but there is generally an extensive involvement of other abdominal lymph nodes, mainly retroperitoneal, that is almost anechoic and usually rounded. In this circumstance, the liver and spleen are enlarged and can show focal lesions [19, 72]. Patients with acquired immunodeficiency syndrome (AIDS) can also show hepatoesplenomegaly and abdominal lymphadenopathy, but the echogenicity of the liver is usually increased. Tumors related to AIDS, such as Kaposi's sarcoma, have also been reported as a cause of lymphadenopathy [73]. Mesenteric and periportal lymphadenomegaly is a common finding in blastomycosis and these are almost anechoic [19].

In the current study, we have also evaluated the liver function of acute schistosomiasis patients by the ALT, AST and γ-GT assessment. Comparing with other studies in literature [15, 16], we did not observe changes in any of the enzymes evaluated, suggesting that the hepatocellular function is preserved or minimally compromised in the study population. In patients presenting intestinal or hepatointestinal clinical form of schistosomiasis,it has been observed that the liver function is maintained. In those patients showing the hepatosplenic form, liver function related to protein metabolism was in general changed, but rarely this function is compromised in a broad spectrum. Only after repeated blood spoliation, in the terminal stage of illness, there is strong involvement of the liver parenchymal. According to some authors, when liver cell damage occurs there is an increase of ALT and AST levels, but in the *S. mansoni* infection this type of injury is not commonly observed [74, 75]. On the other hand, the most important cause of increased

γ-GT levels is the chronic stimulation of the microsomal fraction of hepatocytes and the presence of cholestasis [76]. According to Martins & Borges [77], the chronic stimulation of the microsomal occurs only in patients with hepatosplenic form of schistosomiasis. Kardorff et al. [74] observed that the increase of γ-GT occurs only in patients with severe periportal fibrosis, irregular texture liver and collateral vessels.

5. Conclusion

In conclusion, the data presented here suggest that alterations identified by analyses of clinical/ laboratorial parameters and ultrasonographic features, although not specific, are compatible with the acute phase of Schistosomiasis mansoni and provide a reliable complementary tool for the diagnosis of the infection.

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