

HIGH LEVELS OF IgG4 TO *SCHISTOSOMA MANSONI* EGG ANTIGENS IN INDIVIDUALS WITH PERIportal FIBROSIS

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Abstract. Specific IgG4 and IgE responses to adult worm antigen and soluble egg antigen (SEA) were examined in 267 individuals from an area in which schistosomiasis mansoni is endemic. Based on information obtained from clinical and sonographic examinations of this sample, the individuals were divided in three groups: 1) 204 individuals without periportal fibrosis, and liver and spleen enlargements; 2) 41 individuals without periportal fibrosis, but presenting with organopathy, with or without organomegaly; and 3) 22 individuals with periportal fibrosis, regardless of their status as having hepatomegaly and/or splenomegaly. Levels of IgG4 to SEA were significantly higher in sera from patients with fibrosis compared with the patients from the other two groups. We also found significantly higher levels of IgG4 against SEA in egg-negative patients with fibrosis compared with egg-negative patients from the other two groups. This report demonstrates a specific humoral response in patients presenting with initial fibrosis, a form of schistosomiasis transient between intestinal and severe hepatosplenic.

INTRODUCTION

Schistosoma mansoni has a wide geographic distribution in Africa, South America, and the Caribbean. The disease is due mainly to eggs deposited in host tissue by the adult female. Egg antigens induce granuloma formation and fibrosis, mostly in the intestine and liver (portal system). Most infected individuals living in endemic areas are asymptomatic, but a few develop Symmers' periportal fibrosis of the liver and its sequelae of portal hypertension and esophageal varices. Many investigators attribute the intensity of infection as the main cause of morbidity in schistosomiasis infection.^{1,2} However, the intensity of infection may also be related to behavioral routines (water contact),^{3,4} age,⁵ and sex.^{6,7} Individual susceptibility that is associated with the immunologic^{8,9} and genetic profiles¹⁰⁻¹² of each individual living in the endemic area is also important.

The contribution of each of these factors to the sequential development of pathology in *S. mansoni* infections leading to hepatosplenic disease is not completely understood. Studies of human immunity to schistosomiasis in endemic populations have indicated that parasite-specific antibody isotypes vary in their association with either susceptibility or resistance to infection/reinfection.¹³⁻¹⁶ High levels of IgE and IgG4 are produced during helminth infection. Evidence for a significant correlation between susceptibility to reinfection to *S. mansoni* in humans and increased production of IgG4 antibody was first reported by Auriault and others.¹⁷ In fact, elevated production of IgG4 was consistently associated with increased susceptibility to reinfection,¹⁸ and low production of this isotype was found in patients resistant to infection (endemic normal individuals)¹⁹ and to reinfection.¹³ The association between high levels of IgG4 and susceptibility was thought to result from the blocking effect of this isotype on the protective effect of IgE.¹⁸ More recently, a positive correlation between IgG4 to soluble egg antigen (SEA) and intensity of infection has been reported.²⁰ Previous reports also associated susceptibility with heavy infection as a high risk for the development of periportal fibrosis.^{1,21} However, there are

few studies linking humoral immune response with morbidity.²²⁻²⁴

With the introduction of ultrasound examinations in schistosomiasis field surveys, it has become possible to identify individuals with different grades of periportal thickness due to fibrosis, a typical lesion of hepatic schistosomiasis corroborated by histopathologic studies of liver biopsies.²⁵⁻²⁷

In the present study, we used ultrasonography to grade periportal fibrosis in patients from an area in which *S. mansoni* is endemic in an attempt to correlate the development of hepatosplenic disease with aspects of the humoral response associated with susceptibility to *S. mansoni* infection. This is part of an extensive study to elucidate the contributions of demographic, behavioral, immunologic, and genetic factors on the development of susceptibility and resistance to *S. mansoni* infection and pathology.

MATERIALS AND METHODS

Study design. This investigation was conducted in an area endemic for schistosomiasis, Córrego dos Melquíades, in the Minas Gerais State of Brazil. It was reviewed and approved by the Ethical Committee of Fundação Oswaldo Cruz, Ministry of Health. Local health care personnel explained the study to the village population. It was stressed that all individuals would receive treatment irrespective of whether or not they participated in the study. Participants were enrolled only with their explicit approval, or with that of their parents in the case of children less than 15 years of age. A cross-sectional survey was then conducted, which enumerated individuals and assigned them personal identity numbers and a household identity number. The clinical history of participants was collected, along with their quantitative stool egg counts as determined by duplicate Kato-Katz thick smears. Fecal examinations were completed in May 1998. In September, 340 participants were given physical examinations. Individuals with symptoms of the disease (abdominal pain and diarrhea) or those having liver and/or spleen enlargement detected by abdominal palpation even during deep breaths or

whose abdominal palpation was difficult (expontaneous muscle contraction or an excess of fat) were scheduled for an ultrasound examination. A total of 267 individuals were examined by ultrasonography. The physical examination and ultrasonography were done independently by different physicians. From September to December, 10-ml blood samples were collected in sterile vacutainer tubes (Becton Dickinson, Rutherford, NJ). At the same time infected patients received treatment with oxamniquine at the Brazilian standard dose of 15 mg/kg of body weight for adults and 20 mg/kg of body weight for children. One year after treatment, feces were collected for evaluation of reinfection. Egg-negative individuals and those that did not return a feces sample were not included in the calculation of the reinfection rate. Active infection with *S. mansoni* was defined as the presence of eggs in the stools. Malaria and leishmaniasis, confounders for hepatosplenic disease, are not endemic in the study area.

Detailed methods and the results of the water contact survey undertaken in the area have been previously reported.^{28,29} Questions concerning the frequency of water contact were derived from a direct water contact observation study in the same area.²⁸ Briefly, participants were interviewed individually as to their water contact behavior.²⁹ Questions consisted of the frequency per week for each water contact behavior and the source of the water (e.g., well, bamboo conduit). Parents were asked about their children's water contact behavior only when the children were less than six years old; no proxy responses for water contact behavior for participants more than six years old were accepted. All water contacts were divided into potentially infected (streams, canals, fish ponds, "bicas") or potentially safe sites (springs, wells, water hoses, household faucets, wash basins, and showers sites). The determination of an infected or safe site was based on the presence (infective) or absence (safe) of snail intermediate hosts determined by malacologic surveys undertaken at each water contact site. Water contact frequencies that occurred at potentially infective sites were then multiplied by standardized duration and body immersion values and defined as total body minutes (TBM).²⁹

Ultrasound evaluation. Two hundred sixty-seven subjects were subjected to abdominal ultrasonography using a portable Hitachi EUB-200 machine (Tokyo, Japan). Patients less than five years old were excluded. Liver size, portal-vein diameter, thickness of the walls of peripheral portal branches, spleen size, and splenic vein diameter were assessed as described elsewhere.²⁵⁻²⁷ Liver span was measured both in the midclavicular line and the midline. The liver was also examined for smoothness of surface. The ultrasound criteria for schistosomal hepatic fibrosis are multiple diffuse echogenic areas scattered throughout the liver. Portal vein diameter was measured at its entrance into the *porta hepatis* and its bifurcation inside the liver. The spleen intercostal spaces were evaluated using oblique and longitudinal scanning of the left upper quadrant. The splenic size and texture were noted and the gallbladder was examined for wall thickness and stones. The following classification was adopted to assess the morbidity of *S. mansoni* infection:²⁵ grade 0 = periportal fibrosis thickness less < 3 mm; grade I = thickness of 3-5 mm. There were no significant differences between the control, pathology, and fibrosis groups in relation to portal and splenic vein diameters (cm, mean \pm SD) (portal vein: 1.1 \pm 0.2, 1.2 \pm 0.2, and 1.1 \pm 0.2; splenic vein: 0.7 \pm 0.1, 0.8 \pm 0.2, and 0.7 \pm 0.2,

respectively). The term organopathy was used for individuals with alterations in size, surface or parenchyma (liver or spleen) without periportal echogenicity (periportal fibrosis). Although most of these patients were infected, the liver alterations may not be due to schistosomiasis because they did not have the characteristic periportal thickening limited to the portal vein and its tributaries. The physicians performing ultrasound examinations were not aware of the infection status, results of the clinical examination, and water contact results of the patients undergoing ultrasonography.

Parasitologic examination. Infection intensities were measured by individual fecal egg counts, expressed in eggs per gram of feces (epg) performed by the Kato-Katz³⁰ method on stool samples obtained on three consecutive days. An individual's fecal egg count was the arithmetic mean of these three determinations. Due to the important role of diagnostic sensitivity in causing a truncation in the frequency distribution of egg output,³¹ every attempt was made to standardize the reading of slides, including having the same technicians prepare and read all slides. Quality control was ensured by blinded cross-checking of slides between technicians.

Preparation of crude *S. mansoni* antigens. Sera were examined by enzyme-linked immunosorbent assay (ELISA) for antibody isotypes reactive to a soluble worm antigen preparation (SWAP) and SEA. These antigens were prepared according to methods previously described.^{32,33} The protein concentration was determined with a bicinchoninic (BCA) kit (Pierce, Rockford, IL).

Indirect ELISA. The ELISA was performed using SWAP and SEA. Briefly, plates (Maxisorb; Nunc, Roskilde, Denmark) were coated with 100 μ l of SWAP or SEA at a concentration of 5 μ g/ml in carbonate-bicarbonate buffer (pH 9.6). The plates were then sealed and incubated overnight at 4°C. The next day the plates were washed with 0.15 M phosphate-buffered saline (PBS, pH 7.2). The plates were blocked with 0.15 M PBS, 0.05% Tween 20 (PBST), and 10% fetal bovine serum for 1 hr. The plates were then decanted and 100 μ l of sample sera was added in duplicate two-fold dilutions (from 1:100 to 1:800). The plates were covered and kept overnight at 4°C. The plates were then washed five times in 0.15 M PBST and 100 μ l of biotin-conjugated anti-human IgG4 (Zymed, San Francisco, CA) was added to each well at a dilution of 1:1,000 for IgG4. The plates were incubated for 90 min at room temperature and then washed five times with PBST. One hundred microliters of 1:1,000 streptavidin horseradish peroxidase (Amersham, Piscataway, NJ) was then added to each well and incubated for 90 min at room temperature. One hundred microliters of o-phenylenediamine (OPD) (Sigma, St. Louis, MO) containing 0.03% hydrogen peroxide was then added to each well. The reaction was stopped with 50 μ l/well of 12.5% H₂SO₄ and the optical density (OD) was measured at 450 nm using an automated ELISA reader (Molecular Devices, Sunnyvale, CA). The conditions for the measurement of IgE were the same as described for IgG4 except that 100 μ l of a 1:500 dilution of alkaline phosphate-conjugated IgE (Pharmingen, San Diego, CA) was added to each well and incubated for 90 min at room temperature. The plates were washed five times in PBST and 100 μ l of 10% diethanolamine in 0.01% MgCl₂ (pH 9.8) containing 1 mg/ml of *p*-nitrophenylphosphate was then added. The plates were then covered and stored in the dark for 2 hr and the absorbance was measured at 405 nm.

Quality control of indirect ELISA. Each plate contained three controls to check for non-specific binding of conjugate, chromogen, or streptavidin to the plate. A plate was repeated if one of these controls had an OD > 0.050 nm. In addition, each plate contained duplicates of a 1:100 dilution of sera from three *S. mansoni*-negative individuals from Brazil. A plate was repeated if the OD for one of these three controls was greater than 0.050 nm. Pearson product moment correlations were used to determine if wells on the edge of the plate had higher or lower OD values (e.g., edge effect) than wells in the center of the plate. Sample duplicates with a coefficient of variation greater than 10% were retested. Variation between plates during a single run (interplate variation) was measured by the coefficient of variation between the three positive controls on each plate. If the positive controls between plates on a single run had a coefficient of variation greater than 10%, the entire run was repeated.

Statistical analysis. Data were analyzed by least square analysis of variance technique using general linear models procedures. Scheffe's test was used to determine if a significant difference ($P < 0.05$) could be found between groups related with intensity of infection, TBM, and IgE and IgG4 levels to SWAP and SEA. The levels of antibody isotypes were examined after root square transformation of OD +1 to approximate a normal distribution. For the same reason, we used natural log transformed TBM values. To allow log transformation of zeros, a value of 1 was added to egg counts. Logistic regression analysis was used to examine the relationship between age and sex with status of infection and morbidity (periportal fibrosis). The goal of modeling was not to build a predictive model, but to quantify the association between study variables. For this analysis, age was categorized into two groups (≤ 20 and > 20 years) because of the small number subjects presenting with periportal fibrosis. All analyses were performed using the Statistical Analysis System, version 6.12 (SAS Institute, Inc., Cary, NC).

RESULTS

Characterization of the sample by age, sex, intensity of infection, prevalence, and water contact. Clinical and ultrasound examinations were carried out on 267 individuals. The distribution of patients by age, intensity of infection, prevalence, and water contact before treatment is shown in Figure 1. Individuals less than 20 years of age showed the highest intensity of infection (mean = 72.4 epg, 95% confidence interval [CI] = 28.8–182.1) and prevalence (93%, 95% CI = 87–99). In individuals more than 20 years of age, the intensity of infection varied from 3.5 epg (95% CI = 2.6–6.6) to 17.8 epg (95% CI = 7.9–40.3) and the prevalence varied from 36% (95% CI = 19–54) to 69% (95% CI = 52–86). Water contact ranged from a mean TBM of 128 (95% CI = 113.9–142.2) in the 11–20-year-old age group to a mean TBM of 86 (95% CI = 71.0–104.9) in those more than 60 years old. The TBM was significantly higher in the 11–20-year-old age group only in comparison with the group > 60 years old ($P < 0.05$).

Interestingly, although individuals in the 41–50-year-old age group showed similar TBM levels compared with those in the 11–20-year-old age group, the intensity of infection of this group was 6.3 times less ($P < 0.05$). Males had a higher intensity of infection than females (26.56 epg, 95% CI = 18.1–38.9 versus 14.67 epg, 95% CI = 10.0–21.5) ($P < 0.05$), but

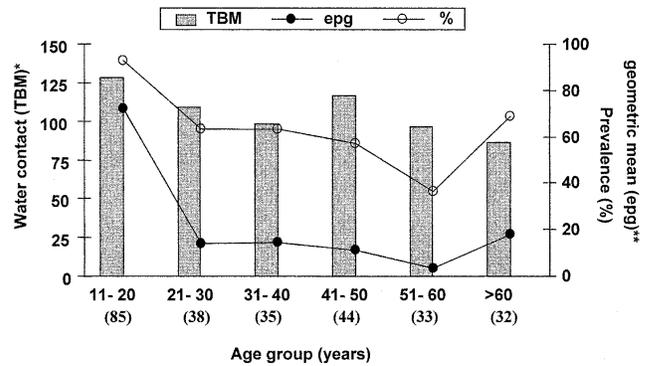


FIGURE 1. Intensity of infection, prevalence, and water contact (total body minutes [TBM]) data for *Schistosoma mansoni* by age group ($n = 267$). *Geometric mean TBM; **Geometric mean eggs per gram of feces (epg) [$\ln(\text{epg} + 1)$]. Values in parentheses are the number of individuals in each age group.

water contact values were not significantly different between genders. Furthermore, water contact values were not significantly different in egg-positive and egg-negative individuals.

Characterization of the study sample based on clinical and sonographic examinations. The association between age and sex with infection or periportal fibrosis was assessed by logistic regression analysis (Table 1). The results show an association between infection and age with the odds of infection 8.16 times greater for individuals ≤ 20 years old compared with older (> 20 years old) individuals. No association was found with gender. Conversely, the presence of periportal fibrosis was associated with gender, since the odds of morbidity were 3.46 times greater for males than for females, with no association with age.

For further analysis, the study sample was divided into three groups based on individual clinical and sonographic records (Table 2). The first group included 204 individuals with no periportal fibrosis, liver or spleen enlargement (control group). The second group included 41 individuals without thickening of the portal vein wall, but presenting with organopathy (cholecystitis, steatosis, hepatic cysts, or renal cysts), with or without organomegaly (pathology group). The third group included 22 individuals with periportal fibrosis (echogenic thickening = 0.3–0.5 cm) with or without organomegaly (fibrosis group).

Our data demonstrate that individuals in the pathology group were significantly older (41.8 years [95% CI = 35.5–48.0]) than individuals in the control group ($P < 0.05$). Although the fibrosis group showed an intensity of infection at least 2.5 times greater than the other two groups, it was not significantly different. There were no significant differences for TBM between the three groups. Moreover, no significant differences in TBM were observed between egg-negative and egg-positive individuals (Table 2), as well as between genders (Figure 2). However, despite no statistical significance, both male and female subjects in the fibrosis group showed a mean intensity of infection at least twice as high as that of the other two groups (Figure 2).

Analysis of antigen-specific IgG4 and IgE in sera from patients with/without periportal fibrosis. To investigate whether humoral immune response would differ between patients with and without periportal fibrosis, the levels of IgG4 (Figure 3) and IgE (Figure 4) antibodies to SEA and SWAP were com-

TABLE 1

Odds ratios (ORs) and 95% confidence intervals (CIs) for age and sex in relation to morbidity or infection with *Schistosoma mansoni*

Variables	Periportal fibrosis		Infection	
	No. (%)	OR (95% CI)	No. (%)	OR (95% CI)
Age group (years)				
> 20	14 (7.8)		104 (57.8)	
≤20	8 (9.2)	1.20 (0.48–2.98)	80 (92.0)	8.16 (3.56–18.68)
Sex				
Female	5 (3.9)		83 (64.3)	1.36 (0.78–2.37)
Male	17 (12.3)	3.46 (1.23–9.71)	101 (73.2)	

pared between individuals from the control, pathology, and fibrosis groups. Our results show that the sera levels of IgG4 against SEA were significantly higher in individuals in the fibrosis group compared with both the control group and the pathology group ($P < 0.05$). No statistic differences were observed for the levels of IgE against SEA or IgG4 or IgE against SWAP between all three groups. When the patients were stratified by gender, the levels of IgG4 to SEA were significantly higher in sera from males than from females in the control and pathology groups ($P < 0.05$). Although the level of IgG4 to SEA was higher in males than in females in the fibrosis group, the difference was not statistically significant. The levels of IgE against SEA were similar between genders in all three groups. For isotypes against SWAP, the levels of IgG4 were substantially higher in the sera from males than from females only in the pathology group ($P < 0.05$) and for IgE in the control group ($P < 0.05$).

Increased levels of IgG4 against SEA in egg-negative patients with periportal fibrosis. To further characterize the humoral immune response in individuals with and without periportal fibrosis, the sera levels of IgG4 and IgE were analyzed after stratifying the patients based on the presence or absence of eggs in their feces (Figure 5). Higher sera levels of IgG4 against SEA and SWAP were found in egg-positive individuals compared with egg-negative individuals in both the control ($P < 0.05$) and the pathology groups ($P < 0.05$), but not in the fibrosis group. However, analysis of sera IgG4 against SEA within the egg-negative subgroup demonstrated higher levels of this antibody only in the fibrosis group compared with the control ($P < 0.05$).

Using the same approach to analyze levels of IgE against SEA and SWAP, we found higher levels of this isotype in the egg-positive individuals only in the control group for SEA ($P < 0.05$). When serum levels of IgE against SEA and SWAP within the egg-negative subgroup were analyzed, we found no differences in these levels between groups. No differences

were observed when the levels of IgG4 and IgE against SEA and SWAP were analyzed in the egg-positive subgroup.

DISCUSSION

In this cross-sectional study, we have performed a detailed descriptive analysis of individuals living in an area where schistosomiasis is endemic to evaluate the relationship between demographic features, parasitologic status, behavior routines, and the immune response in patients with periportal fibrosis.

By characterizing the sample by age, sex, intensity of infection, prevalence, and water contact, we have found a typical relationship between age and either prevalence or intensity of infection. Individuals less than 20 years old showed the highest prevalence and intensity of infection compared with other age groups. The decrease in prevalence and intensity of infection with age has been explained as a multifactorial phenomena that, in part, results from changes in exposure to cercariae due to decreased contact with infected water,^{3,34} development of acquired immunity,^{35,36} and differences in hormones levels or physiologic features, such as thickening of cutaneous fat observed with aging.³⁷ Our data are consistent with this multifactorial hypothesis, since the decrease in prevalence and intensity of infection in older subjects could not be explained by the lower exposure to infected water in all age groups. The lower TBM (water contact) observed in the 31–40-, 51–60-, and < 60-year-old age groups could account for their lower prevalence and intensity of infection. However, individuals in the 21–30- and 41–50-year-old age groups, regardless of similar TBM, showed decreased prevalence and less intensity of infection compared with individuals less than 20 years of age (Figure 1). Thus, a difference in TBM does not explain an age effect in prevalence and intensity of infection in the Melquíades study sample. As has been suggested by other studies in areas where schistosomiasis is en-

TABLE 2

Descriptive analysis for individuals infected with *Schistosoma mansoni* stratified by periportal fibrosis, age, intensity of infection, and water contact

Group	Echogenicity†	Organomegaly (%)	No.	Age (years)	epg‡	TBM (n)*		
						Total	Egg +	Egg -
Control	≤0.2	–	204	33.9	19.74	107	109 (141)	102 (63)
Pathology	≤0.2	55.0	41	41.8§	12.93	124	130 (25)	118 (16)
Fibrosis	0.3–0.5	36.4	22	32.2	48.62	104	107 (18)	93 (4)

* Geometric mean of total body minutes (ln [TBM]). n = number of individuals.

† Periportal fibrosis (cm).

‡ epg = geometric mean eggs per gram of feces (ln [epg + 1]).

§ Difference between pathology and control groups ($P < 0.05$).

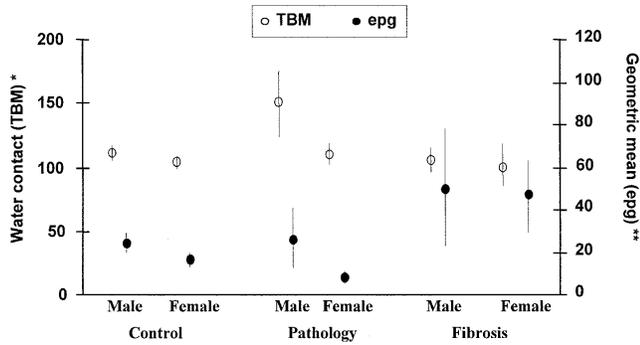


FIGURE 2. Intensity of infection (eggs per gram of feces [epg]) and water contact (total body minutes [TBM]) data for *Schistosoma mansoni* in individuals from the control, pathology, and fibrosis groups separated by sex. *geometric mean [ln(TBM)]; **geometric mean epg [ln(epg + 1)]. Data points and vertical lines show the mean ± standard error.

demic, age-acquired immunity could also play a role in the prevalence and intensity of infection.^{8,38-42}

Periportal fibrosis detected by ultrasonography has been considered the most important indicator for morbidity in human schistosomiasis.^{43,44} In this study, data from clinical and ultrasonographic examinations allowed us to identify in a population of 267 a group of 22 individuals with echogenic lesions indicating severe chronic schistosomiasis and another group of 41 individuals with organopathy but without periportal fibrosis. Using a logistic regression model, we did not find any association of age with morbidity, but we observed an association between age and infection as previously described for human schistosomiasis.⁴⁵ However, 77% of the

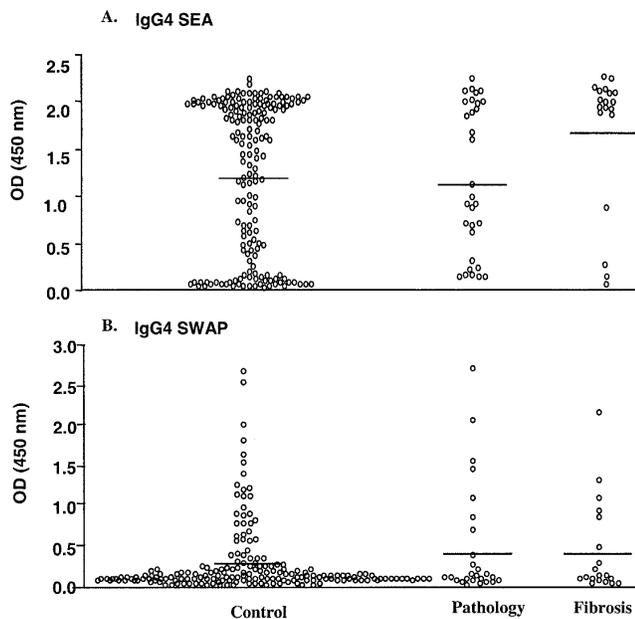


FIGURE 3. Levels of IgG4 against *Schistosoma mansoni* soluble egg antigen (SEA) (A) and an adult worm antigen preparation (SWAP) (B) in sera from patients from the control, pathology, and fibrosis groups. The levels of IgG4 against SEA from the fibrosis group were significantly higher than those in the control and pathology groups ($P < 0.05$). Data points are optical density (OD) values at 450 nm. The horizontal lines indicate the arithmetic mean.

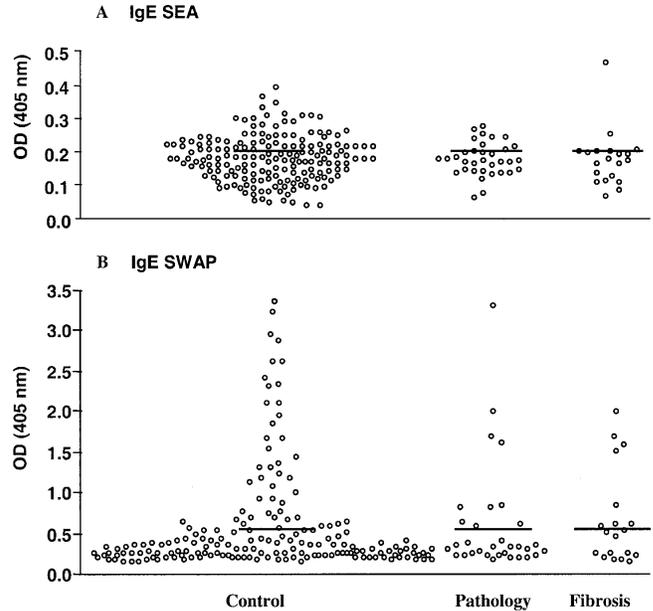


FIGURE 4. Levels of IgE against *Schistosoma mansoni* soluble egg antigen (SEA) (A) and an adult worm antigen preparation (SWAP) (B) in sera from patients from the control, pathology, and fibrosis groups. Data points are optical density (OD) values at 405 nm. There was no difference between the groups. The horizontal lines indicate the arithmetic mean.

individuals with periportal fibrosis were male, with an odds ratio of 3.46, compared with females (Table 1). Interestingly, TBM was similar for both genders (Figure 2). Similar associations between gender and morbidity have been reported for *S. mansoni* and *S. haematobium* infections.⁴⁵⁻⁴⁷

In addition to gender, it has also been proposed that severe schistosomiasis is more often observed in subjects with a higher intensity of infection.^{1,21} In this regard, our results show that patients in the fibrosis group have increased intensity of infection in comparison with the pathology and the control groups (Table 2). Moreover, we observed that one

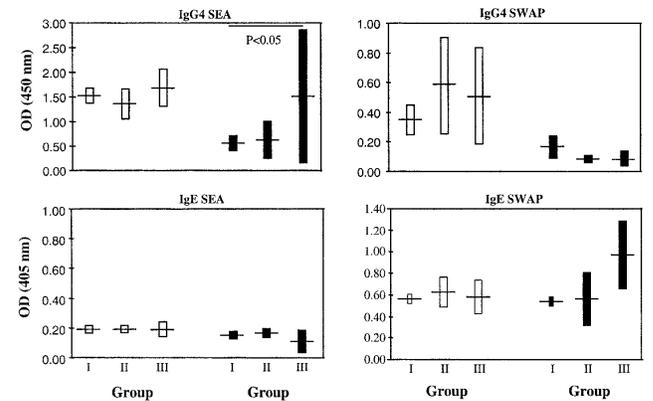


FIGURE 5. Levels of IgG4 and IgE against *Schistosoma mansoni* soluble egg antigen (SEA) and an adult worm antigen preparation (SWAP) in sera from patients from control (I), pathology (II), and fibrosis (III) groups after subgrouping the patients based on the presence (open bars) and absence (solid bars) of eggs. Bars show the mean ± 95% confidence intervals of optical density values read at 450 and 405 nm.

year after treatment, 23.1% of the patients in the fibrosis group had become reinfected in contrast with 9.8% and 6.9% of patients from control and pathology groups, respectively.

In general, it has been difficult to demonstrate a relationship between intensity of infection and morbidity. Both positive and negative associations have been reported.^{48–51} Interpretation of this discrepancy has been extensively discussed and includes the reduction in egg excretion leading to an underestimation of parasite load in patients with periportal fibrosis, the chronic evolution of the disease that does not synchronize morbidity and the peak of infection intensity, and differences on the clinical phenotypes counted as morbidity markers, such as hepatosplenomegaly or fibrosis. We suggest that the intensity of infection, although important in disease progression, is not the only critical factor in the development of fibrosis and other components such as the immune response and host genetic factors may play critical roles.

Most immunologic studies on *S. mansoni* have focused on the relationship between the immune response and development/maintenance of different clinical forms of the disease or the mechanisms of resistance and susceptibility to infection or reinfection.^{13,15,16} The current study adds to these studies by examining the association between humoral immune response and the development of periportal fibrosis.

Early studies comparing anti-SEA IgG4 before and after chemotherapy demonstrated that the level of this isotype decreased following a decrease in egg output.⁵² In this paper, we are dealing with two supposedly causal variables associated with the intensity of infection (Figure 3): fibrosis and the levels anti-SEA IgG4. To address this confounding question, the anti-SEA IgG4 levels in our study was examined after stratifying the population into two groups: egg-negative and egg-positive. As shown in Figure 5, the association between anti-SEA IgG4 and fibrosis still remained significant among individuals without eggs in their stool. The current literature links levels of anti-SEA IgG4 with susceptibility,^{13,18,19} but our data suggest that the levels of IgG4 may also be involved in the development of fibrosis. These patients, although egg-negative, live in same endemic area, have similar water contact as the infected individuals, and presented with high peripheral blood mononuclear cell proliferative responses to adult worm and eggs antigens, as well as with cytokine synthesis in their cell culture supernatants (A. M. S. Silveira, G. Gazzinelli, L. F. Álvares-Oliveira, J. Bethony, A. Gazzinelli, C. Carvalho-Queiroz, M. C. B. Alveres, F. C. L. Silva, A. Prata, P. T. LoVerde, and R. Correa-Oliveira, unpublished data). Furthermore, the coefficient of variability of the Kato quantitative method for patients with less than 20 epg is very high (89.55);⁵³ thus, patients with a low intensity of infection may be not detected. These data suggest that these egg-negative individuals are infected or previous sensitized by *S. mansoni*. In relation to fibrotic, egg-negative patients, the possibility that the abnormal echogenicity reported is caused by *S. mansoni* infection is very high because 8–12 months after chemotherapy, the lesions detected by ultrasonography disappeared (T. V. B. Magalhães, A. M. S. Silveira, M. C. B. Álvares, and G. Gazzinelli, unpublished data).

In conclusion, IgG4 specific for SEA was associated with the initial stages of fibrosis, which was graded by the echogenic pattern of the periportal tree by ultrasound. This allowed the identification of the critical step in the progression of disease from mild to severe fibrosis.

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