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Diagnosing schistosomiasis-induced liver morbidity: implications for global control

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SUMMARY

Background: Subclinical morbidity due to schistosomiasis was evaluated in 565 patients, and the enhanced liver fibrosis (ELF) test was assessed for the first time as a potential screening tool for disease.
Methods: The prevalence and intensity of infection were determined by Kato–Katz thick smear stool examination at baseline and 2 years after curative treatment. The degree of hepatic fibrosis was assessed by ultrasound. Non-invasive serum biomarkers of hepatic fibrosis were also evaluated.
Results: The baseline human prevalence and infection intensity were found to be moderately high at 34% and 123 eggs per gram, respectively. However, hepatic parenchymal fibrosis occurred in 50% of subjects, with grade II fibrosis in 19% and grade III in 6%. The ELF score and higher serum levels of tissue inhibitor of metalloproteinase 1 (TIMP-1) and hyaluronic acid (HA) correlated with the grade of liver fibrosis.
Conclusions: The findings of this study demonstrated that praziquantel treatment had a short-term impact on both the prevalence and intensity of infection, but less of an impact on established morbidity. Higher TIMP-1 and HA serum levels, and an ELF cut-off score of 8 were found to be correlated with the grade of liver fibrosis; these values may, therefore, assist physicians in identifying individuals at greater risk of disease.

1. Introduction

Schistosomiasis is a major public health problem that afflicts approximately 240 million people worldwide[1] and causes approximately 70 million disability-adjusted life years lost.[2] Preventive chemotherapy has been endorsed and advocated by the World Health Organization (WHO) for the global control of schistosomiasis.[3] Since its inception in 1979, much optimism has surrounded mass drug administration (MDA) for the worldwide control of schistosomiasis, for which praziquantel (PZQ) has served as the cornerstone drug. Numerous studies have claimed that preventive chemotherapy (i.e., 40 mg/kg PZQ) given once or twice yearly can significantly reduce the prevalence and intensity of infection and control morbidity in the long term.[3]

Schistosomiasis was first reported in the Philippines in 1906. Approximately 865 000 people are currently infected and a further 12 million are at risk of infection.[3,4] Major endemic foci (80%) are in the poorest regions of the Visayas (Samar and Leyte) and Mindanao.[3,4] The current national control program comprises annual free MDA (40 mg/kg PZQ) in all schistosomiasis-endemic communities with a prevalence of >10%. The Philippines National Schistosomiasis Control Program has recently reported that the human prevalence has declined to less than 3% nationally.[5] However, contradictory reports claim the program is failing because of poor drug compliance, poor drug coverage, infrequent monitoring and evaluation, and rapid re-infection rates.[4–6] Moreover, newly published data have revealed very high prevalence rates in both humans and bovines in endemic areas

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throughout the country.[4–6] There are now advanced schistosomiasis cases and disease-related deaths being reported by the National Department of Health for Mindanao, Samar, Leyte, and Oriental Mindoro.[4]

Hepatic fibrosis is the major cause of morbidity and mortality among people with chronic schistosomiasis. Schistosomiasis-induced liver fibrosis in the field setting is best assessed with a portable ultrasound.[6] The instrument has been reliable for detecting and assessing the degree of parasite-induced liver abnormalities and for monitoring pathology regression following anti-schistosome treatment.[6] Despite inter-observer variability related to its use,[8] several studies have confirmed its usefulness in evaluating hepatosplenic schistosomiasis in the field over the past 30 years.[6,9–17] The potential of non-invasive markers to complement the imaging assessment of organ morbidity has been studied before.[5] Among the most promising for assessing schistosome-induced liver fibrosis are hyaluronic acid (HA), collagenous proteins, matrix metalloproteinases, and intercellular adhesion molecules.[5,14,15]

In this study, the clinical morbidity due to schistosomiasis at baseline and at 2 years after curative treatment was evaluated among 565 residents of a known endemic area in Northern Samar, the Philippines. Furthermore, the enhanced liver fibrosis (ELF) test was assessed as a potential field screening tool for advanced disease.

2. Methods

2.1. Study area

The study area comprised 18 schistosomiasis-endemic barangays in the municipalities of Laoang and Palapag, Northern Samar, the Philippines. Residents of this area are typically poor rice farmers with family incomes far below the national average. Over 50% of the population lives below the poverty line, and the water supply, sanitation, and hygiene are rudimentary.[7] The area is non-endemic for malaria but has had an active schistosomiasis control program for more than over three decades, including an MDA program that commenced in 2008. All individuals aged 5–65 years are offered free annual treatment (40 mg/kg of PZQ) in accordance with the Department of Health Administrative Order 2007–0015.[7]

2.2. Study procedures

A cross-sectional schistosomiasis survey involving approximately 20 000 individuals was conducted in 2012 in order to determine the prevalence, intensity of infection, and morbidity associated with the disease (Figure 1).[7] The baseline prevalence and intensity of infection were determined by Kato–Katz thick smear stool examination. Individuals were asked, over the course of a week, to provide two stool specimens from which six 50 g Kato–Katz thick smears were prepared on microscope slides according to established methods.[20] Slides were examined under a light microscope by experienced laboratory technicians, who counted the number of *Schistosoma japonicum* eggs per slide. For quality control, 10% of all slides were randomly selected and re-examined by a senior microscopist at the Research Institute for Tropical Medicine, Manila. *S. japonicum* egg counts were expressed as eggs per gram (epg) of stool.[18] The intensity of infection was graded according to WHO criteria: light infection, 1–99 epg; moderate infection, 100–399 epg; heavy infection, ≥400 epg.[19]

For a more accurate assessment of schistosome-induced hepatosplenic morbidity, ultrasonographic studies were performed on a subset of individuals with symptoms and signs suggestive of schistosomiasis. All subjects (n = 736) who reported gastrointestinal and/or neurological symptoms (i.e., fatigue, malaise, abdominal pain, blood per rectum, hematemesis, diarrhea, jaundice, dizziness, headache, and seizures) and/or were clinically assessed to have morbidity (i.e., palpable liver and spleen, varices, ascites, etc.) based on physical examination were initially selected for the ultrasound investigation. However, only those with baseline stool and ultrasound examination results were included in the final analysis (n = 565). The degree of hepatic fibrosis was assessed by ultrasound examination using a portable gray-scale ultrasonogram equipped with 3 MHz curve array transducer (SONOACE X1; Madison Co., Ltd, Seoul, South Korea). Liver size was measured in millimeters along the mid-sternal line (MSL) and mid-clavicular line (MCL) for the left lobe and right lobe, respectively. Spleen size was measured in millimeters along the left mid-axillary line (MAL). Hepatic fibrosis grading was adopted

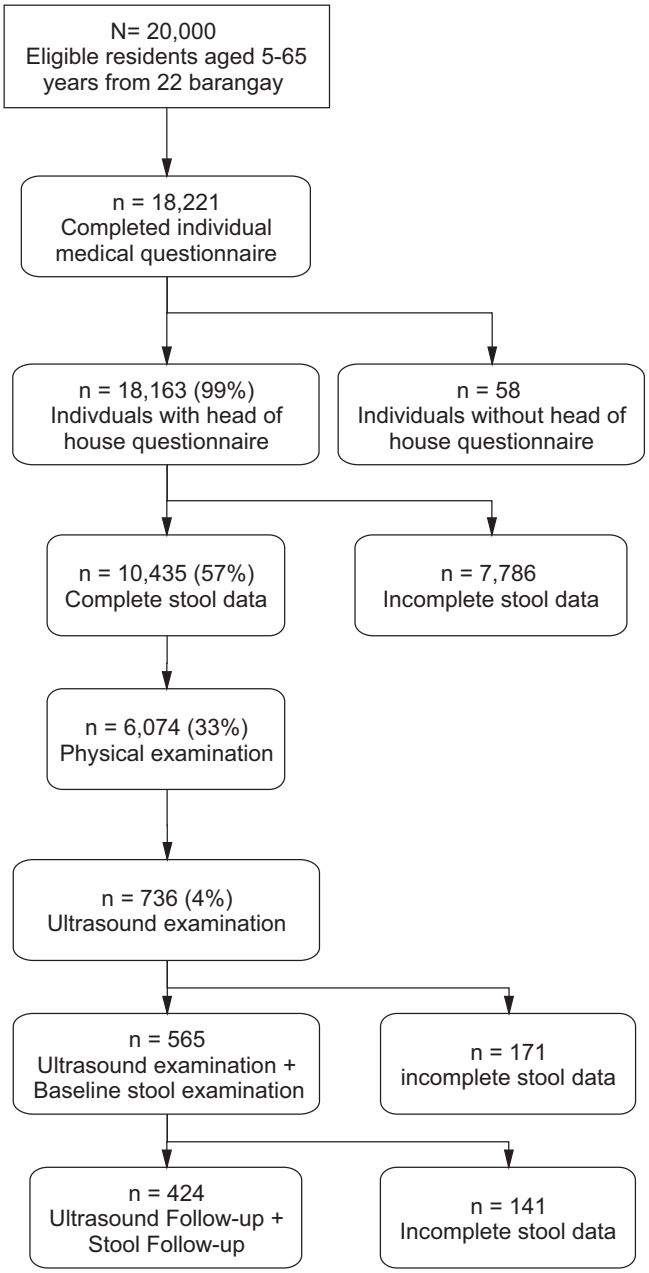

Figure 1. Study profile and compliance among 18 221 residents from 22 schistosomiasis-endemic barangays in Palapag and Laoang, Northern Samar, the Philippines. All inhabitants aged 5–65 years were invited to participate in the questionnaire and provide two stool samples for parasitological examination. A subset of patients (n = 736) were selected for ultrasound investigations.

from the practical guidelines established for ultrasonography by the WHO/TDR in 2000 (WHO Special Programme for Research and Training in Tropical Diseases).[20] Ultrasound findings were arrived at by consensus agreement of two experienced ultrasonographers. Ten percent of the ultrasound images were independently validated by a radiologist in Manila.

Serum biomarkers of hepatic fibrosis were also evaluated on a random subset of patients. The selection of the sample subset from each fibrosis grade category was done using computer-generated random numbers. Other laboratory investigations were conducted to rule out etiologies of known liver pathology (e.g., hepatitis B and alcoholism). Blood (10 ml) was collected from each subject enrolled. Serum was separated from the whole blood and refrigerated at 2–8 °C. Collected sera were then transferred to a 4 °C cooler box and shipped to the Research Institute for Tropical Medicine (RITM) clinical laboratory in Manila. Each serum sample was divided into equal aliquots. Half of the serum specimens were used for liver function and hepatitis B surface antigen (HBsAg) testing and the other half was kept at −80 °C for use in fibrosis marker assays. Hepatitis C testing was excluded due to the very low prevalence (<3%) reported for rural remote communities in the Philippines. Liver function (including tests for hepatocellular injury, parenchymal damage, capacity for protein synthesis, and cholestasis) and HBsAg tests were performed on the samples using an automated machine (A15; Biosystems S.A., Barcelona, Spain) and commercial enzyme immunoassay kits (MONOLISA; Bio-Rad, Marnes-la-Coquette, France), respectively. The serum samples for fibrosis marker testing were transferred to Siemens, Australia who evaluated HA, procollagen type III (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) serum levels using the ELF test.[21] The ADVIA Centaur CP XP Immunochemical Analyzer Bayswater, (Victoria, Australia) was used according to the manufacturer's instructions. The ELF score was calculated directly by the instrument using the following equation:

$$\text{ELF score} = 2.278 + 0.851\ln(C_{HA}) + 0.751\ln(C_{P3NP}) + 0.394\ln(C_{TIMP-1})$$

The serum levels of soluble intercellular adhesion molecule 1 (sICAM-1) were measured at QIMR Berghofer Medical Research Institute using a separate ELISA kit (R&D systems, Minneapolis, MN, USA).

2.3. Study oversight

Ethical consent for the study was obtained from the ethics review boards of the Department of Health in the Philippines (Institutional Review Board Number 2012-13-0), Griffith University, Australia, and QIMR Berghofer Medical Research Institute, Australia. Written informed consent was obtained from each individual or from the parents/legal guardians of those aged <15 years. Stool-positive subjects were treated orally with 60 mg/kg PZQ in two divided doses, followed by another treatment of the same dose 2 weeks later. Stool-negative individuals were treated with a single oral dose of 40 mg/kg.

2.4. Statistical analysis

FoxPro version 6.0 (Microsoft Corp) was used for double-entry of data. Data were cross-checked and analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). The statistical analysis was performed using Stata version 11 (StataCorp LP, College Station, TX, USA). Data were summarized as frequencies or the mean ± standard deviation, as appropriate. All categorical variables were evaluated using the Chi-square test. Wilcoxon signed-rank and McNemar tests were used for paired analysis of infection intensity and prevalence, respectively, across different age groups. With regards to the impact of treatment on infection intensity, the *S. japonicum* egg count + 1 was $\log_{10}$-transformed to bring observed distributions closer to normality. The Kruskal–Wallis test was used to detect significant differences in the level of serum marker types across fibrosis grades. Receiver operating characteristics (ROC) analysis was performed to assess the clinical utility of the three serum fibrosis markers (HA, TIMP-1, and PIIINP), both individually and as their combined quantitative result (ELF score), in identifying subjects with high-grade liver fibrosis (grade II–III). A significance level of 5% was used for statistical inference.

3. Results

3.1. Study descriptors

Fifty-three percent of the study sample (n = 565) was male; the mean age of the study subjects was 39.4 years (95% confidence interval (CI) 38–41 years). Most were poor rice farmers with a high degree of exposure. There was no statistical difference in the sex profile of the sample. However, most of the study participants belonged to age groups 36–45 years (24%) and 46–55 years (27%). Only 1.0% of the population reported having been diagnosed with hepatitis B; however, 38.4% drank alcohol moderately (consumed 1–2 drinks per day).

3.2. Prevalence, intensity, and morbidity outcomes

The study communities have participated in an active schistosomiasis program for the past 20 years. However, the prevalence of *S. japonicum* infection among the pre-treatment cohort subjects (n = 565) was 34% (Figure 2). Males had a higher prevalence of infection (46%) than females (20%). *S. japonicum* infection was more prevalent (49%) among subjects less than 20 years of age. The mean intensity of infection was 123.1 epg (95% CI 78.9–167.3 epg) (Figure 2).

Twenty-five percent had light intensity infections, 6% had moderate intensity infections, and 2% had heavy infections. Hepatic parenchymal fibrosis occurred in 50% of subjects, with grade II fibrosis in 19% and grade III in 6% (Table 1). The prevalence of fibrosis was 52.7% (298/565) in males and 47.3% (267/565) in females. The degree of liver fibrosis correlated significantly (p = 0.0002) with the intensity of infection. The left and right hepatic lobe lengths increased with increasing fibrosis grade. The mean left lobe lengths of those with grade II (mean length 93.7 mm, 95% CI 92.5–94.8 mm) and III (mean length 98.4 mm, 95% CI 96.4–100.4 mm) fibrosis were significantly longer (p = 0.0001) than the lengths of those without fibrosis (mean length 87.7 mm, 95% CI 86.4–88.9 mm). However, only the mean length of the right lobe of individuals with grade III fibrosis (122.3 mm, 95% CI 120.7–124.0 mm) was significantly (p = 0.04) longer compared with those lacking fibrosis. Likewise, the mean length of the spleen increased with increasing degree of fibrosis, although not significantly (p = 0.21).

Four hundred and seventy-three subjects had both baseline and follow-up stool examination results; these individuals were utilized in the final analysis to determine the impact of PZQ treatment on the prevalence and intensity of infection. The prevalence dropped significantly 2 years post-treatment, especially among those aged 16–25 years (p = 0.008) and 46–55 years (p = 0.001) (Figure 2). Similarly, the intensity of infection across all age groups dropped compared with pre-treatment levels (Figure 2). The prevalence decline observed among the 16–25 years age group may be due in part to limited exposure to infection. On the other hand, acquired immunity to re-infection may partially

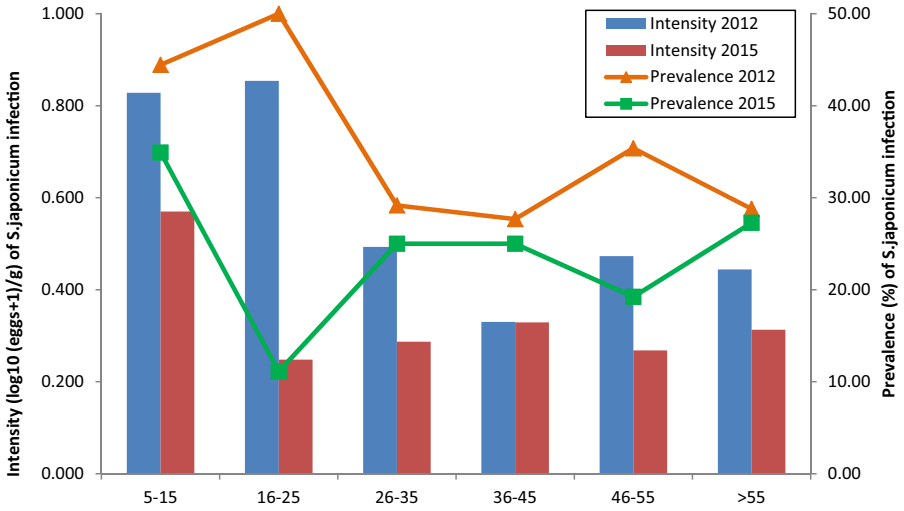

Figure 2. Prevalence and intensity of infection in subjects with *Schistosoma japonicum* by age before (2012) and after (2015) 2 years of praziquantel treatment (i.e., 40–60 mg/kg; $n = 473$).

Table 1
Dynamics of schistosomiasis liver parenchyma grading before and after praziquantel treatment in a cohort of 424 subjects from Northern Samar, Philippines, from 2012 to 2015

	Parenchyma grading 2015				Total (n)	Total (%)	Progressed (%)	Regressed (%)
Parenchyma grading 2012	Normal	Grade I	Grade II	Grade III				
Normal	156	54	3	0	213	50.24	26.8	0.0
Grade I	40	40	17	7	104	24.53	23.1	38.5
Grade II	8	17	33	22	80	18.87	27.5	31.3
Grade III	2	1	14	10	27	6.37	0.0	63.0
Total (n)	206	112	67	39	424	100	103	82

account for the prevalence drop among those aged 46–55 years. However, only subjects aged 46–55 years showed significant decreases in the intensity of infection compared with the baseline findings ($p = 0.003$). The prevalence of grade II–III fibrosis remained relatively unchanged at 25.2% (107/424) on follow-up (Table 1). The data suggest that a 2-year follow-up period may be too short a time frame to assess disease regression following treatment.

3.3. Liver function tests, hepatitis B, and serum fibrosis markers

Liver function tests were performed on a selected subset of patients ($n = 136$). The total number of individuals was derived via age-matched specific grouping, resulting in 34 subjects for each of the four fibrosis clinical grades. Sixty percent (81/136) of the subjects had normal liver function tests, with the proportion being uniform across all fibrosis grades. Mild alanine aminotransferase elevation was noted in 1.5% (2/136) of the sample. Alkaline phosphatase and total bilirubin results were similarly distributed among the four fibrosis groups.

HBsAg testing was done on all 565 subjects. The HBsAg carrier rate was 4.2% (24/565). The carrier rates in those with grades I, II, and III fibrosis were 1.4% (2/145), 2.8% (3/105), and 2.9% (1/34), respectively. The carrier rates in those with fibrosis were actually lower than the rates in those without fibrosis (6.4%). Thus, the prevalence of both alcohol and chronic hepatitis B-induced liver fibrosis were demonstrated to be low in the cohort.

The serum fibrosis marker levels and infection intensity before and after PZQ treatment were also assessed. Only baseline mean TIMP-1 levels showed cumulative results with increasing intensity of infection ($p = 0.001$). The baseline and endpoint serum levels of the fibrotic markers were also compared with parenchymal grading. The ELF marker score and two of three ELF markers correlated with the severity of fibrosis. The mean baseline and follow-up ELF scores correlated significantly with increasing liver fibrosis grade ($p = 0.002$, $p = 0.049$). Before treatment, baseline TIMP-1 levels were increased in all three increasing fibrosis grades ($p < 0.001$). At the endpoint, TIMP-1 levels were also significantly higher in grade III compared with grade I ($p = 0.034$). The HA levels were higher in grade III compared with grade 0 ($p = 0.031$) and grade I ($p = 0.0429$). sICAM-1 was also correlated with the grade of fibrosis ($p = 0.0013$).

Table 2
Area under the receiver operating characteristics curve (AUC) and diagnostic sensitivities and specificities for ELF score cut-off values and individual serum markers of fibrosis at baseline and 2 years after treatment to distinguish between severe fibrosis (grade II–III) and mild fibrosis (grade 0–I)

Fibrosis marker	AUC	p-Value	Sensitivity	Specificity	Cut-off
Baseline ($n = 177$)					
ELF	0.68	<0.001	64	71	9.2
Hyaluronic acid	0.69	<0.001	66	72	30.7
PIIINP	0.56	0.515	61	51	10.2
TIMP-1	0.65	<0.001	63	68	253.6
Follow-up ($n = 180$)					
ELF	0.62	0.004	62	62	8.9
Hyaluronic acid	0.63	0.043	73	53	26.3
PIIINP	0.57	0.475	58	57	9.4
TIMP-1	0.62	0.001	73	51	183.7

ELF, enhanced liver fibrosis test; PIIINP, procollagen type III; TIMP-1, tissue inhibitor of metalloproteinase 1.

3.4. Diagnostic performance of ELF

Table 2 shows the baseline and follow-up ROC curve analysis results of the ELF score and individual markers to differentiate subjects with grade II–III fibrosis (severe) from those with grade 0–I fibrosis (mild). The diagnostic accuracies of HA and the ELF score were slightly higher than those of the other two ELF markers assessed based on the area under the curve (AUC) at baseline. At follow-up, HA, TIMP-1, and the ELF score had higher diagnostic accuracy than PIIINP. The cut-off value of 30.7 ng/ml for HA (AUC 0.69, $p < 0.001$) and 9.2 for the ELF score (AUC 0.68, $p < 0.001$) to discriminate grade II–III fibrosis from grade 0–I among 177 subjects at baseline showed sensitivities of 66% and 64%, respectively, and specificities of 72% and 71%, respectively. At follow-up, the cut-off value of 26.3 ng/ml for HA (AUC 0.63, $p < 0.043$), 183.7 ng/ml for TIMP-1 (AUC 0.62, $p < 0.001$), and 8.9 for the ELF score (AUC 0.62, $p < 0.004$) to discern grade II–III fibrosis from grade 0–I among 180 subjects showed sensitivities of 73%, 73%, and 62%, respectively, and specificities of 53%, 51%, and 62%, respectively. PIIINP was uninformative, having the lowest AUC for both time points.

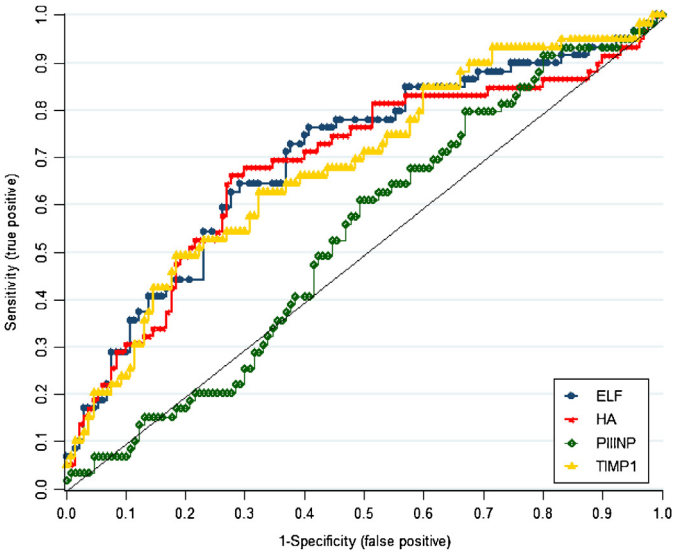

Figure 3. Baseline ROC curves ($n = 177$) for the enhanced liver fibrosis (ELF) score and serum concentrations of hyaluronic acid (HA), procollagen type III (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) for differentiating grade II–III liver fibrosis from grade 0–I fibrosis..

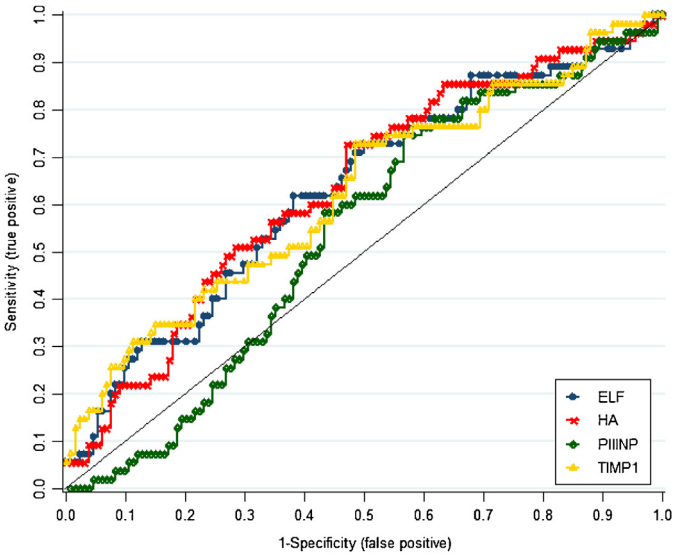

Figure 4. Follow-up ROC curves at 2 years post-treatment ($n = 180$) for the enhanced liver fibrosis (ELF) score and serum concentrations of hyaluronic acid (HA), procollagen type III (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1) for differentiating grade II–III liver fibrosis from grade 0–I fibrosis.

Table 3
Mean fibrosis marker value by intensity of infection and fibrosis grade

Intensity of infection	*n*	ELF	HA	PIIINP	TIMP-1
Grade 0					
Negative	68	8.8	31.6	12.1	199.8
Mild	10	8.9	23	14.7	252.5
Moderate	2	8.5	21.4	8.2	278.1
Heavy	0				
Grade 1					
Negative	0				
Mild	7	8.8	23.2	13.7	295.3
Moderate	4	9	27.3	13.6	290.1
Heavy	1	9.4	12	27.6	594.5
Grade 2					
Negative	14	9.3	41.1	12.3	294.6
Mild	17	9.1	46.5	10.3	274.6
Moderate	4	9.4	59.2	10	241.1
Heavy	1	7.7	5.6	10.5	277.1
Grade 3					
Negative	8	10	85.5	21.4	302.8
Mild	10	9.6	64.6	11.2	279.5
Moderate	3	10.2	76.2	14.2	370.4
Heavy	2	8.7	24.1	10.3	457.8

ELF, enhanced liver fibrosis test; HA, hyaluronic acid; PIIINP, procollagen type III; TIMP-1, tissue inhibitor of metalloproteinase 1.

Figures 3 and 4 illustrate the ROC curves for the three individual ELF markers and the curves for the ELF score. The ELF score and HA showed the highest diagnostic performance in detecting severe schistosome-induced liver fibrosis. When sex was considered, no significant difference was observed in the cut-off values at baseline and at 2 years of follow-up. Cut-off values of 8.9 ng/ml and 9.2 ng/ml were noted for males (baseline: AUC 0.69, $p = 0.003$; follow-up: AUC 0.62, $p = 0.342$) and females (baseline: AUC 0.64, $p = 0.226$; follow-up: AUC 0.69, $p = 0.042$), respectively, suggesting that the same cut-off value can be utilized for both sexes. However, when age was taken into account, the cut-off value at baseline was noted to be higher among subjects younger than 18 years of age (9.9 ng/ml, AUC 0.96, $p = 0.192$) compared with those aged 18–40 years (8.9 ng/ml, AUC 0.71, $p = 0.087$), 41–50 years (8.9 ng/ml, AUC 0.74, $p = 0.005$), and over 50 years (9.2 ng/ml, AUC 0.61, $p = 0.046$). This suggests that age may influence the ELF test results. On follow-up, the cut-off values could not be determined for the adolescent (<18 years) cohort because very few of these individuals had moderate to severe fibrosis.

Overall, the mean values of the fibrosis markers seemed to increase across the fibrosis grades, except for PIIINP (Table 3). However, within each grade, there seemed to be no apparent pattern across intensity of infection. This observation may be due to the small sample size within each category.

4. Discussion

In this study, the prevalence of schistosomiasis, level of infection intensity, and level of morbidity was evaluated among 565 subjects residing in 18 moderately endemic barangays in Northern Samar, the Philippines. The study communities have participated in the National Department of Health human treatment program for schistosomiasis over the past three decades. Nevertheless, the baseline human prevalence and infection intensity were 34% and 123 epg, respectively. Baseline ultrasonography revealed a high level of schistosomiasis-induced morbidity among subjects with signs and symptoms suggestive of

schistosomiasis. Eighty-nine percent of the patients had left lobe liver enlargement (≥70 mm) and 25% had grade II or III liver fibrosis. Splenomegaly (≥100 mm) was seen in 13% of the population. Infection intensity was significantly correlated with grade III fibrosis ($p < 0.0002$). Hepatitis B virus, alcoholism, and malaria were excluded as possible causes. The results suggest that the national control strategy for schistosomiasis (i.e., human treatment with 40 mg/kg PZQ) is inadequate in the study area. Low drug coverage (<36%), low drug compliance (<40%), and the inability of PZQ to prevent re-infection given the zoonotic nature of the disease may have contributed to the findings.[22]

As expected, a reduction in both the prevalence and intensity of infection were noted at 2 years after chemotherapy (i.e., 40–60 mg/kg PZQ) compared to baseline. However, treatment had less of an impact on lowering schistosomiasis-induced morbidity. Overall, hepatic fibrosis (grades I–III) regressed in only 24.3% of those who received a single treatment and in only 19.3% of those who received two PZQ doses. This outcome is similar to that reported for *S. japonicum* morbidity in China.[9] The baseline prevalence of grade II–III fibrosis was 25.2%, but this remained unchanged at 2 years post-treatment. Individuals with significant morbidity despite standard treatment will need a longer treatment course at a higher dose (i.e., 60–80 mg/kg) to reverse fibrosis and improve clinical outcomes. Approximately 10% of residents in *Schistosoma mansoni* and *S. japonicum* endemic areas develop severe hepatosplenic disease.[23–25] These observations have been explained in part by genetic factors regulating disease development.[23–25]Liver fibrosis is a complex process involving the production and deposition of insoluble components that comprise the extracellular matrix (ECM).[9] Quantitative and qualitative ECM changes in liver fibrosis can be measured in blood or urine using indirect and direct biomarkers.[5] These biomarkers of hepatic matrix metabolism have also been drawn together into multi-parameter scores to produce promising results.[21,26] The ELF test is an ECM marker set comprising an algorithm of three direct liver fibrosis markers (HA, TIMP-1, and PIIINP). The ELF test was one of the first commercially available serum fibrosis tests,[21] and its diagnostic potential has been explored in identifying and staging liver fibrosis among patients with hepatitis C virus infection,[21,27] alcoholic liver disease,[26] and non-alcoholic fatty liver disease (NAFLD).[28] The test is said to be comparable to FibroScan (transient elastography)[29,30] and more accurate or more reproducible than simple panels (such as aspartate aminotransferase to platelet ratio and Fibrosis-4) in detecting advanced fibrosis.[31,32]

The present study assessed four promising direct biomarkers (HA, PIIINP, TIMP-1, and sICAM-1) based on their performance in previous studies.[5,9,14,15] To the best of the authors' knowledge, this is the first time that the ELF score has been deployed in the evaluation of liver fibrosis due to schistosomiasis. The best correlation between serum marker level and ultrasonographic fibrosis grading was demonstrated with TIMP-1. TIMP-1 levels increased as the degree of fibrosis increased both at baseline and at endpoint. The marker levels also decreased in all fibrosis grade categories at endpoint (after treatment), suggesting that serum levels of TIMP-1 can be used to accurately assess liver fibrotic disease due to schistosomiasis. Also, TIMP-1 serum levels correlated positively with the intensity of infection, suggesting that the marker may also respond to inflammatory reactions to eggs deposited in the liver. HA and sICAM-1 also showed some correlation, but not as significant as shown for TIMP-1. Moreover, the ROC analysis revealed that HA as an individual marker showed the highest sensitivity and specificity in discerning higher fibrosis grades from lower grades. The diagnostic performance of the ELF score was equally as useful as that shown for HA alone. However, the AUC values generated using both the ELF score and serum biomarkers individually indicated only moderate discrimination between grades II–III and 0–I fibrosis (i.e., AUC 0.6–0.7).[33] Moreover, consistent with other studies, age should be considered as a factor when generating ELF scores.[21] An unadjusted ELF score may misclassify normal individuals younger than 18 years of age as having moderate-to-severe schistosome-induced liver fibrosis. If the diagnostic value of the ELF test can be improved as a reliable stand-alone test and suitable for field application, then it could potentially be deployed as a component of the diagnostic and treatment algorithm for *S. japonicum*-infected patients (Figure 5).

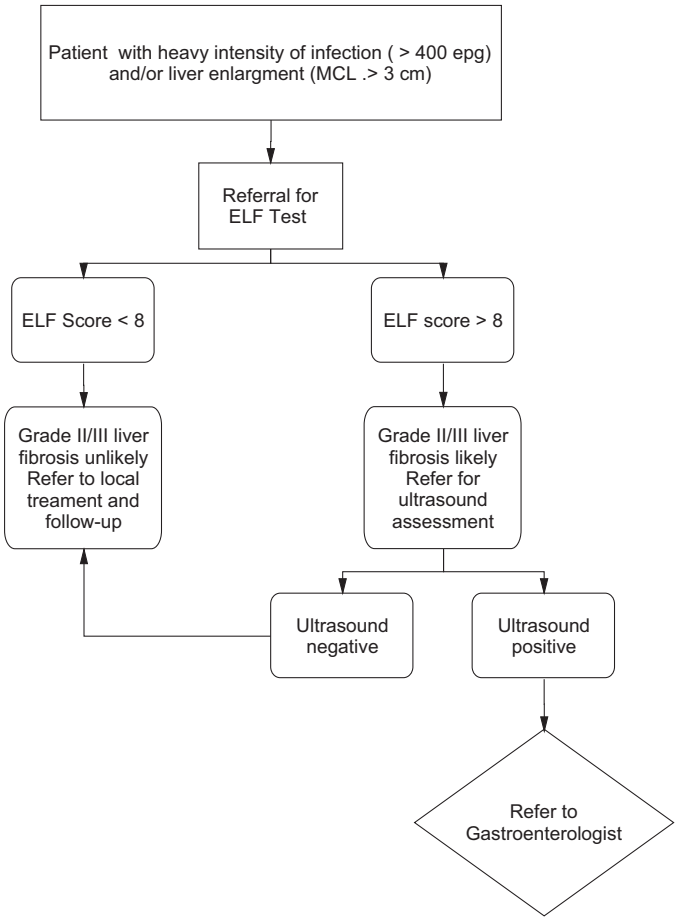

Figure 5. Algorithm for the recommended diagnosis and management of severe hepatic fibrosis (grade II–III) due to gastrointestinal schistosomiasis infection.

In conclusion, a high level of schistosomiasis-induced morbidity was demonstrated among subjects with signs and symptoms suggestive of the disease in a moderately endemic area of the Philippines that has been under active community-based chemotherapy for over three decades. Hepatic fibrosis, the main cause of hepatosplenic schistosomiasis, takes time to reverse with chemotherapy. Individuals with hepatosplenic schistosomiasis should be identified and treated as early as possible to prevent disease progression and possible death. Serum fibrosis markers, particularly high TIMP-1 and HA levels, and ELF scores above 8 may potentially guide program managers on where to direct limited health care resources for schistosomiasis control. More specifically, if made affordable for field application, the ELF test may assist clinicians in assessing patients with heavy intensities of infection (>400 epg) and/or with physical signs of advanced disease (e.g., MCL >3) in determining their risk of severe liver fibrosis.

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