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Review article

Recent advances in the diagnosis of human schistosomiasis

Jones MK

Australian Centre for International and Tropical Health, Queensland Institute of Medical Research, Herston, Queensland, Australia, School of Veterinary Science, The University of Queensland, St Lucia, Queensland, Australia

Abstract

Schistosomiasis, caused by parasitic bloodflukes of the genus Schistosoma, is a major source of human disease in developing tropical nations. This paper reviews the diagnostic various assays for schistosomiasis, with particular reference to recent advances in assays incorporating direct parasitological examination, serology, antigen detection, and polymerase chain reaction detection of parasite genetic material.

Keywords: schistosomiasis; diagnosis; parasitology; serology

INTRODUCTION

A sensitive and specific assay for diagnosis of parasitic disease is a prerequisite for guiding treatment of affected individuals. Since recent schistosomiasis control strategies are directed towards mass treatments of populations in areas endemic for specific parasites[1], the need for accurate diagnostic tools has becomes more urgent, particularly in areas where parasite prevalence and infection intensities have diminished. This review will examine the effectiveness of the current means for diagnosis of schistosomiasis, both within endemic regions, and for travellers and immigrants entering non-endemic regions. In addition, attention will be drawn to recent advances in diagnostic assays.

Schistosomiasis is a chronic helminth disease of humans, occurring in 74 countries worldwide. The disease is linked with rural poverty, with most vulnerable populations including school aged children and women of reproductive ages[2]. Transmission of the parasite arises through poor sanitation[1,3]. Humans are infected through contact with contaminated water in endemic areas. Some 207 million people are infected with 5 primary species of schistosomes (*Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi* and *S. intercalatum*) in Africa, Middle East, South America, the Caribbean and southern Asia[3]. Approximately 750 million people are at risk of infection and recent assessment indicates that the disease burden is up to 15 times greater than previously estimated[4]. A recent analysis of disability weight due to schistosomiasis japonica in one region of China has raised concerns that global burden of schistosomiasis may still be underestimated[5].

Control strategies for schistosomiasis advocated by international agencies include evidence-based approaches, relying on estimates of parasitological burdens in specific communities. The control measures vary depending on schistosome prevalence in the populations. In areas of low endemicity prevalence [1], for example, affected individuals are targeted for drug treatments. In areas where populations experience moderate to high endemicity prevalence, mass treatments are employed. This single approach is not universally applicable [6,7], but they have been successful in reducing parasite burdens in some regions of high endemicity. Thus, a major consequence of mass treatment is that low burden infections become more common, and correspondingly, more difficult to detect. All diagnostic procedures for schistosomiasis have significant limitations in terms of specificity and sensitivity [8], and diagnostic problems increase as community parasite burdens decrease. The life-

Correspondence to: Malcolm k. Jones, PhD, Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Herston Qld 4029, Australia malcolm. jones@ qimr. edu. au
Tel: +61-7 33620406 Fax: +61-7 33620104

cycle of schistosomes, however, is extremely efficient, and the association between miracidium and snail host is ancient. Reduced infection intensities do not necessarily result in cessation of transmission. Indeed chemotherapy for schistosomiasis may only transiently reduce the infection risk to humans in some endemic areas[6].

The need for robust, reliable and cost-effective diagnostic assays clearly remains. What assays are available to us? Doenhoff *et al* [8] have summarised the methods as direct and indirect methods. Direct assays incorporate either morphological observation of parasite eggs in excreta or in tissues, the detection of parasite excretory/secretory molecules in the circulation or excreta, or the detection of specific anti-parasite antibodies in the circulation. Indirect methods of detection rely on self identification of symptoms by the patient through the use of questionnaires, or by symptomatic diagnosis by qualified practitioners.

SCHISTOSOME EGGS

Direct observation of schistosome eggs in excreta is diagnostic for all species of schistosomes. The parasite eggs are large, of large mass and thus sink in isotonic and moderately hypertonic media. The larvae hatch in hypotonic solutions, and this observation forms the basis of the miracidial hatching assay, a diagnostic procedure still employed in some studies, particularly for *S. japonicum* diagnostics[9]. Eggs of medically-important schistosomes have distinct morphology[10]. The primary species infecting humans, *S. mansoni*, *S. japonicum* and *S. haematobium*, can be readily distinguished from each other. Of the other two schistosomatids infecting humans, *S. mekongi* has an egg with similar morphology to that of *S. japonicum*, but can be distinguished on size and geographical history of the patient. Similarly, *S. intercalatum* eggs have a similar morphology to those of *S. haematobium*, but appear in the faeces, whereas those of the latter occur in the urine. The most commonly used morphological assay for schistosomiasis for *S. mansoni* and *S. japonicum* remains the Kato-Katz thick smear, while sedimentation of urine is used for *S. haematobium* diagnostics. While direct evidence of eggs in faeces or urine is diagnostic of an active infection, considerable difficulties arise because of inconsistencies in detection. Kato-Katz has poor diagnostic sensitivity, but remains a common procedure in field studies because of its low cost and cost-effectiveness[11], relative simplicity and ease of use. The Kato-Katz assay has a sensitivity cut-off of approximately 20 eggs per gram of faeces (epg)[8]. This low sensitivity is problematic in areas of low endemicity, such as endemic regions for schistosomiasis mansoni in Venezuela, where over 80% of infected individuals excrete fewer than 100 egg/g faeces per day[12]. Efficiency of the Kato-Katz method of diagnostics is 60% at 100 epg[13] indicating that the method underestimates prevalences in field situations. Accordingly, the examination of multiple smears for a patient is advocated as a means to address underestimation, although this adds to the cost of analysis. It has been recommended for *S. mansoni* infections that either 5 replicate slides, or sets of triplicate slides made from stools collected on 2 successive days, be used for assessment of infection status in field trials[14]. Testing of multiple smears, however, adds to the cost of the assay. Direct observation, however, allows for concurrent assessment and diagnosis of other parasites, particularly hookworms, that might be present in affected humans.

An alternative to the Kato-Katz method is the miracidial hatch assay. This method relies on the ability of schistosome miracidia to hatch upon exposure to freshwater and has been applied widely in surveys of *S. japonicum* infections in China[9], but is also applicable to other schistosomes as well[8]. A recent comparison of diagnostic techniques in China found that the hatching test was much less sensitive than the Kato-Katz thick smear method[9], although others have found greater concordance between the two assays [15]. Yu *et al*[9] attributed the lack of sensitivity to a number of factors including water quality and temperature. The authors noted further that the method was time-consuming and laborious. They argued that previous reports of high sensitivity of the hatching assay might reflect poor quality "gold standard" assays used for comparison.

An interesting modification of direct parasitological examination of schistosome eggs from faecal samples was described by Fagundes Texiera *et al*[6]. These authors found that paramagnetic beads were able to bind eggs of *S. mansoni* under the influence of an external magnetic field. The interaction between

eggs may arise from the inclusion of iron in the egg-shell matrix[16]. The increased sensitivity with the use of the beads was 100% at 1.4 epg, a major improvement over Kato-Katz thick smear analysis [16]. This magnetic bead assay will be more expensive than the use of the Kato-Katz alone and cost-benefit analysis may be necessary to determine whether this adjunct in diagnosis will be of value, particularly in many endemic nations [17].

Urine is used for morphological diagnosis of *Schistosoma haematobium* infections. Terminal urine of at least 10 ml is collected and allowed to sediment, or is filtered through a 12-20 μm nylon filter in a Luer-lock assembled filter. After filtration, the filter is placed on a slide, stained with Lugols´iodine and examined. Sensitivity of urine sedimentation was approximately 70% in one study in one study in Egypt (Talaat, 1996), cost benefit analysis suggesting that advanced methods of urine filtration did not improve the diagnostic sensitivity.

SCHISTOSOME ANTIGENS

The development of antigen detection methods for schistosomiasis has been reviewed extensively by Hamilton *et al*[18]. Although a number of antigens have been tested, most studies have been based on a group of proteoglycans, the so-called circulating anodic antigen (CAA), and the circulating cathodic antigen (CCD). The antigens gain their name from their migratory patterns in electrophoresis [18]. Both antigens contain a number of repeated glycan residues, and this feature allows for detection of *S. mansoni* and *S. haematobium*. Both CAA and CCA are excreted with urine, allowing for less invasive assays than serum analysis[4]. A variety of assays exist for the antigens, including, indirect hemagglutination, immuno-urometric assay, magnetic bead immunoassay, reagent strips and ELISA [8]. One of the benefits of detection of the antigens is that, since the antigens are released by living worms, the levels in serum correlate well with worm burden.

A major problem with detection of schistosome antigens is the lack of sensitivity. Doenhoff *et al*[8] indicated that the sensitivity of an CAA-ELISA assay was equivalent to direct parasitological observation. Similarly, commercially-available reagent strips using CCA have positive predictive value in regions of high endemicity[19,20], but are of less value when parasite burdens are low and are of limited value in detecting infection in a clinical setting[21]. Using CAA as antigen for detection, Cortsjens and colleagues[21], developed a lateral flow assay for schistosomiasis diagnosis. The detection system consists of a monoclonal serum raised against CAA coupled to up-converting phosphor reporters. These are sub-micron particles that emit in infra-red wavelengths upon excitation. The authors showed that signal intensity correlated with infection intensity in a series of selected and characterised cases. The system was far more sensitive than ELISA and more suitable for single case identification in laboratory diagnosis, suggesting that this technique may have excellent application in clinical settings. Current test strips for schistosome antigen detection cost approximately USD $2 per strip[19]. Such costs do not compare favourably with that of the much less expensive Kato-Katz thick smear. the lateral flow system appears more expensive again, thereby preduding the use of this method in field situation unless external funding sources meet the increased costs.

SEROLOGY ASSAYS

Patent schistosome infections are highly immunogenic because of the intense host responsiveness to entrapped schistosome eggs in host tissues. A range of antibody detection assays have been developed for schistosomiasis diagnosis. These assays include hypersensitivity reactions to intradermally-introduced antigen, complement-fixation tests (CFT), indirect fluorescent antibody tests (IFAT), indirect hemagglutination (IHA), radioimmunoassay, various flocculation tests, the circum-oval precipitin test (COPT), enzyme-linked immunosorbent assay (ELISA) and the Cercarienhuellen reaction (CH) [8,18]. Of these IHA and ELISA are most commonly used, with either soluble egg extract (SEA) or adult worm extract (AWA) as antigen[22].

As a means to enhance specificity of the reaction, a number of schistosome antigens, including microsomal extract, gut-associated polysaccharide, heat-shock protein 70, the CEF6 fraction and worm antigens Sm31 and Sm32 have been tested as diagnostic markers[8]. Wu *et al*[22] report some additional purifications of SEA, but none appear to provide an

advantage over SEA. The recent publication of the egg secretome of *Schistosoma mansoni* may reveal new targets for specific serology based on egg antigens [23]. Keyhole-limpet haemocyanin (KLH) has been advocated as an antigen for diagnosis, because of strong cross-reactivity with carbohydrate epitopes of schistosomes [24].

Serological tests for diagnosis of schistosomiasis are highly sensitive. Some authors note a lack of specificity and attribute this, in part, to the quality and host source of egg and adult worm antigen [19]. ELISA-SEA is a preferred assay for clinical diagnosis [8], although IHA has also been commonly used[22], often in field assessments of schistosomiasis burden. With ELISA diagnostics of *S. japonicum* infection, non-specific reactions due to false positives do occur, although cross-reactivity with other parasitic infections can be more problematic[22]. A colloidal dye immunofiltration assay for detection of *S. japonicum* infection has been developed recently, with encouraging results in areas of low transmission[25]. This visually-read test can be completed in a few minutes and is applicable in field situations.

A major issue for serological testing is that elevated antibody levels against schistosomes remain in hosts months, if not years, after (apparent) parasitological cure [8, 16, 22, 24]. This is of particular importance in the clinic, where serology can be of little value in detecting treatment failures[24]. Although serological assays remain the tool of choice for diagnosis, especially in the clinic, there is clearly a need for new antigen preparations[22]. In *S. mansoni* infection, antibody titre against CEF6 decreases after parasitological cure, while those of crude SEA do not, suggesting that the use of purified antigen has potential for progression as a serological marker of schistosomiasis.

MOLECULAR DIAGNOSTICS

PCR tests for schistosomes in stools use high-repeat nuclear genomic[26] or mitochondrial sequences [27]. Both tests report high sensitivity, at times exceeding that of direct coprological examination. The mitochondrial genomic probes, which amplify sequences spanning the *Cox*2/*nad*6 genes for *S. japonicum* and *S. mansoni*, *nad*1-2 for *S. japonicum* and *nad*5 for *S. mansoni*, show absolute specificity and a reported absence of false positive reactions [27]. A multiplex PCR diagnostic test for *S. haematobium* and *S. mansoni* infections has recently been reported[28]. This test amplifies sequences of the cytochrome C oxidase gene. In that study, the PCR threshold values correlated significantly with microscopic egg counts for both species. PCR amplification was also as specific as duplicate Kato-Katz smear analysis[28]. A limitation of molecular techniques remains the high costs of PCR reactions relative to other diagnostic methods. However, as ten Hove and colleagues [28] argue, instrumentation for molecular techniques is now more readily available in a variety of developing nations. If schistosome diagnostics could be run in combination with other molecular diagnostic assays, then the prospect for further development of this form of assay is more attractive.

FUTURE PROSPECTS

Diagnostic stringency in the clinic and the field differs. In the clinic, the focus is on individual patients, for whom there is a requirement for a highly specific and sensitive test that is sufficiently robust to perform in isolated cases. In the field, where the focus is on communities, sensitivity and specificity may have to be traded off against expediency and efficiency. In clinical situations, especially in developed nations where patients are primarily returned travellers, the recent advances in a number of techniques associated with direct parasitological identification of parasites, together with new evidence on parasite antigens gained from proteomic analyses, suggest that there are excellent prospects for improvements and standardization of diagnostic assay. What is needed is a highly sensitive assay that can distinguish active from past infection.

For field studies or clinical parasitology in endemic countries, a major limitation on deployment of these recent advances remains cost of the assay [12]. The principle of "ASSURED" diagnostics (diagnostics that are "affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users") [29] remains a major requirement in these countries. In the absence of support from external agencies, it may be that less sensitive methods, such as direct parasitological examination of faeces or urine, fulfil the "ASSURED" principle.

This remains particularly the case where soil-transmitted and food-borne helminth infections are co-endemic with schistosomiasis.

It is clear that as conditions of low schistosomiasis transmission increase in endemic regions, the need for assays with higher sensitivities will increase. At the present time, serology, despite its limitations represents an excellent suite of assays. Most likely, a uniform "gold-standard" assay that can meet all requirements for clinical diagnosis and surveillance, will not be available in the new future. A combination of assays, however, and in particular those incorporating the recent technological advances reviewed here, may go a long way to meet the ASSURED standards. These advances require further field testing. In addition, it will be necessary to undertake detailed cost-benefit analysis, such as has been performed by Carabin and colleagues[11] for African schistosomiasis, to weigh their benefits against improved disease surveillance.

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