ULTRASOUND AND CLINICAL INVESTIGATION OF HEPATOSPLENIC SCHISTOSOMIASIS: EVALUATION OF SPLENOMEGALY AND LIVER FIBROSIS FOUR YEARS AFTER MASS CHEMOTHERAPY WITH OXAMNIQUINE

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Abstract. The course of hepatosplenic schistosomiasis after mass chemotherapy with oxamniquine has been rarely reported. We report the effect of treatment in patients with advanced schistosomiasis mansoni living in area of Brazil highly endemic for this disease. A total of 739 inhabitants of a village were subjected to clinical and abdominal ultrasound examinations and were treated with oxamniquine. We have identified 84 individuals with hepatosplenic schistosomiasis. Alcohol abuse was associated with periportal thickening. Four years after treatment, 42 of the 84 individuals were re-examined and regression of splenomegaly was observed in 59% and of periportal thickening in 32%. Our data indicate that mass chemotherapy can lead to reduction of schistosomiasis morbidity but a significant group of patients (68%) will not improve. The association with alcohol abuse should be further evaluated. Thickening of the gallbladder wall can be a useful predictor of no involution of liver fibrosis after treatment.

INTRODUCTION

Reduction of the prevalence of Symmer’s fibrosis is central to controlling morbidity and mortality from schistosomiasis. The impact of anti-schistosomal chemotherapy on periportal fibrosis has not been sufficiently explored. Ultrasound examination effectively defines those with and without periportal thickening and can be used to detect the presence of Symmer’s fibrosis. It allows investigators to determine the factors that favor development, progression, or regression of this complication. There is experimental evidence of regression of fibrosis and portal hypertension after treatment of schistosomiasis. However, although there are many reports concerning the effects of chemotherapy, most of them were based on clinical measures of S. mansoni morbidity. Few investigators have followed-up the same patients, using ultrasound, for a long period. The objective of the present study was to evaluate the effect of mass chemotherapy with oxamniquine on the behavior of periportal fibrosis and splenomegaly over a four-year period.

PATIENTS AND METHODS

This study was conducted in Jequitinhonha’s Vale in northeastern Minas Gerais, Brazil, an area endemic for schistosomiasis. The study was divided in two phases. Initially, 739 (83%) of 890 inhabitants of the study area more than five years of age were given physical, parasitologic, and ultrasonographic examinations in 1999. After initial examination, all individuals were treated with a single dose of oxamniquine (15 mg/kg of body weight for adults and 20 mg/kg of body weight for children). Alcohol abuse was defined as the ingestion of more than 60 grams of alcohol per day (15 mg/kg of body weight for adults and 20 mg/kg of body weight for children). Alcohol abuse was associated with periportal thickening. Four years after treatment, 42 of the 84 individuals were re-examined and regression of splenomegaly was observed in 59% and of periportal thickening in 32%.

Our data indicate that mass chemotherapy can lead to reduction of schistosomiasis morbidity but a significant group of patients (68%) will not improve. The association with alcohol abuse should be further evaluated. Thickening of the gallbladder wall can be a useful predictor of no involution of liver fibrosis after treatment.

 examiners. On the basis of two stool examinations with the Kato-Katz technique, the overall prevalence of infection with Schistosoma mansoni in this area was 74% before mass chemotherapy with oxamniquine. We used a previous defined protocol with anthropometric and clinical variables such as massive hematemesis and a history of blood transfusions. Other control activities against schistosomiasis transmission such as education and sanitation were conducted in the area during the study period. The study was reviewed and approved by the Ethical Committee of the Federal University of Minas Gerais, Brazil.

We initially identified 84 hepatosplenic individuals (11.4% of the examined population). The diagnosis was based on the presence of periportal thickening on ultrasound and/or the presence of a palpable spleen. Only 21% of the patients identified (18 individuals) exhibited the classic presentation of hepatosplenic schistosomiasis with Symmer’s fibrosis and a palpable spleen (group 1). Fifteen individuals (18%) had Symmer’s fibrosis detected by ultrasound with a non-palpable spleen (group 2) and 51 patients (61%) had splenomegaly without liver fibrosis (group 3). In the second phase of the study four years later, 42 of the 84 hepatosplenic individuals were re-examined in a tertiary hospital in Belo Horizonte (the capital of the state of Minas Gerais), after informed consent has been obtained. They were subjected to the same tests as in the first phase of the study plus upper endoscopy to diagnose esophageal varices.

Abdominal ultrasound was performed at baseline using real time equipment (EUB 200 ultrasound unit; Hitachi, Tokyo, Japan) with electronic linear 3.5-MHz transducers and at follow-up with an ATL-HDI 1500 scanner with a 2–5-MHz transducer (Philips, Bothell, WA) by the same radiologist who was unaware of the previous ultrasonographic results and patient’s clinical status. The degree of periportal fibrosis was graded as reported by our group: 1 = slight echogenic thickening of the wall of less than one-third of peripheral portal branches or normal liver; 2 = moderate-to-severe wall thickening (> 2 mm) of more than one-third of peripheral portal branches. We measured the size of the right and left lobe of the liver, the spleen length in cranio-caudal extension,
the diameter and the wall thickness of the gallbladder, and the portal vein system, including the first, second, and third portal vein branches. Splenomegaly was established when the spleen length in the cranio-caudal extension exceeded the height-adjusted normal value published by the World Health Organization.12

All 84 hepatosplenic individuals were invited to be examined four years after treatment. We obtained the follow-up data on 42 (50%) of the 84 patients. The clinical and ultrasonographic profiles of these 42 patients and the original 84 patients were compared to ensure that this sample was not biased (Table 1). The distribution of deaths (6 of 84 patients in the 4 years of follow-up) was similar among the three clinical ultrasonographic groups.

Statistical analysis was conducted using the Student’s t-test, analysis of variance, the Wilcoxon signed-rank test (for non-parametric variables), the chi-square test, and the kappa coefficient whenever appropriate for comparison of proportions and means. Multivariable logistic regression analysis was used to adjust for covariates. A receiver operator characteristic (ROC) curve was used to evaluate the accuracy of gallbladder thickness in predicting fibrosis involution. A P value < 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of the study population are shown in Table 1. Using the classification previously described, we observed no clinical or ultrasonographic differences between patients with periportal thickening independent of the presence of a palpable spleen (group 1 versus group 2). Alcohol abuse was associated with periportal thickening (groups 1 and 2), but not associated with splenomegaly (group 3) (odds ratio [OR] = 2.5, 95% confidence interval [CI] = 1.6–4.1). Patients with splenomegaly and without Symmer’s fibrosis were younger when compared with other clinical presentations (mean ± SD age = 21.9 ± 14 years versus 35.8 ± 16.1 years; P = 0.0001). Esophageal varices were described in 3 of the 42 re-examined patients. All had severe periportal thickening and a palpable spleen in the two examinations conducted four years apart.

Clinical and ultrasonographic changes in the study groups are shown in Table 2. Of 11 patients with periportal thickening and a palpable spleen, 5 showed no changes. Four patients had no change in periportal thickening but the spleen became non-palpable. All had a smaller longitudinal spleen length than in the first examination. Two other cases showed an absence of periportal thickening, and one showed a palpable spleen.

Of the 8 patients with Symmer’s fibrosis and a non-palpable spleen, 3 remained stable throughout this period. One patient showed enlargement of the spleen, which was palpable at the follow-up. Two patients showed a lower degree of Symmer’s fibrosis with a non-palpable spleen and two patients showed a marked decrease of Symmer’s fibrosis with a palpable spleen. However, none showed enlargement of the spleen by ultrasound.

Changes in the degree of fibrosis of the study groups are shown in Table 3. Regardless of the clinical and ultrasonographic presentation, periportal fibrosis was absent in 32% of the patients on repeat ultrasound examination four years after treatment. None of the patients evolved from a state of non-detectable fibrosis to one of detectable fibrosis or showed an increased degree of fibrosis after its initial detection. The presence of gallbladder wall thickening was the only predictive factor of non-regression of fibrosis (OR = 4.3 95% CI = 1.2–18.1) (Figure 1). There were no patients with gallbladder wall thickness more than 5 mm in the first examination who showed involution of liver fibrosis. The gallbladder wall thickness cut-off point was 4.5 mm (sensitivity = 69% and specificity = 100%), and the area under the ROC curve was 85% (95% CI = 0.65–1.02) (Figure 2).

A significant difference was observed in spleen palpation in the two phases of the study (Table 4). There was a decrease in the size of the spleen in 20 (58.8%) of 34 patients. However, only 12 (35%) of 34 splenomegalic patients on clinical

### Table 1

Comparison of baseline characteristics of patients with hepatosplenic schistosomiasis, Brazil, 1999

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hepatosplenic individuals</th>
<th>Hepatosplenic individuals re-examined 4 years later</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 42</td>
<td>n = 42</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>45 (53.6%)</td>
<td>27 (64.3%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Age, years (median)</td>
<td>22 (14–42)</td>
<td>25 (15–43)</td>
<td>0.45</td>
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<tr>
<td>Skin color</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>29 (34.5%)</td>
<td>15 (35.7%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Non-white</td>
<td>55 (65.5%)</td>
<td>27 (64.3%)</td>
<td></td>
</tr>
<tr>
<td>Palpable spleen</td>
<td>69 (82.1%)</td>
<td>34 (80.9%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Periportal thickening (ultrasound)</td>
<td>33 (39.3%)</td>
<td>19 (45.2%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Palpable spleen ultrasound proven</td>
<td>29 (34.5%)</td>
<td>13 (30.9%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Schistosoma mansoni eggs excreted (median)</td>
<td>88 (18–178)</td>
<td>54 (16–120)</td>
<td>0.69</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>19 (22.6%)</td>
<td>7 (16.7%)</td>
<td>0.44</td>
</tr>
</tbody>
</table>

### Table 2

Clinical and ultrasonographic classification of hepatosplenic schistosomiasis in an area endemic for schistosomiasis in northeast Minas Gerais, Brazil, 1999

<table>
<thead>
<tr>
<th>Clinical and ultrasonographic classification at baseline</th>
<th>PT (+)</th>
<th>Spleen (+)</th>
<th>PT (+)</th>
<th>Spleen (+)</th>
<th>PT (+)</th>
<th>Spleen (+)</th>
<th>PT (+)</th>
<th>Spleen (+)</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>PT (+)</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Spleen (+)</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>PT (+)</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>15</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen (+)</td>
<td>6</td>
<td>7</td>
<td>11</td>
<td>18</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*PT = periportal thickening; Spleen (+) = palpable spleen; Spleen (−) = non-palpable spleen.
examination at baseline had spleen enlargement by the definition of the World Health Organization. Only one of three patients with a non-palpable spleen at baseline and a palpable spleen four years later had confirmed organ enlargement by ultrasound. In the follow-up, 9 (53%) of 17 clinically proven splenomegalic patients had splenomegaly confirmed by ultrasound. Analysis of clinically and ultrasonographically proven splenomegaly showed a significant discordance between the two methods, especially in the field examination (kappa coefficient = 0.26 in field and 0.57 in the follow-up examination).

Ultrasonographic results showed that 7 (54%) of 13 patients with splenomegaly had similar measurements four years later (Table 5). Three (10%) of 29 patients without splenomegaly at baseline were characterized as splenomegalic in the follow-up. All showed an increase in spleen length, but the ultrasound did not detect any change in the degree of fibrosis in the liver.

**DISCUSSION**

In the study area, the prevalence of schistosomiasis decreased from 74% to 25% after mass chemotherapy with oxamniquine and the introduction of environmental control measures. There was also a decrease of 83% in the stool egg counts in the group not cured when the population was re-examined one year after the first treatment. No patient with normal liver findings by ultrasound at baseline developed perportal fibrosis during the four years of follow-up. After treatment, there was a reduction of 32% in perportal thickening in the patients. Gallbladder wall thickening was a good marker of non-reversal of perportal fibrosis. A correlation was also observed between alcohol abuse and the degree of perportal thickening. We have followed-up three groups of hepatosplenic patients based on a previously reported classification of schistosomiasis, and the regression of fibrosis or splenomegaly was not influenced by the baseline classification.

It has been repeatedly demonstrated that gallbladder wall thickening is associated with perportal fibrosis in the absence of a calculous cholecystitis. In our study, a positive correlation was also observed between gallbladder wall thickening and non-reversing of fibrosis four years after treatment for schistosomiasis. This is the first step in the search for quantitative predictors of non-reversing of fibrosis using imaging techniques. We have no explanation for these findings, but this may be an indication of disease so advanced that involution of fibrosis is no longer possible.

**TABLE 3**

| Grades of periportal thickening during the baseline study and 4 years later in an area endemic for schistosomiasis in northeast Minas Gerais, Brazil, 1999* |
|---|---|---|
| Periportal thickening at baseline | Periportal thickening 4 years after treatment |
| Absent | Present | Total |
| Absent | 23 | 0 | 23 |
| Present | 6 | 13 | 19 |
| Total | 29 | 13 | 42 |

* P = 0.03 by McNemar test.

**FIGURE 1.** Median thickness and interquartile range of the gallbladder wall in patients who showed regression of fibrosis (responders) compared with those with no regression of fibrosis (non-responders) four years after treatment in Brazil, 1999.

**FIGURE 2.** Receiver-operator-characteristic curve for gallbladder wall thickness in the prediction of no involution of liver fibrosis in Brazil, 1999.

**TABLE 4**

| Splenomegaly by clinical evaluation at baseline and 4 years later in an area endemic for schistosomiasis in northeast Minas Gerais, Brazil, 1999* |
|---|---|---|
| Splenomegaly at baseline | Palpable | Non-palpable | Total |
| Palpable | 14 | 20 | 34 |
| Non palpable | 3 | 5 | 8 |
| Total | 17 | 25 | 42 |

* P = 0.00001 by McNemar test.
Our results tend to confirm that the decrease in patient worm burden after chemotherapy favors involution of liver fibrosis.\textsuperscript{15} Sixty-eight percent of the patients showed no regression of liver fibrosis at follow-up. With regard to fibrosis, the response or lack of response of the patients did not depend upon age, sex, pre-treatment egg count, the presence or absence of a palpable spleen, or a history of alcohol abuse. Re-infection caused by continuous transmission may be a possible explanation, but our data do not support this. The prevalence decreased after treatment and egg counts decreased accordingly in the uncured patients. Only 10 (24\%) of 42 patients re-examined four years later had eggs in their stools, which shows that re-infection in this group was not important. In addition, some investigators have recently confirmed that treatment of schistosomiasis increases resistance to re-infection, and that it takes time for most individuals, even when re-infected, to reach the level of egg counts found before any treatment.\textsuperscript{16} Therefore, the immunologic status and genetic background of our patients should be further evaluated to find explanations for this poor response to treatment. Serologic markers of fibrosis may also help to separate responders from non-responders.\textsuperscript{17,18}

Studies on the involution of fibrosis in schistosomiasis can be divided in two types: 1) those based on clinical examination\textsuperscript{19,20} and 2) those based on ultrasonography.\textsuperscript{5-9} In Brazil, splenomegaly diagnosed by abdominal palpation has been the classic diagnostic tool in field-based studies,\textsuperscript{21} but the limitations of this method have been previously demonstrated.\textsuperscript{22} Ultrasound is also an operator-dependent procedure and does not evaluate the texture of the organs, which is considered important in identifying hepatosplenic schistosomiasis. To overcome such problems, we have been using both methods (clinical and ultrasound examinations) for the past 10 years.\textsuperscript{23}

In the present study, ultrasound identified 18 individuals with periporal thickening and a non-palpable spleen among 739 persons examined. Patients with such severe schistosomiasis have not been included in previous field-based studies of morbidity of \textit{S. mansoni} infection, except for one report from Africa.\textsuperscript{24} In 51 (61\%) patients in our study the spleen was palpable but there was no evidence of fibrosis by ultrasound. Our findings show the inaccuracy of spleen palpation in assessing liver fibrosis. Nevertheless, splenomegaly in patients without periporal fibrosis may represent the reactive hyperplastic stage of schistosomiasis. The lower median age of the patients in our study corroborates the hypothesis. Also, there was no report of malaria or visceral leishmaniasis in our study area. The prevalence of chronic hepatitis B and hepatitis C in a previous study in the same area was 5\% and less than 1\%, respectively.\textsuperscript{25} In a sample of our study population, no correlation was observed between antibodies against \textit{Leishmania}, hepatitis B or C, and periporal thickening.

No patient in our study area with splenomegaly without periporal fibrosis at baseline developed periporal fibrosis after four years of follow-up and this fact has been attributed to mass chemotherapy with oxamniquine. Since the Brazilian program of schistosomiasis control started in 1975, eight million people have been treated with oxamniquine and the efficacy of this drug, which is comparable to that of praziquantel, has been repeatedly shown.\textsuperscript{5,22,26,27} For the last four years oxamniquine has been replaced in Brazil, by the more cost-effective praziquantel. Thus, we believe that the observations in our study are not specifically dependent on the use of oxamniquine, i.e., they would have occurred after any similar treatment. For ethical reasons, we did not follow a control group of patients with splenomegaly without treatment.

To the best of our knowledge, the correlation between alcohol abuse and the intensity of periporal thickening has not been reported in patients with schistosomiasis mansoni. The few available studies on alcohol consumption and schistosomiasis have produced conflicting results. Orrego and others showed a reduction of fibrosis in mice infected with \textit{S. mansoni} and receiving toxic quantities of alcohol.\textsuperscript{28} Houston and others found no association between lifetime alcohol consumption and the presence of periporal thickening in a rural community in Zimbabwe.\textsuperscript{29} Conversely, Liu and others reported an association between liver fibrosis and alcohol abuse among patients infected with \textit{S. japonicum}.\textsuperscript{30} New studies should address this subject.

Our data indicate that mass chemotherapy can lead to a reduction in schistosomiasis morbidity, but a significant group of patients will still have periporal fibrosis and splenomegaly. A second treatment after four years may further reduce morbidity and this is being evaluated. More studies on the association of alcohol abuse and liver fibrosis in schistosomiasis are needed. Gallbladder wall thickening can be a useful predictor of no involution of liver fibrosis after mass chemotherapy.

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REFERENCES


\begin{table}[h]
\centering
\caption{Splenomegaly by abdominal ultrasound at baseline and 4 years after treatment in an area endemic for schistosomiasis in northeast Minas Gerais, Brazil, 1999*}
\begin{tabular}{|c|c|c|c|}
\hline
Abdominal ultrasound at baseline & Abdominal ultrasound 4 years after treatment & & \\
\hline
Splenomegaly & Normal spleen & Total & \\
\hline
6 & 7 & 13 & \\
3 & 26 & 29 & \\
9 & 33 & 42 & \\
\hline
\end{tabular}
\end{table}

* \( P = 0.34 \) by McNemar test

† Normal spleen: spleen not enlarged by World Health Organization recommendations for ultrasound.

\textsuperscript{5}