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Long-term effect of mass chemotherapy of *Schistosoma mansoni* on infection rate and diagnosis accuracy

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SUMMARY

Objectives: To assess the performance of microscopic stool examination, which is used widely for the diagnosis and assessment of infection rates of *Schistosoma mansoni* in Egypt, for the evaluation of chemotherapy efficacy after a decade of regular mass treatment.
Methods: A total of 651 individuals from Lower Egypt (55 children and 596 adults) were examined for *S. mansoni* ova by microscopic stool examination (MSE) alone (n = 166; 111 adults and 55 children), rectal biopsy (RB) alone (n = 32 adults), or both MSE and RB (n = 453 adults).
Results: Infection detection rates were significantly lower in the MSE alone group (9%; 15/166) compared to the RB alone group (40.6%; 13/32) and to the RB+MSE group (37.7%; 171/453). Out of all positive cases in the MSE+RB group, only 23/171 patients (13.5%) were positive by stool examination, of whom 21 were also positive by RB, in contrast to 169/171 patients (86.5%) positive by RB in the same group. It was noted that adding MSE to RB did not increase the prevalence compared to RB alone: 37.3% in the MSE+RB group vs. 40.6% in the RB only group. Using the summation of both MSE and RB tests as the gold standard, the sensitivity of MSE was significantly lower than that of RB: 13.5% vs. 98.8%.
Conclusions: The implementation of mass treatment programmes has resulted in a new era of light infection, for which conventional parasitological methods for the diagnosis and monitoring of infection can miss many patients.

1. Introduction

Schistosomiasis, also known as bilharziasis, has been and remains one of the most prevalent parasitic diseases worldwide. It affects millions of people in the developing world, mainly in Africa, and has substantial public health and economic impacts.[1] Egypt is one of the major countries affected by schistosomiasis. It has been an area endemic for schistosomiasis since at least the Middle Kingdom period (1500 BC).[2] Early in the 20th century, between 1932 and 1934, Scott performed a wide-ranging survey of two million individuals in government treatment centres and a house-to-house survey of 40 000 subjects throughout rural areas in Egypt. He found the prevalence of infection of both *Schistosoma haematobium* and *Schistosoma mansoni* to be equally about 60% in the rural population residing north and east of the Nile Delta.[3]

About 15 years ago, the Egyptian Ministry of Health and the United States Agency for International Development (USAID) performed a huge schistosomiasis research project investigating the prevalence and intensity of infection of *Schistosoma* species.[4] As part of the study, they collected stool samples from governorates in Lower Egypt (Kafr El-Sheikh, El-Gharbia, El-Menoufia, El-Qalubia, and El-Ismailia) almost exclusively endemic for *S. mansoni*. The total screened population was 49 950. Quantitative microscopic counting of Schistosoma ova in the stool using the modified Kato technique was performed to diagnose these patients. Results showed that the prevalence ranged from 17.5% to 42.9%, with an average of 36.4%. Males had higher infection rates and ova counts than females in all age groups.

In 2001, the World Health Assembly adopted Resolution 54.19, which set a global target for all endemic countries for the year 2010. The World Health Organization (WHO) strategy for schistosomiasis control was based on regular mass treatment

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with praziquantel. The rationale came from the availability of safe and effective drugs, which made it feasible to implement large-scale preventive chemotherapy. Many countries benefited from the WHO treatment programme, including Brazil, Burkina Faso, Cambodia, China, Mauritius, Morocco, Oman, and Saudi Arabia. As for many other countries, the WHO strategy was endorsed by the Egyptian Ministry of Health. It authorized the distribution of praziquantel to high-risk individuals even prior to laboratory diagnosis. Praziquantel became available free of charge in almost all primary care units, including general practice centres, and at a cheap price even over the counter. The media has played a major role in population awareness about this treatment.

The outcome appeared excellent. In El-Beheira Governorate, one of the most endemic areas in the Nile Delta, the prevalence of schistosomiasis was reduced from 26% in 1996 to 3.8% in 2003.[5] In 2003, the mass distribution of praziquantel targeting mainly school children was continued and achieved a further reduction in prevalence to 1.9% in 2007.[6]

More recently, however, the long-term benefits of this strategy have been the subject of debate. Estimates of the prevalence of schistosomiasis have relied on the use of well-established, but imperfect, diagnostic tests.[7] The main method used to diagnose these patients has been simple microscopic stool examination (MSE), which has several drawbacks in the process of diagnosing *S. mansoni* due to the daily variations in egg excretion levels and the intensity of infection. MSE has been shown to have low sensitivity in cases with light infection.[6]

The use of diagnostic assays with a low sensitivity and/or specificity can be misleading in the evaluation of schistosomiasis control programmes, such as those aimed at morbidity reduction through mass human chemotherapy. This study was designed to assess the performance of parasitological microscopy, which is used widely for the diagnosis of active infection and infection rates in Egypt, after a decade of regular mass chemotherapy of schistosomiasis.

2. Subjects and methods

This study was conducted and coordinated by the National Liver Institute, Menoufia University, Egypt. The National Liver Institute is located in Menoufia Governorate, in the middle of the Nile Delta region of Egypt, which is a well-known endemic area for *S. mansoni* infection. Individuals were recruited from nine villages of three governorates in the Nile Delta region: Menoufia, El-Gharbia, and El-Beheira. All children included in the study were recruited from Al-Raheb village primary school, Menoufia Governorate, through a collaborative work with the Al-Raheb General Practice Medical Unit, Egyptian Ministry of Health.

Individuals from rural areas with a history of continuous canal water contact suggesting a risk of infection were included. However, none of them was clinically symptomatic. All individuals were screened for *S. mansoni*. They were divided into groups according to age and the method of diagnosis. Adults were classified into three groups: group 1 had MSE only, group 2 had a rectal biopsy (RB) only, and group 3 had both MSE and RB. All children were allocated to group 4 and underwent MSE only (Table 1).

Full history-taking and a complete clinical examination were performed by a consultant and/or hepatology registrar to exclude any manifestation of hepatic decompensation, defined as clinical evidence and/or a history of bleeding oesophageal varices, hepatic encephalopathy, jaundice, prolonged prothrombin time, or ascites.

2.1. Diagnosis of Schistosoma mansoni

Two different procedures were used to diagnose active infection: (1) simple microscopic stool examination, and (2) rectal biopsy. Some patients had only one procedure, while others had both (Table 1). The RB technique was not used for children. Praziquantel therapy was offered to all Schistosoma-infected cases.

Table 1
Prevalence of *Schistosoma mansoni* in all patients' groups

	Test performed	No. of patients (F/M)	Positive cases (F/M)
Adults	Group 1: MSE only	111 (43/68)	13 (2/11)
	Group 2: RB only	32 (9/23)	13 (4/9)
	Group 3: RB + MSE	453 (106/347)	171 (23/148) Stool positive = 23 (9/14) Rectal biopsy positive = 169 (23/146)
Children	Group 4: MSE only	55 (27/28)	2 (0/2)
Total		651 (185/466)	199 (29/170)

F, female; M, male; MSE, microscopic stool examination; RB, rectal biopsy.

2.2. Microscopic stool examination (MSE)

The conventional method was employed for the examination of stool samples: simple stool sedimentation by centrifugation followed by detection under a microscope with a ×10 objective lens; any entity seen was then further confirmed with a ×40 objective lens. The test was considered positive if ova were present in at least one of the two slides prepared.

2.3. Rectal biopsy (RB)

Rectal biopsies were performed in the endoscopy unit of the National Liver Institute. Subjects from El-Gharbia governorate had their RB performed in the Al-Mahalla City Charitable Medical Centre for convenience. For this procedure, the patient was positioned in the left lateral position with extension of the left knee and flexion of the right knee. A proctoscope with a light source was inserted gently with lubricant into the rectum. Three to five fragments of superficial tissue were obtained with biopsy forceps. Biopsies were taken from sites where small haemorrhages or other suspicious lesions were seen. These tissue specimens were placed between two glass slides and examined immediately under light microscopy. All rectal biopsies were performed and analyzed by trained clinical research fellows of the National Liver Institute, Menoufia University, Egypt.

2.4. Sample size and power calculation

Prior data indicated that the infection rate among individuals in the Nile Delta region, as determined using MSE, is 1.9%.[6] If the true infection rate of the same subjects increased to 3% by implementing RB, 510 individuals would be required for screening to be able to reject the null hypothesis that the infection rates for MSE and RB subjects are equal. Analyses determined the minimum effect size that may be detected with 90% power and type I error probability of 0.05 using Fisher's exact test to evaluate the null hypothesis. Six hundred and fifty-one subjects were recruited to allow for occasional individual preference to have a single diagnostic test instead of having both tests and to confer a safe margin for dropouts.

The study was approved by the Ethics Committee of the National Liver Institute and Menoufia University according to the Declaration of Helsinki. Informed consent was obtained from all adults recruited and from the guardians of all children enrolled.

3. Results

The study recruited individuals from different villages of three governorates in the Nile Delta region of Lower Egypt. Fifty-five

children and 596 adults (total 651 subjects; 185 female and 466 male) with a clinically suspected infection were screened using MSE, RB, or both. Adult subjects ranged in age from 18 to 61 years (mean 28.9 years), and 71.6% were male; the children ranged in age from 7 to 9 years, and 51% were male.

The *S. mansoni* infection rate changed according to the method of diagnosis. When conventional MSE was used alone in group 1 (adults) and group 4 (children), the infection rate was 11.7% (13/111) and 3.6% (2/55), respectively. This increased substantially with the use of RB alone for diagnosis (group 2): 40.6% (13/32) (Table 1).

In group 3, each individual had both MSE and RB. The overall positivity was 37.7% (171/453). Only 23 patients (23/453; 5.1%) were positive by MSE, while 169/453 (37.3%) were positive by RB (148 with RB only positive plus 21 patients with both MSE and RB positive) (Figure 1). Of note, adding MSE to RB (group 3) did not significantly increase the prevalence compared to the use of RB alone (group 2): 37.3% vs. 40.6%, respectively.

Table 2 shows the significant statistical difference in prevalence in group 3 according to the method of diagnosis. As expected, the overall prevalence in females was lower than that in males (21.7% vs. 42.7%).

To statistically translate these results, survey tables were plotted to assess the sensitivity of MSE and RB. The sum of positive cases for both MSE and RB was used as the gold standard assuming a specificity of both tests of 100%. The results showed MSE to have a sensitivity of 13.5%, a negative predictive value of 65.6%, and an accuracy of 67.3%. In contrast, RB was found to have a sensitivity of 98.8%, negative predictive value of 99.3%, and accuracy of 99.5% (Tables 3 and 4).

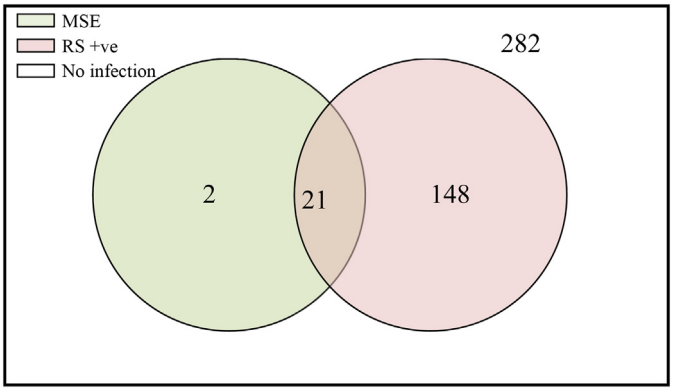

Figure 1. Number of positive cases by each test for subjects in group 3. The overall *Schistosoma mansoni* positivity was 37.7% (171/453), out of which only 23 patients (5%) were positive by stool examination. Of 169 (37.3%) patients with a positive rectal biopsy, 148 were positive only by rectal biopsy and 21 patients had both stool examination and rectal biopsy positive. Overall negativity was 62.3% (282/453).

Table 2
Sex-specific prevalence of *Schistosoma mansoni* by rectal biopsies and stool examination in group 3

Sex	No. examined	Number (%) positive for *Schistosoma mansoni*				*p*-Value
		Both stool and biopsies positive	Stool only positive	Rectal biopsies only positive	Total positive	
Male	347	12 (3.5%)	2 (0.6%)	134 (38.6%)	148 (42.7%)	<0.01
Female	106	9 (8.5%)	0 (0%)	14 (13.2%)	23 (21.7%)	<0.01
Total	453	21 (4.6%)	2 (0.4%)	148 (32.7%)	171 (37.7%)	

Table 3
Group 3 screening test results: the relationship between microscopic stool examination and the gold standard[a]

	Gold standard test: RB+MSE positive		
	Positive	Negative	Total
MSE			
Positive	23	0	23
Negative	148	282	430
Total	171	282	453

MSE, microscopic stool examination; RB, rectal biopsy.
[a] The summation of rectal biopsy and stool examination was used as the gold standard to determine the sensitivity and specificity of microscopic stool examination: sensitivity = 23/(23 + 148) = 13.5%; specificity = 282/(282 + 0) = 100%; positive predictive value = 23/23 = 100.0%; negative predictive value = 282/430 = 65.6%; accuracy = (23 + 282)/453 = 67.3%.

Table 4
Group 3 screening test results: the relationship between rectal biopsy and the gold standard[a]

	Gold standard test: RB+MSE positive		
	Positive	Negative	Total
RB			
Positive	169	0	169
Negative	2	282	284
Total	171	282	453

MSE, microscopic stool examination; RB, rectal biopsy.
[a] The summation of rectal biopsy and stool examination was used as the gold standard to determine the sensitivity and specificity of rectal biopsy: sensitivity = 169/(169 + 2) = 98.8%; specificity = 282/(282 + 0) = 100%; positive predictive value = 169/169= 100.0%; negative predictive value = 282/284= 99.3%; accuracy = (169 + 282)/453= 99.5%.

4. Discussion

These data suggest that the true prevalence in endemic communities following mass treatment programmes may be considerably underestimated. The conventional MSE method failed to diagnose 86.5% (148/171) of positive cases. This apparently striking result indicates that the use of simple microscopic examination for the monitoring of infection is ineffective because of the low sensitivity.

From the present results, it is believed that the mass treatment strategy implemented by the WHO more than 10 years ago has led to a new era of light Schistosoma infection for which simple MSE can miss many cases. This would explain the significant contradiction in infection rates according to the method of diagnosis in the present study. It would also clarify the high prevalence found in the present study in which RB was implemented, in comparison to the lower prevalence reported by other studies in which various MSE methods were applied.[8,9] To the authors' knowledge, this study is the first to provide an evaluation of the performance of the routinely used MSE and an estimation of the prevalence of infection in Egypt following the mass treatment 'light infection era'. It also highlights the importance and need for reliable and practical tests for the diagnosis of *S. mansoni* to overcome the diagnostic dilemma expected from ongoing mass treatment strategies.

Although the current data relate mainly to adults, MSE was also evaluated in children aged 7–9 years, showing comparable results to those obtained in adults using the same method of diagnosis (Table 1). A similar underestimation of infection rates in school-aged children as in adults is expected. Doctors and healthcare workers should be aware that a negative stool examination does not automatically mean that the patient is free of the disease.

The value of the current study originates from the importance of diagnosing patients with light infection. A light infection may have substantial public health and economic impacts. It has been assumed that a reduction in infection intensity has remained the main objective of schistosomiasis control and morbidities.[10] Schistosomiasis is a significant chronic inflammatory disease that contributes substantially to chronic morbidity whether of light or heavy intensity.[11] The relationship between light infection and morbidity and/or mortality is not clear. The well-known infection-associated advanced morbidities that occur late in infection, i.e. liver fibrosis, intestinal bleeding, urinary tract obstruction, superinfection, and cancer, seem to represent only a small fraction of Schistosoma infection-associated disease. Chronic complications, such as anaemia, under-nutrition, short stature or underweight, abdominal and pelvic pain, and possibly infertility, may place a significant health burden on affected individuals and populations.[12] Evidence suggests that the patient's immune response to the parasite and/or eggs indicates tissue inflammation in schistosomiasis.[13,14] This means that individuals who have a light infection are still at significant risk of schistosomiasis-associated disease. Furthermore, it is known that children who are exposed to their mother's helminth infections in utero are born with anti-parasite immune responses that polarize to either a hyperactive or significantly suppressed (i.e., hyperimmune or tolerized) status. An important consequence of this skewing of the immune response is that children with highly active anti-helminth (Th2-type) immune responses are less responsive to routine childhood vaccination against pathogens that require Th1 immunity, such as tuberculosis.[12]

The benefits of mass treatment are still questionable; the results of the present study and others show that the benefits of these programmes as a definitive solution remain in doubt. These programmes are unable to break the transmission cycle of Schistosoma.[9] Recurring low-level re-infection is likely to be associated with subtle but persistent morbidity. Furthermore, broad-scale chemotherapy alone will not reliably affect local levels of parasite transmission and the consequent risk of re-infection with Schistosoma.[15,16] The cessation of drug treatment for even a few years could result in the recurrence of high levels of schistosome infection.[17,18]

The results of this study demonstrate that in adults, RB for the detection of parasite eggs in tissue is the best currently available diagnostic tool for active infection. Nevertheless, caution should be applied. The inclusion of RB examination in the process of diagnosing and monitoring human mass chemotherapy has limitations. As a relatively invasive procedure, the lack of financial resources and need for well-trained medical staff will not easily allow this option to replace the simple and quick MSE. The relatively high operational costs compared with MSE make it an unsuitable alternative test. Another disadvantage of RB is that the intensity of the infection cannot be quantified. Furthermore, RB requires relatively sophisticated equipment. In areas of high endemicity, personnel with only basic training are available. More importantly, establishing a RB unit would also require the establishment of a well-standardized sterilization unit, otherwise RB could become another source of serious blood-borne infection, particularly hepatitis C and B, which are highly prevalent in the region. In areas with a general lack of medical services, having a RB may not be practical. The authors believe that a simple, cheap, sensitive and specific assay for the routine diagnosis of schistosome infection is not yet available and is seriously needed.

In conclusion, current thinking on schistosomiasis should change. The level of infection is actually higher than originally thought. The implementation of mass treatment programmes in the last decade has resulted in a new era of light infection, for which conventional parasitological methods for the diagnosis and monitoring of infection are not effective. People who are stool-negative for parasites cannot be assumed to be non-infected. Improved diagnostic agents and therapeutic strategies should be the main vital topic for further applied research on schistosomiasis.

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