Diagnostic Efficacy of Indirect Hemagglutination Test in Relation to Kato Method for Diagnosis of Schistosomiasis Mansoni

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ABSTRACT

BACKGROUND: Sensitive and specific diagnostic tools are very central to decide on individual case management and at all stages of control programs in schistosomiasis. This study was undertaken to assess the diagnostic efficacy of a commercial indirect hemagglutination test in detection of schistosomiasis mansoni in relation to the Kato method.

METHODS: In a cross sectional study undertaken in May 2004, blood and stool samples were collected from 134 children in Bochesa Elementary School around Ziway town. The stool specimens were processed using Kato method and examined microscopically. Eggs per gram of stool were computed as geometric mean. Sera were separated and transported to laboratory. Indirect hemagglutination test was performed following procedures given by the manufacturers. The diagnostic performance of indirect hemagglutination was evaluated using Kato as the gold standard.

RESULTS: The overall prevalence of intestinal schistosomiasis as detected by indirect hemagglutination test and Kato method was 74.6% and % 76.1%, respectively (p = 0.284). The respective prevalence of infection as detected by indirect hemagglutination tests and Kato method among male students was 82.6% and 85.0 % where as the corresponding value for females was 60.4% for both indirect hemagglutination test and Kato method. The sensitivity and specificity of the indirect hemagglutination test were 83% and 53%, the positive predictive value being 85%. However, there was discrepancy between indirect hemagglutination test and Kato method in determining the intensity of infection. Only 5% of the examined individuals had light infection with a cut off titer of ≤1: 256 as detected by IHA test while 95% of the cases had heavy infection with a cut off titer of ≥ 1: 512. On the other hand, the Kato method revealed that 18.5% of the children was heavily infected (≥400 epg) while 81.5 % of them had light to moderate infection (≤399 epg).

CONCLUSIONS: The present findings indicate that indirect hemagglutination test can be used as an adjunct to the Kato method for field use in the diagnosis of intestinal schistosomiasis. Nevertheless, the fact that the test is expensive and also not a rapid test may limit its use in the field for epidemiological application.

KEY WORDS: Schistosomiasis mansoni, diagnosis, indirect hemagglutination test, Ethiopia

INTRODUCTION

Schistosomiasis represents a serious public health and socioeconomic problems in tropical countries of the world including Ethiopia. Although the mortality due to the disease is low, usually less than 1%, the chronic nature of the disease and substantial morbidity that occurs in the productive age group causes incapacity to work in farmers, laborers and fishermen thereby seriously affecting socioeconomic development of the disadvantaged developing country (1).

Chemotherapy has been spearheading schistosomiasis control programs since the advent of safe and effective drug (praziquantel) in the 1980s (2). Along with safe and effective drugs, the availability of cheap and reliable diagnostic tools is crucial for detection of infected cases and evaluation of successful control measures (3).
The standard method for the diagnosis of schistosomiasis is usually by microscopic demonstration of schistosome eggs in excreta, but this is a laborious task particularly when used for community diagnosis and is also aesthetically unpleasant. Furthermore, the high day-to-day fluctuation in egg excretion necessitates examining stool samples on consecutive days (4 - 6). Attempts have been made to adopt a wide variety of methods for antibody detection (7).

Since diagnosis of human schistosomiasis is very central to individual case management and control programs, continuation of research for development of new diagnostic procedure or validation of the developed procedure is very crucial. Hence, the aim of the present study was to assess the diagnostic efficacy of a commercial (indirect hemagglutination) IHA test for the detection of schistosomiasis mansoni relative to the Kato method.

**MATERIALS AND METHOD**

The study was carried out in Bochesa village near Ziway town, located about 160 km south of Addis Ababa, in May 2004. Systematic random sampling was used to select 134 children (86 males and 48 females, 7 to 22 years of age, mean 12.4 year) from a total of 423 school children in Bochesa Elementary School.

To obtain stool specimens, small plastic sheets were distributed to the selected children who were then informed to bring about 3 gm samples of their own stool. During stool collection, names, sex and ages of each study participant were recorded. After carefully labeling the slides for each study participant, the specimens were processed using single Kato slide employing a template delivering a plug of 41.7mg of stool as previously described (14). The specimens were qualitatively examined on the spot for helminths ova by two experienced laboratory technicians and children who were found positive for schistosome infection were treated with praziquantel while those were positive for soil-transmitted helminths were treated with albendazole. Quantitative egg count was made later at the Aklilu Lemma Institute of Pathobiology, Addis Ababa University, within a week after specimen collection. For positive subjects excreting *Schistosoma mansoni* ova, egg count per slide was transformed into eggs per gram (EPG) of stool and intensity of infection was expressed as a geometric mean.

In parallel with stool collection, two ml of venous blood was drawn aseptically from the study subjects by certified technicians using disposable syringes. The blood was allowed to clot, centrifuged, and sera separated and kept frozen at -20°C in labeled cryotubes until assay.

A commercial IHA kit (Cellognost Schistosomiasis H) manufactured by Dade Behring Marburg GmbH, Germany, was purchased and the test was performed following procedures given by the manufacturer.

The level of agreement between the IHA test and the Kato method was calculated using kappa test following standard epidemiological procedure (15).

Ethical issues were strictly handled according to the International Ethical Guidelines for Biomedical Research. Prior to stool and blood collection the purpose of the study was explained to the community leaders, school teachers and the study participants/school children. Then, about 2 ml of venous blood was drawn from each student aseptically by qualified technicians using disposable syringe. A physician treated all children found positive for intestinal schistosomiasis with praziquantel in a single dose at 40 mg/kg body weight and for other intestinal helminthiasis with albendazole.

**RESULTS**

The prevalence of *Schistosoma mansoni* infection as determined by IHA test and single Kato method was 74.6% and 76.1%, respectively. The difference between the two diagnostic techniques in detecting *S. mansoni* infection was not statistically significant (p= 0.284). Statistical analysis also showed that there was a fair agreement (kappa = 36%) between the two diagnostic techniques (Table 1). The sensitivity and specificity of the IHA test taking Kato as the gold standard were 83% and 53%, respectively, whereas the positive predictive value was 85%.

Fig. 1 presents the prevalence of *Schistosoma mansoni* infection by age as determined by IHA test and Kato method. The two methods clearly demonstrated high schistosomiasis infection peak in the age group 11 to 14 and decline thereafter.

<table>
<thead>
<tr>
<th>Method/Test</th>
<th>IHA test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Kato method</td>
<td>85</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
</tr>
</tbody>
</table>

*Kappa = 0.36*
The prevalence of *Schistosoma mansoni* infection among male children was 82.6% and 85% by IHA and Kato methods, respectively, while the corresponding value for females was 60.4% for both IHA test and Kato method. There was a fair agreement (kappa = 0.39) between IHA test and Kato method in determining the prevalence of schistosomiasis among females but the agreement was weak for males (kappa = 0.23).

No relationship was observed between egg output determined by Kato method and serum antibody levels determined by IHA test at light (1 – 99 epg), moderate (100 – 399 epg), and heavy (≥ 400 epg) classes of intensities. Only 5% of the examined individuals were found with light infection with a cutoff titer of 1: 256 or lower by IHA. About 95% of the cases were heavily infected with a cutoff titer of ≥ 1: 512. The Kato method revealed that 18.5% of the population had heavy infection (≥ 400 epg), 81.5% of them were with light to moderate infection (≤399 epg).

<table>
<thead>
<tr>
<th>Method/Test</th>
<th>Male</th>
<th>IHA test</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kato method</td>
<td>Positive</td>
<td>63</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td><em>Kappa</em></td>
<td>0.23</td>
<td></td>
<td>0.39</td>
</tr>
</tbody>
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**DISCUSSION**

Indirect Hemagglutination (IHA) tests using adult worm antigens were previously evaluated for diagnosis of schistosomiasis by few investigators (16, 17). With a few exceptions such as the ones used by van Gool et al. (18), most of the previously used IHA test kits were not commercially available for use in epidemiologic application. These previous works have reported sensitivity ranging from 71 to 100% and specificity ranging from 80 to 100%. In the present study, the sensitivity of IHA test for the diagnosis of schistosomiasis mansoni was found to be 83% and this fall within the range previously reported while the specificity was lower (53%) as compared with the previous reports (18).

The prevalence of *Schistosoma mansoni* infection as detected by IHA test was 74.6% while the
corresponding prevalence detected by a single Kato smear was 76.1%. Nevertheless, the agreement between the two methods was not strong (kappa = 0.36). There was also no relationship between the number of eggs per gram of feces as detected by Kato method and the level of serum antibody as detected by IHA test. According to the manufacturer protocol, a cut off titer of $\leq 1: 256$ suggest weak titer while a cut off titer of $\geq 1: 512$ suggests strong titer. Accordingly, only 5% of the examined individuals had light infection with a cut off titer of $\leq 1: 256$ and 95% of the cases had heavy infection with a cut off titer of $\geq 1: 512$. On the other hand, the Kato method revealed that 18.5% of the children was heavily infected ($\geq 400$ epg) while 81.5 % of them were with light to moderate infection ($\leq 399$ epg). This discrepancy might be attributable to several factors. In prepatent and chronic infection, antibody detection may be preferable to parasitological Kato method but in our study this cannot explain the disparity because the two methods were comparable in detecting the infection. The other possible explanation may be that the classification of light, moderate and heavy infection by the Kato method as well as weak and strong titer by the IHA test is subjective and may cause problem to compare classes of intensity using the two methods.

Diagnosis of human schistosomiasis is very central to decision on individual case management, at all stages of control programs and for comparing control programs (13, 19). Although the gold standard to diagnose intestinal schistosome infection is by detection of eggs in faeces, failure to recover eggs does not rule out the possibility of infection. Hence, a diagnostic tool should be sensitive enough to detect all infected individuals irrespective of their infection status. In early or chronic infection as well as in lightly infected individuals only passing few or no eggs in their stool, the parasitological method may or may not always detect eggs in feces. The low specificity of the IHA test observed in this study may necessitate the need for comparative studies of commercially available IHA tests and antigens prepared from local parasites for diagnosis to see if there are variations in specificity between different parasite strains. It is also suggestive to assess the degree of cross-reactions with other helminth parasites in schistosomiasis non-endemic areas whenever such assays are carried out. The criteria that should be considered important in a diagnostic test include a high level of sensitivity and specificity, an indication of the intensity of infection, the ability of a test to differentiate between past and present infections, the expense, the time taken for the test, the potential use of untrained personnel, the reproducibility of the test, and the simplicity and social acceptability of sample collection (20). In the light of these criteria, the sensitivity of IHA test is relatively high and it is also not demanding in terms of equipment. Other criteria such as high level of specificity, quantification of intensity of infection, the cost and the ability to distinguish between past and present infections may limit its large scale application for epidemiological use. Nevertheless, IHA test can still be used as an adjunct to the Kato method for the diagnosis of intestinal schistosomiasis.

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