Are we ready for a global pandemic of Ebola virus?

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The chronic enteropathogenic disease schistosomiasis

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S U M M A R Y

Schistosomiasis is a chronic enteropathogenic disease caused by blood flukes of the genus Schistosoma. The disease affects approximately 240 million individuals globally, causing approximately 70 million disability-adjusted life years lost. Chronic infections with morbidity and mortality occur as a result of granuloma formation in the intestine, liver, or in the case of Schistosoma haematobium, the bladder. Various methods are utilized to diagnose and evaluate liver fibrosis due to schistosomiasis. Liver biopsy is still considered the gold standard, but it is invasive. Diagnostic imaging has proven to be an invaluable method in assessing hepatic morbidity in the hospital setting, but has practical limitations in the field. The potential of non-invasive biological markers, serum antibodies, cytokines, and circulating host microRNAs to diagnose hepatic fibrosis is presently undergoing evaluation. This review provides an update on the recent advances made with respect to gastrointestinal disease associated with chronic schistosomiasis.

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1. Introduction

Schistosomiasis (bilharzia) is a chronic enteropathogenic disease caused by blood flukes of the genus Schistosoma. It affects approximately 240 million people and is considered the third most devastating tropical disease in Africa, South America, the Caribbean, the Middle East, and Asia. More than 78 countries are affected, and nearly 800 million people are at risk of infection. Of the five species that infect humans, Schistosoma mansoni, Schistosoma haematobium, and Schistosoma japonicum cause the most morbidity. The disease burden due to these three species is estimated to be as high as 29 million disability-adjusted life years (DALYs).

The lifecycles of the five schistosome species are similar and involve a snail intermediate host (Figure 1). Chronic infections with all Schistosoma species, with the exception of S. haematobium, can cause significant morbidity and mortality as a result of granuloma formation in the intestine and liver. However, cases of liver and intestinal disease from S. haematobium have also been reported. This review emphasizes the pathogenesis of the intestinal and hepatosplenic forms of the disease – the major causes of morbidity in schistosomiasis mansoni and japonica. The evaluation of hepatic fibrosis with diagnostic imaging and the performance of different direct serum biomarkers and potential use of circulating microRNAs (miRNAs) for disease staging and predicting the risk of hepatic fibrosis are also discussed.

2. Chronic schistosomiasis

2.1. Intestinal disease

One of the common manifestations of the chronic form of this enteropathogenic disease is intestinal schistosomiasis. The infection is caused by S. mansoni, S. japonicum, S. mekongi, and S.
of calcified eggs, atrophy of the overlying mucosa ensues and it acquires a granular dirty yellowish appearance. Polyps, which are said to be the most common among the spectrum of intestinal lesions, may result from an immune-mediated inflammatory process associated with continued egg deposition and ova entrapment leading to a foreign body reaction with progressive inflammation and fibrosis. Schistosomal eggs are deposited in the superficial layers of the submucosa where reactive cellular debris and vascular granulation tissue accumulate. Eggs will then produce a cell-mediated inflammatory response with granuloma formation and necrosis. The subsequent healing of the necrotic foci will lead to the formation of fibrous connective tissue and hypertrophy of the muscularis mucosa. The fibrous connective tissue in the submucosa and the hypertrophied muscularis mucosa form a barrier to the ova transiting from the mesenteric veins to the gut lumen. The trapped ova then elicit further inflammation and fibrosis. This continuous process elevates the hypertrophied muscularis mucosa to form a nodule which is the earliest detectable polyp.

Clinical manifestations of intestinal schistosomiasis include abdominal pain, altered bowel habits, and bloody stools. Iron-deficiency anemia and eosinophilia are also present. Polyposis from intestinal schistosomiasis does not appear to be related with colorectal carcinoma, but a recent study has shown that a history of chronic schistosomiasis japonica is a probable independent risk factor for the development of colorectal neoplasias.

Appendiceal schistosomiasis was first documented in 1909, and the most frequent species associated with this condition are S. haematobium and S. mansoni. In one case report, schistosomiasis haematobia presented as acute appendicitis in a 26-year-old Israeli male who developed symptoms 2 years after visiting Africa; tissue sections showed extensive inflammatory areas and fibrosed granulomas. This rare condition was also reported recently in a 30-year-old male UK resident from Ghana; histological sections of his appendix revealed luminal pus associated with numerous S. mansoni egg masses transmurally and within the subserosal adipose tissue. The usual granulomatous response around the eggs was evident. Eggs in the submucosa produce an obstructive type of appendicitis, while serosal lesions produce inflammation and adhesion formation.

In Saudi Arabia, an unusual case of disseminated peritoneal S. japonicum has also been reported in a 32-year-old Filipino female who presented with signs and symptoms of acute appendicitis. However, a right iliac fossa mass was also seen on diagnostic laparoscopy. Microscopic sections of both the appendiceal wall and the adherent omental mass showed suppurative inflammation and multiple foci of schistosomal ova highly indicative of the S. japonicum species. Interestingly, a granulomatous response was not seen in the sections examined.

2.2. Hepatosplenic disease

Hepatic schistosomiasis represents the best known form of chronic disease with a wide range of clinical manifestations, and its pathogenesis is related to the host cellular immune response. The mechanisms involved in granuloma formation and fibrosis have been documented extensively in experimental models and humans infected with S. mansoni and S. japonicum. Eggs trapped in the pre-sinusoidal portal venules secrete soluble egg antigens which are taken up by antigen-presenting cells such as macrophages. Subsequently, antigen presentation stimulates Th1 cells (CD4+ T lymphocytes) to secrete interleukin (IL)-2, interferon gamma (IFN-γ), and tumor necrosis factors (TNF), which in turn drive a cell-mediated response and attract more immune cells around the ova. As the granuloma becomes more organized, the Th1 cells are gradually replaced by Th2 cells, which produce IL-4, IL-5, IL-10, and IL-13, completing granuloma maturation.
Towards the late stage of granuloma formation, the fibroblasts are stimulated by egg products and by T lymphocyte cytokines to proliferate, replacing most of the cellular elements, and mediating fibrotic collagenous material deposition around the portal vein tributaries. The pathogenesis of hepatic fibrosis leading to hepatosplenic schistosomiasis is illustrated for *S. japonicum* in Figure 2.

Fibrosis, leading to portal hypertension, is the major cause of disease morbidity and mortality. Grossly, whitish plaques known as ‘clay-pipestem’ fibrosis are evident on cut sections, contrasting with the intact liver acinar architecture.27 Lesions commence as eosinophilic infiltrates surrounding trapped eggs, which may subsequently lead to abscess formation. A Splendore–Hoeplli reaction (asteroid body formation) may sometimes occur.28 Peri-ovular granulomas develop and the eggs inside degenerate and calcify over time. As older granulomas involute, macrophage-predominant granulomas begin to form.27 Eventually, granulomas are replaced by surrounding fibrous tissue. As more new eggs arrive, resultant damage to larger diameter veins occurs, along with periportal granulomatous inflammation and inter-granulomatous fibrosis. In the absence of a coexisting hepatotropic viral infection, the liver of patients with periportal fibrosis secondary to schistosomiasis retains its hepatocellular function, differentiating the disease from cirrhosis and other liver diseases.7–29 Severe schistosome-induced hepatic fibrosis causes portal vein obliteration leading to the development of portal hypertension, and lethal complications of the hepatosplenic form of the disease include pulmonary hypertension,30,31 glomerulopathy,32,33 splenomegaly, and thrombocytopenia.34

Despite the general mechanism of granuloma formation leading to hepatic fibrosis in schistosomiasis, studies have shown some peculiarities regarding this pathogenesis among schistosome species. For instance, in a study involving mouse models, tissue studies were done to shed light on the characteristics of granulomas caused by *S. mekongi* compared with *S. japonicum*-induced granulomas. In the murine livers, it was shown that *S. mekongi*-induced granulomas were initially cellular, formed by foam cells, and continuously appeared in the intralobular areas, while *S. japonicum*-induced granulomas were fibrous and did not continuously appeared in the intralobular areas. Portal fibrosis was also not observed in *S. mekongi*-infected murine livers, while this lesion appeared later in livers infected with *S. japonicum*. It was thought that the absence of portal fibrosis in *S. mekongi* infection allowed the eggs to infiltrate the interlobular areas continuously, which may have accounted for the absence of echogenic pattern on ultrasonographic evaluation (a feature noted with *S. japonicum* species).35 On the other hand, in another study, the intensity of liver fibrosis among patients with *S. mekongi* infection was assessed by liver biopsy. Liver biopsies revealed complete disorganization of hepatic architecture with fibrous enlargement of portal tracts and some portal–portal bridging fibrosis, but there was no cirrhosis. There was blood vessel congestion and thrombosis with inflammation in the portal areas. Numerous eggs of *S. mekongi* were observed mostly in fibrous areas and more rarely in the parenchyma. Some eggs were surrounded by epithelioid and giant cell reaction. A high degree of fibrosis was observed among young adults in that study and this contrasts with the findings observed with other schistosome species.36

![Figure 2. Pathogenesis of hepatic fibrosis leading to hepatosplenic schistosomiasis japonica](image-url)
The clinical manifestations of *S. mekongi* are similar to those of *S. mansoni* and *S. japonicum* infections. Clinical hepatomegaly, splenomegaly, reported blood in stool, abdominal pain, diverted circulation, and ascites are frequent reported clinical signs in studies assessing hepatosplenic morbidity from *S. mekongi*.

Although several studies have clearly shown that schistosomous egg-induced granulomas are pathogenic to the host, there are some current studies suggesting that the lesions may have some protective functions. In an *S. mansoni* study involving transgenic mice, it was thought that granuloma formation around the egg offered some protection against schistosome-related hepatotoxicity (an occurrence that is currently observed only in *S. mansoni*-infected mice). Mice that were not able to produce the necessary cytokines to form a functional granuloma died early due to egg-induced endotoxemia. The granuloma is not only thought to act as a physical barrier, but also functions to sequester the antigenic products secreted by the egg. Nevertheless, it is also thought that the parasite takes advantage of this functional granuloma by facilitating excretion of its eggs, without killing the host, and therefore continuing its lifecycle.

Recently, hepatic stellate cells (HSCs) have been acknowledged to be major players in the liver fibrotic process. The interactions of the HSCs with *S. japonicum* and *S. mansoni* eggs have been investigated; both studies supported the contributory role of these cells in schistosome-induced hepatic fibrosis. Further, it has been demonstrated that the activated HSC myofibroblastic phenotype can be reversed to its quiescent phenotype (manifested by lipoprotein storage in cells) by *S. mansoni* egg antigens, an interaction not observed with *S. japonicum* eggs. This inability of the *S. japonicum* eggs to induce a quiescent myofibroblastic phenotype has been suggested to explain in part why *S. japonicum* is more pathogenic to the liver compared with *S. mansoni*.

### 3. Evaluation of hepatic fibrosis

The pathology of schistosome-induced liver disease has been studied extensively since its first description in 1904. The term ‘Symmers fibrosis’ was originally adopted to describe the unusual pattern of collagen and glycosaminoglycan (GAG) deposition observed in an autopsy. This term was changed in 1947 to clay-pipestem fibrosis in recognition of the fact that the hepatic parenchyma was spared in schistosomal-induced liver disease and appeared fundamentally different from ‘cirrhotic’ deposits of collagen and GAGs.

Currently there are a number of methods that can be used to diagnose and evaluate the severity of liver fibrosis. Liver biopsy is considered the gold standard but causes significant discomfort and possible post-procedural risk. The evaluation of tissue morphology may be informative but does not have sufficient sensitivity to diagnose periportal fibrosis (PPF) and cannot explain the disease dynamics occurring between sampling periods. Imaging modalities like ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), on the other hand, are used not only to support the diagnosis of schistosomiasis but also to accurately assess and detect target organ damage that can develop due to chronic infection with schistosomiasis. Research to identify non-invasive markers for hepatic fibrosis is also underway and the combination of these biomarkers along with comprehensive history and physical examination, basic laboratory tests, and imaging methods seems to offer the best approach for evaluating patients with this disease.

#### 3.1. Diagnostic imaging

The reliability of US has made it the routine imaging method in the evaluation of hepatosplenic schistosomiasis for the past 30 years. In the diagnosis of *S. mansoni*, US can demonstrate PPF appearing as echogenic tubular shadows with an anechoic lumen that radiates from the porta hepatis. When the tubular structure is viewed crosswise, it appears as a ring of concentric fibrosis surrounding portal venous vasculature, and is known as the ‘bull’s eye lesion’. Other US findings include hypertrophy of the left hepatic lobe, atrophy of the right hepatic lobe, gallbladder wall thickening, granulomas, and splenic nodules. The above lesions can also be seen in liver pathology associated with *S. japonicum* infection. However, the demonstration of a septal formation by high echogenic bands like a mosaic or network pattern in the liver by US is typical only for *S. japonicum* infection.

CT, on the other hand, is not routinely used in the evaluation of schistosomiasis due to its cost and the utilization of ionizing radiation. CT scans show similar imaging findings to US, including atrophy of the right hepatic lobe, hypertrophy of the left hepatic lobe, splenomegaly, and ascites. PPF is seen in CT as a band of low attenuation around portal vein branches throughout the liver, with enhancement following the intravenous administration of contrast medium. The ‘bull’s eye’ lesion in the liver can be demonstrated on CT as concentric layers of periportal enhancement and is thought by some authors to be a more specific indicator of schistosomiasis than the periportal enhancement. The network pattern seen in the liver in *S. japonica* appears on CT as a ‘turtleshell’ or ‘tortoiseshell’ lesion. This lesion is thought to be due to septal calcification of schistosomal ova.

In the diagnosis of hepatosplenic schistosomiasis using MRI, the most frequent findings are accentuation of periportal signal in T2-weighted sequences, and a hypointense signal in relation to the normal liver parenchyma in T1-weighted sequences with fat suppression. The periportal signal is accentuated on T1-weighted sequences following contrast administration. It has been suggested that periportal inflammation may be differentiated from fibrosis by the hyperintense signal observed in T2-weighted sequences. Portal vein thrombosis (PVT), which may be due to hepatosplenic schistosomiasis, is best diagnosed by MRI. Cavernous portal vein transformation due to PVT is described on MRI as small enhancing flow voids around the right portal vein in a T1-weighted image. Although MRI uses a gadolinium-based contrast, its higher cost precludes routine use.

The utility of imaging techniques in the diagnosis of schistosomiasis is demonstrated in Figure 3. A 12-year-old Filipino boy from a known schistosomiasis endemic area in the central Philippines was diagnosed by portable gray-scale US to have moderate PPF with severe splenomegaly due to schistosomiasis japonica. Further examination with MRI confirmed the PPF and showed an additional finding of PVT and cavernous transformation of the right branch of the main portal vein. Massive splenomegaly was also demonstrated on MRI. The patient was successfully treated by splenectomy.

#### 3.2. Serum biological markers

The supporting framework of the normal and fibrotic liver comprises a group of macromolecules called extracellular matrix (ECM). In advanced stages of fibrosis, the liver contains approximately six times more ECM than normal, including collagen I, III, and IV, fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycan. Qualitative and quantitative ECM changes in liver fibrosis can be measured in the blood or urine using indirect and direct biomarkers. Direct biomarkers are classified into three groups: (1) those that measure matrix deposition: procollagen I
carboxy terminal peptide (PICP), procollagen III amino terminal peptide (PIIINP), tissue inhibitors of metalloproteinase (TIMPs), transforming growth factor beta (TGF-B), tenasin; (2) those that reflect matrix removal or degradation: procollagen IV C peptide, procollagen IV N peptide (7-S collagen), collagen IV, metalloproteinase MMP, undulin, urinary desmosine, and hydroxylysylpyridinoline; and (3) those that cannot clearly determine the relationship to the matrix deposition or removal: hyaluronic acid, YKL-40 (Chondrex), and laminin.63,64 Many of these biomarkers have been evaluated and found useful in the diagnosis and grading of liver fibrosis caused by several conditions, including chronic viral hepatitis, alcoholic cirrhosis, non-alcoholic steatohepatitis, and schistosomiasis.65 However, their ability to identify and grade liver fibrosis in schistosomiasis at the community level in endemic areas needs further evaluation. The performance of some serum markers in the evaluation of schistosome-induced hepatic fibrosis is discussed below and is summarized in Table 1.

3.3. Collagen

Collagen and its metabolic products have been examined in the blood and urine of patients with different etiologies of fibrotic liver disease.66 Collagen types I, III, and IV have been used extensively in the evaluation of hepatic fibrosis. Serum PIIINP has also gained wide acceptance as a blood test for collagen metabolism.67 Type III collagen is secreted in the extracellular space as a procollagen molecule. The amino terminal portion of the molecule is then cleaved by enzymatic cleavage by two specific endopeptidases.68 This releases the helical type III collagen molecule to combine with other ECM macromolecules to form a collagen fibril. This short peptide fragment (PIHIP) diffuses from fibrotic loci, circulates in the blood, and is metabolized further to form a fragment termed Col-1 (procollagen type I peptide (PIP)).69 The PIHIP assay appears to have its greatest utility in the early stages of fibrosis when type III collagen biosynthesis predominates.70 In the late stages of most cirrhotic conditions, PIP is the dominant peptide synthesized.71 A study on PICP in schistosomiasis mansoni showed that the levels of this biomarker were higher in infected patients than in uninfected controls and that serum levels decreased during the first year post-treatment with the anti-schistosome drug praziquantel; however, no correlation was established between PIP and fibrosis scores by US examination.72 On the other hand, PIIINP levels in schistosomiasis mansoni were elevated in patients with hepatic disease compared with normal or uncomplicated control subjects. In another study it was shown that PIIINP levels returned to normal levels 18 months after patients received praziquantel.73 Higher levels of PIIINP were also observed in subjects with advanced PPF diagnosed by histology or with more severe hepatic disease.73

Serum type IV collagen has also been examined in schistosomiasis patients. Levels of procollagen IV peptide did not correlate with the presence or intensity of infection, but they correlated significantly with liver fibrosis, splenomegaly, portal vein dilatation, and the presence of portosystemic collaterals.4,74 In another study, a positive correlation between type IV collagen and advanced schistosomiasis was noted, with a significant reduction being observed in serum levels following splenectomy, but there was no correlation with the grade of PPF as determined by US.79,77

3.4. Hyaluronic acid

Hyaluronic acid (HA), a high molecular weight GAG, is an essential ECM component. It is synthesized by HSCs and is degraded by the sinusoidal endothelial cells.76,77 Increased levels are due either to decreased hepatic removal or to increased production by stellate cells, or to both processes.80 Several studies have examined HA serum levels in a number of chronic liver
Table 1
Performance of serum markers in the evaluation of hepatic fibrosis due to schistosomiasis

<table>
<thead>
<tr>
<th>Serum marker</th>
<th>Species</th>
<th>Studies undertaken</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>S. mansoni</td>
<td>Correlation of levels of HA in patients with intense, moderate, light, and without fibrosis by US in 79 subjects</td>
<td>HA was able to separate individuals with fibrosis from those without, and light from intense fibrosis. The HA diagnostic accuracy for fibrosis was 0.89. With a cutoff level of 115.4 ng/mL sensitivity and specificity were 0.98 and 0.64, respectively</td>
<td>Marinho et al., 2010 (Brazil)⁶⁴</td>
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<td>A study of 61 patients with schistosomiasis mansoni and 16 healthy individuals</td>
<td>A serum HA level of 20.2 µg/l was observed that differentiated between patients with milder PPF (patterns C+D) and those with more severe PPF by US, with a sensitivity of 60% and a specificity of 65%</td>
<td>Silva et al., 2011 (Brazil)⁷⁷</td>
</tr>
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<td></td>
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<td>Correlation of HA level in hepatoplenic schistosomiasis and with mild to advanced fibrosis</td>
<td>Higher levels of HA were noted in more advanced forms of schistosomiasis</td>
<td>Ricard-Blum et al., 1999 (Madagascar)⁷²</td>
</tr>
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<td></td>
<td></td>
<td>Serum levels in advanced forms of the disease</td>
<td>Serum HA levels were increased</td>
<td>Pascal et al., 2000⁵⁵, and Eshombou et al., 2005 (Sudan)⁸⁰</td>
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<tr>
<td></td>
<td></td>
<td>Levels of HA in 153 patients in Senegal with schistosomiasis hepatic fibrosis</td>
<td>No changes in patient levels of HA. However 60% of patients showed early stages of hepatic involvement with US, while only 3% presented with HS disease</td>
<td>Burchard et al., 1998 (Senegal)⁹⁰</td>
</tr>
<tr>
<td></td>
<td>S. japonicum</td>
<td>HA levels in 38 cases with portal hypertension</td>
<td>Levels of HA are higher in those with portal hypertension than the normal range</td>
<td>Guanqin et al., 2002 (China)⁷⁷</td>
</tr>
<tr>
<td></td>
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<td>HA levels in 193 individuals exposed to endemic Schistosoma japonicum</td>
<td>HA levels correlated with US findings</td>
<td>Li, Sleigh et al., 2000 (China)⁹⁰</td>
</tr>
<tr>
<td></td>
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<td>Patients were identified with either mild (n = 30) or severe (n = 30) hepatic fibrosis due to S. japonicum infection</td>
<td>HA levels in normal, mild, and severe cases of hepatic fibrosis were 83.0 ± 35.7, 216.1 ± 77.9, and 212.6 ± 80.9 µg/ml, respectively. HA levels did correlate well with the degree of fibrosis.</td>
<td>Zheng et al., 2005 (China)⁹²</td>
</tr>
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<td>TIMPs</td>
<td>S. japonicum</td>
<td>611 S. japonicum-infected Filipinos were treated with praziquantel; FibroPlex analytes produced by peripheral blood mononuclear cells stimulated with schistosome egg antigen 4 weeks after praziquantel treatment were measured and these levels related to the risk of fibrosis 1 year after praziquantel treatment</td>
<td>Individuals with detectable TIMP-1 had a 3.5-fold greater risk of fibrosis 1 year after praziquantel treatment compared to individuals with undetectable levels (OR 3.48; 95% CI 1.41–8.43; P = 0.007)</td>
<td>Fabre et al., 2011 (Philippines)⁷⁹</td>
</tr>
<tr>
<td>Laminin</td>
<td>S. mansoni</td>
<td>Correlation of levels with portal hypertension</td>
<td>Laminin levels correlated with portal hypertension</td>
<td>Ricard-Blum et al., 1999 (Madagascar)⁷²</td>
</tr>
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<td>Field studies evaluating the levels of laminin in individuals with milder forms of schistosomiasis</td>
<td>Results did not reveal a correlation between laminin levels and milder forms of PPF</td>
<td>Kardorff et al., 1997, 1999 (Tanzania)⁷⁴,⁷⁵</td>
</tr>
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<td></td>
<td></td>
<td>Correlation of serum laminin levels with infection (egg-positive infected patients)</td>
<td>Serum laminin level was significantly higher in egg-positive infected patients than in endemic controls</td>
<td>Tanabe et al., 1989 (Brazil)⁹³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum levels of laminin in hepatoplenic and hepatointestinal patients</td>
<td>Serum levels of laminin were higher in hepatoplenic than in hepatointestinal patients and were also higher in hepatointestinal patients than the controls</td>
<td>Parise and Rosa, 1992 (Brazil)⁷⁵</td>
</tr>
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<td></td>
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<td>Laminin levels were determined in cases with initial stages of disease in hepatointestinal cases and in advanced disease</td>
<td>Progressive increase of laminin levels in those with initial to advanced cases of hepatointestinal schistosomiasis were observed</td>
<td>Wyszomirska et al., 2005 (Brazil)⁷⁶</td>
</tr>
<tr>
<td></td>
<td>S. japonicum</td>
<td>Laminin levels in 193 individuals exposed to endemic S. japonicum</td>
<td>Levels of laminin are correlated with re-infection</td>
<td>Sleigh et al., 2000 (China)¹²⁸</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>S. mansoni</td>
<td>Correlation of procollagen IV peptide levels and intensity of schistosomiasis, liver fibrosis, splenomegaly, portal vein dilatation, and the presence of portosystemic collaterals</td>
<td>Levels of procollagen IV peptide did not correlate with the presence or intensity of schistosomiasis infection, but were significantly correlated with liver fibrosis and signs of portal hypertension. Type IV collagen had a good specificity (over 90%) but poor sensitivity because more than half of those with severe liver involvement exhibited normal levels of type IV collagen</td>
<td>Kardorff et al., 1997, 1999 (Tanzania)⁷⁴,⁷⁵</td>
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<td>Correlation between type IV collagen and advanced forms of schistosomiasis mansoni</td>
<td>A positive correlation between type IV collagen and advanced forms of schistosomiasis mansoni. However, no correlation to the grade of PPF assessed by US examination as the gold standard method</td>
<td>Wyszomirska et al., 2005, 2006 (Brazil)⁷⁶</td>
</tr>
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<td></td>
<td>S. japonicum</td>
<td>Collagen IV levels in 193 individuals exposed to endemic S. japonicum</td>
<td>Levels of collagen IV correlated with re-infection</td>
<td>Sleigh et al., 2000 (China)¹²⁸</td>
</tr>
</tbody>
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Table 1 (Continued)

<table>
<thead>
<tr>
<th>Serum marker</th>
<th>Species</th>
<th>Studies undertaken</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procollagen type III</td>
<td>S. mansoni</td>
<td>Levels of PIIP in 82 individuals infected with S. mansoni without liver enlargement and 20 with hepatic or hepatosplenic disease</td>
<td>PIIP was elevated in patients with hepatic disease, but normal in uncomplicated cases</td>
<td>Zwingenberger et al., 1988 (Zaire)</td>
</tr>
<tr>
<td></td>
<td>S. mansoni</td>
<td>Correlation of levels of PIIP with the presence or intensity of infection or fibrosis scores</td>
<td>No significant correlation evident between PIIP levels and the presence or intensity of infection or fibrosis scores</td>
<td>Kardorf et al., 1997, 1999 (Tanzania)119; Tanabe et al., 1989 (Brazil)117; Burchard et al., 1998 (Senegal)118; Ricard-Blum et al., 1999 (Madagascar)119; Guanjin et al., 2002 (China)120; Zheng et al., 2005 (China)116</td>
</tr>
<tr>
<td>YKL-40</td>
<td>S. japonicum</td>
<td>PIIP levels in 38 cases with portal hypertension. Levels of YKL-40 were determined in 60 patients with mild, moderate, or severe hepatic fibrosis due to S. japonicum infection</td>
<td>Levels of PIIP were higher in those with portal hypertension than the normal range Levels of YKL-40 were 40.0 ± 10.4, 92.3 ± 18.5, and 172.1 ± 35.0 µg/ml, respectively for mild, moderate, and severe hepatic fibrosis. Serum levels of YKL-40 correlated with the stage of hepatic fibrosis</td>
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HA, hyaluronic acid; US, ultrasound; PPF, perportal fibrosis; HS, hepatosplenic; TIMP, tissue inhibitor of metalloproteinase; OR, odds ratio; CI, confidence interval; PIIP, aminoterminal procollagen III-peptide; PIINP, procollagen III amino terminal peptide.

diseases and have suggested that it may be a candidate marker for detecting fibrosis in cirrhosis, chronic hepatitis B and C, and alcoholic and non-alcoholic fatty liver diseases.76,81–84 Studies in patients with different degrees of fibrosis due to schistosomiasis mansoni have suggested a correlation between HA serum levels and disease severity. More severe cases of PPF had much higher levels of HA, while subjects with mild fibrosis had low HA levels.85–87 The evaluation of serum levels of HA and type IV collagen with respect to US patterns of PPF showed that only the former was capable of separating patients with mild fibrosis from those with intense fibrosis. Moreover, HA correlated positively with portal hypertension and PPF, and collateral circulation predicted HA increase.88 It is also noteworthy that, as with serum type I collagen, the high HA levels in patients with hepatic fibrosis were reduced following praziquantel treatment.72 In another study involving advanced schistosomiasis japonica patients and controls, serum HA and TIMP-1 levels were elevated in the former, with HA outperforming TIMP-1.38

3.5. Chitinase-3-like protein 1

Chitinase-3-like protein 1 (YKL-40) is a novel liver fibrosis marker. This recently described glycoprotein belongs to the chitinase family and is expressed in human liver and arthritic articular cartilage.89–91 Although its precise physiological function is not known, YKL-40 is thought to contribute to tissue remodeling or degradation of the ECM in liver fibrosis. In a study of patients with hepatitis C virus (HCV)-associated liver disease comparing type IV collagen, PIINP, HA, YKL-40, and biochemical parameters in the assessment of hepatic fibrosis, it was concluded that HA and YKL-40 were more useful than the other markers for assessing the fibrosis stage; in particular, YKL-40 was the most useful for monitoring the fibrosis of liver disease and for distinguishing extensive from the mild stage of liver fibrosis assessing the fibrosis stage.92 Additionally, YKL-40 was more sensitive than HA in measuring the degree of hepatic fibrosis due to schistosomiasis; the serum levels increased in patients infected with S. japonicum and correlated with the hepatic fibrosis stage.92

3.6. Matrix metalloproteinases and inhibitors

The matrix metalloproteinases (MMPs) and their inhibitors are involved in controlling matrix degradation. MMPs are enzymes that are produced intracellularly and secreted as proenzymes, which require cleavage by cell surface mechanisms for functional activity. The action of MMPs is counteracted by TIMPs.93 As such, they act to degrade ECM and to permit new matrix deposition. It has been suggested that the imbalance between MMPs and TIMPs affects the rate of fibrosis progression and that their levels correlate with the fibrosis stage, but the results have been variable and dependent upon the MMP/TIMP being assessed.94–96

The diagnostic accuracy of TIMP-1 and pro-MMP-2 (the free precursor molecule of MMP-2) and their relationships to histological inflammatory scores have been evaluated in patients with HCV infection; both assays performed either as well as or better than HA for the diagnosis of cirrhosis, but only TIMP-1 showed diagnostic value for the identification of patients with early stages of fibrosis. The levels of MMP-2 exhibited no relationship with the stage of fibrosis, thereby limiting its value for staging liver disease.95,97,98 Serum MMP-1 and MMP-3 levels have also been shown not to have any diagnostic value.94,96 On the other hand, one study has demonstrated that TIMP-1 can predict the risk of hepatic fibrosis in patients after 1 year of praziquantel treatment. This highlights the importance of identifying biomarkers for fibrosis risk because fibrosis can occur despite effective treatment.39

3.7. Laminin

Laminin is a major non-collagenous glycoprotein of basement membranes. Synthesized by HSCs, serum levels of laminin correlate with portal hypertension.72 Field studies evaluating individuals with milder forms of schistosomiasis did not, however, reveal a correlation between laminin and PPF or the presence or intensity of infection.72,74,75,100 However, some studies noted that the mean value of serum laminin was significantly higher in egg-positive schistosome-infected patients than in endemic controls.72,101 In another study, serum laminin levels were shown to be higher in hepatic schistosomiasis subjects than in hepatointestinal patients and were also higher in the latter compared with controls.102 A progressive increase in laminin has also been reported in the initial stages of hepatointestinal schistosomiasis and in advanced cases.76,103

3.8. Cytokines

Cytokines have been evaluated as biomarkers of hepatic fibrogenesis in a limited number of studies on schistosomiasis, with conflicting results. TGF-β is the dominant stimulus for producing ECM by HSCs. In a study of 88 patients who had chronic
hepatitis C, there was a correlation between total TGF-β1 and the degree of hepatic fibrosis. The role of cytokines in the development of hepatic fibrosis before the hepatoplenic and early hepatoplenic stages of schistosomiasis mansoni was evaluated in a group of patients with different degrees of hepatic fibrosis determined by US. Peripheral blood mononuclear cells (PBMCs) from schistosomiasis japonica patients were stimulated by S. japonicum antigens and the levels of IL-5, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β determined in the PBMC supernatants. Of significance, higher levels of IL-5, IL-10, and IL-13 were found in the supernatants of soluble egg antigen-stimulated PBMCs from subjects with stage III hepatic fibrosis compared to patients with stage I or II fibrosis. Significant increases in IL-5 and IL-13 levels were also observed in some of the subjects who remained untreated for 1 year following initial assessment and who developed more serious fibrosis during this period. In a study of hepatoplenic schistosomiasis mansoni, there was no significant difference in the mean serum concentrations of IL-10 and IL-13 between the different categories of hepatoplenic disease.

3.9. Circulating miRNAs

miRNAs comprise a family of conserved small non-coding RNAs (approximately 22 nucleotides) that can be detected in a wide range of body fluids, including blood plasma/serum. The high level of stability of miRNAs in biofluids has been attributed to two mechanisms: (1) formation of a protein–miRNA complex with argonaute proteins (mainly Ago2) and (2) incorporation into exosomes. miRNAs are being developed as novel biomarkers for various cancers and other diseases.

For example, serum levels of liver-specific miR-122 and miR-34a were suggested to be correlated with fibrosis, steatosis, and inflammatory activities in a study of chronic HCV infection and non-alcoholic fatty liver disease (NAFLD). Liver fibrosis and/or cirrhosis were also shown to be associated with increased serum levels of miR-571 and miR-513-3p and reduced serum levels of miR-29 and miR-652. A recent study in HCV-infected patients has suggested that serum miR-20a may serve as a predictive biomarker for HCV-mediated fibrosis. It is noteworthy that the serum exosomal miRNA expression profile was linked to grade and stages of liver fibrosis in patients with chronic HCV infection. The expression levels of two miRNAs (miR-483-5p and miR-671-5p) increased significantly and the expression levels of 14 miRNAs (let-7a, miR-106b, miR-1274a, miR-130b, miR-140-3p, miR-151-3p, miR-181a, miR-19b, miR-21, miR-24, miR-375, miR-548i, miR-93, and miR-941) were progressively reduced as liver fibrosis increased.

As schistosomal egg-induced immunopathology is an unusual type of chronic liver disease distinguishable from many other liver disease types, it is tempting to speculate that a unique set of circulating miRNAs may be defined to potentially serve as sensitive molecular signatures for assessment of the severity of the fibrotic pathology of schistosomiasis. To date, a set of miRNAs of parasite origin have been identified in both S. japonicum and S. mansoni and miRNA expression profiles at different developmental stages of S. japonicum have been characterized.

However, the abundance of schistosome-specific miRNAs in the plasma of the definitive mammalian host has been shown to be relatively low, which will likely limit its utility for the diagnosis and staging of schistosomiasis. Encouragingly, however, alterations of specific host miRNAs within different tissues have been shown to be associated with S. japonicum infection. The expression of several host miRNAs was shown to be altered rapidly in the lungs, liver, and spleen of BALB/c mice as early as 10 days post infection with S. japonicum. A broad array of miRNAs in liver was dysregulated and associated with the progression of S. japonicum infection. These results raise the possibility that circulating host miRNAs may be dynamically altered during the course of schistosome infection; this provides an avenue for their development as useful diagnostic and pathogenic biomarkers for schistosomiasis, because of the strong correlation between the expression profile of miRNAs and the status/progression of the disease.

4. Conclusions

Schistosomiasis can cause significant pathology and chronic morbidity in humans infected with numerous egg granulomas and this typically results in fibrosis in target organs. The intestinal form of the disease is caused by the deposition of eggs within the bowel wall, while the hepatoplenic form of the disease is due to the eggs trapped in the liver pre-sinusoids. In the former, the eggs cause bowel lesions ranging from colitis to polyp formation, whereas in the latter, granuloma formation and subsequent fibrosis involve immune responses initially mediated by Th1 and later by Th2 lymphocytes. Severe fibrosis leads to portal hypertension-related complications causing significant illness or death.

There are various methods to diagnose and evaluate schistosome-induced liver fibrosis. Liver biopsy is still considered the gold standard, but the procedure is clinically impractical in the field. Ultrasonography is invaluable in assessing pathology, but is not readily available in many endemic communities, and the results among users can vary widely. Although costly and limited to the hospital setting, CT and MRI show distinct imaging features associated with hepatoplenic schistosomiasis and aid in the diagnosis and clinical management of patients.

Attention has recently been given to non-invasive biological markers that can determine the severity of hepatic fibrosis and monitor quantitative and qualitative changes following treatment. The most promising serum markers for the evaluation of schistosome-induced hepatic fibrosis appear to be hyaluronic acid, collagen type III, YKL-40, and laminin. More studies are needed to evaluate the utility of matrix metalloproteinases and inhibitors, and cytokines. Circulating host miRNAs show promise as useful biomarkers for schistosomiasis diagnosis due to the strong correlation between the expression profile of miRNAs and the disease status/progression. Biological markers are presently limited to research investigations and may prove too costly for broader clinical application, but their potential role in the diagnosis of schistosomiasis warrants further investigation.

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