Evaluating the Performance of Diagnostic Methods for *Schistosoma mansoni* Against the “Gold” Standard in Amhara National Regional State, Northwest Ethiopia, 2020

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Abstract: Background: *Schistosoma mansoni* is a parasitic worm that can infect humans throughout the world. It is more prevalent in Africa including Ethiopia. Proper detection of *Schistosoma mansoni* using sensitive diagnostic methods is crucial in the national *Schistosoma mansoni* prevention and control strategies. However, direct wet mount microscopy with its low sensitivity has been used as a diagnostic technique in Ethiopia. Alternative diagnostic methods are not yet implemented. Therefore, the aim of this study was to evaluate the performance of diagnostic methods for *Schistosoma mansoni* against the “Gold” standard in Amhara region, Ethiopia. METHODS: A cross-sectional study among 520 school children was conducted from October to December, 2019 in Amhara region. The study participants were selected by using systematic random sampling technique. Stool samples were processed via formol ether concentration, Kato-Katz and spontaneous tube sedimentation techniques. Data was entered into Epi-data version 3.1 and analysis was done using SPSS version 20.0. The sensitivity, specificity, positive predictive value and negative predictive value were calculated against the combined result as “Gold” standard. Strength of agreement of the diagnostic methods was determined by Kappa value. Results: The Overall prevalence of *Schistosoma mansoni* was 20.2% using a combination of three methods. The prevalence 8.3%, 12.9%, and 16.3%, respectively was recorded by using formol ether concentration, Kato-Katz and spontaneous tube sedimentation. The spontaneous tube sedimentation method (81.0%) had better sensitivity as compared to Kato-Katz (63.8%) and formol ether concentration (41.0%) methods in *Schistosoma mansoni* detection. Conclusion: Spontaneous tube sedimentation was the best method as compared to the other methods to detect *Schistosoma mansoni* infections. In addition, Kato-Katz method was more sensitive than formol ether concentration method in *Schistosoma mansoni* detection.

Keywords: *Schistosoma mansoni*, Diagnostic Performance, School Children, Ethiopia

1. Introduction

*Schistosoma mansoni* (*S. mansoni*), *Schistosoma hematobium* (*S. hematobium*) and *Schistosoma japonicum* (*S. japonicum*) are the schistosome species of major public health importance [1]. The global burden of disease due to schistosomiasis is estimated at 3.3 million disability-adjusted life years (DALYs) [2]. In Ethiopia, the number of people living in Schistosoma endemic areas is estimated at 37.3 million [3, 4]. The regional prevalence within the Amhara region was 6.9% for *S. mansoni* [5]. *Schistosoma* parasite completes its life cycle in two different hosts, namely, humans and freshwater snails [6]. The human host acquires the infection via the active
penetration of the skin by cercariae up on contact with fresh water [7, 8]. Once Schistosoma enters the human body system, it chronically infected children and results in chronic malnutrition and anemia [9].

Infections of S. mansoni can be diagnosed by several diagnostic techniques including: direct wet mount, Kato-Katz (KK), Spontaneous tube sedimentation (STS), Formol-ether concentration (FEC), immunodiagnostic and molecular techniques. However, these methods vary in sensitivity, cost, simplicity and applicability [10, 11]. The KK technique is recommended by the world health organization (WHO) to detect S. mansoni infections [12, 13]. To be effective, diagnostic methods must be accurate, simple and affordable for the whole population. They must also provide a result in a short period of time to effective prevention and control measures [14]. Likewise, diagnostic methods play an important role in the assessment of treatment efficacy and in patient management [15]. Diagnostic methods with low sensitivity such as the wet mount technique leads under diagnosis of S. mansoni infections may mislead the physicians [16-19]. Proper detection of S. mansoni using sensitive diagnostic methods is crucial in the national S. mansoni prevention and control strategies. However, direct wet mount is still used as a routine diagnostic method for S. mansoni infections in Ethiopia. This has a negative impact on the prevention and control plan. Updating and applying better sensitive, specific and cost effective diagnostic methods as a routine diagnostic approach is ideal in Ethiopia. Therefore, the aim of this study will be to evaluate the performance of FEC, KK and STS for S. mansoni detection against the “Gold” standard in Amhara region.

2. Method

2.1. Study Design, Period and Area

A cross-sectional study was conducted among primary school children from October to December, 2019 in Amhara National Regional State (ANRS). The ANRS consists of 10 zones and 157 districts and is divided into three major ecological zones: the highlands, midlands and the lowlands. The annual mean temperature is between 15°C and 21°C. The mean annual rainfall is also 1,165 mm. According to the economic and finance office of the ANRS 2017 report, the total population of children (5-14 years) in the region is 5,996,074 [20].

2.2. Sampling Techniques

Six districts and twelve primary schools (two primary schools in each district) were selected by simple random sampling technique and a systematic random sampling technique was used to select the study participants in each selected primary schools as class roster was used as a sampling frame. The sample size (520) was proportionally allocated for each school by taking the total number of students in each category into consideration.

2.3. Inclusion and Exclusion Criteria

Students who fall in the age range 6-14 years, gave consent and volunteered to participate were included whereas, students who took anti-helminthic drugs for the last 2 months prior to data collection or during data collection time were excluded from the study.

2.4. Data Collection and Processing

Study participants were informed about the purpose of the study. Approximately, 10 g of fresh stool sample was collected with 25.0 ml stool cup from each study participant and transported to the nearby health institution within an hour. The fresh stool samples were processed with FEC, KK and STS to detect S. mansoni.

In the modified Richie’s method, approximately 0.5 g fresh stool sample was added in the sample collecting tube containing 2.5 ml of formalin and 1 ml of ethyl acetate. The test tube was mixed well and centrifuged at 1500 revolutions for three minutes. Finally, the supernatant was discarded and the sediment was mixed and put on the microscope to detect the ova of S. mansoni [21].

In the KK technique, stool sample was pressed through a mesh screen to remove large particles. About 41.7mg of sieved stool was transferred to the templates which was put on a slide till the template whole is filled. Then, the template was removed and the stool sample was covered and pressed with cellophane which is previously immersed with Glycerol-malachite green. The Kato - Katz thick smears were examined for S. mansoni ova [13].

In the STS technique, approximately 3g of fresh stool sample was weighed and homogenized in 10 ml of normal saline solution. The mixture was filtered through surgical gauze into a 50 ml plastic tube which was then filled with more saline solution to 50 ml gauge, plugged, and shaken vigorously. The tube was left to stand for 45 minutes, and then discard the supernatant. A sample was taken from the bottom and put on a microscope slide and seen for the ova of S. mansoni [11].

2.5. Performance Evaluation

The detection rate and performance of FEC, STS and KK methods to S. mansoni was checked. The diagnostic agreements of methods were evaluated by Kappa value, number of observed agreements, number of agreements expected by chance and standard error of Kappa. Kappa result was interpreted as follows: values ≤ 0 as indicating no agreement and 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement [22].

2.6. Data Quality Control

Training was given for laboratory personnel about the study, data collection, and detection. The quality of reagents and instruments were checked. The stool samples were also checked for serial number, and quantity. To eliminate observer
bias, each stool sample was examined immediately by two laboratory personnel. To maintain the reliability of the study findings, 15% of KK slides were randomly selected and re-examined by a third laboratory personnel who was blind for the first stool examination. The principal investigator checked the discordant results and put the final result.

2.7. Data Analysis

Data was entered in Epi-data version 3.1 and analyzed using SPSS version 20.0 statistical software for descriptive statistics. The sensitivity, specificity, negative predictive value and positive predictive values for each diagnostic method in S. mansoni detection were calculated against the combined results as a “Gold” standard method. Kappa values were estimated at 95% CI to determine the strength of agreement of the diagnostic methods. P-value <0.05 were considered as statistically significant.

2.8. Ethical Consideration

Ethical clearance was obtained from College of Medicine and Health Science ethical review committee, Bahir Dar University and permission letter was obtained from ANR health bureau. Supporting letters were also obtained from ANR education bureau, Zonal and district education offices. Written informed consent was secured from parents/guardians of each study participant. Study participants infected with intestinal parasites were referred to doctors at the nearby health institution.

3. Results

3.1. Socio-demographic Characteristics of the Study Participants

A total of five hundred twenty (n=520) students were enrolled in this study. The mean age was 10.14 years ranged from 6 to 14 years with a standard deviation of 1.66 years. The male participants accounted for 266 (51.2%) and four hundred ninety seven (95.6%) participants were rural dwellers.

3.2. Prevalence of S. mansoni

The overall prevalence of S. mansoni was 20.2% with a combined method. The detection rate 16.3%, 12.9% and 8.3% to S. mansoni infections was obtained using STS, KK, and FEC techniques, respectively (Table 1).

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Diagnostic technique</th>
<th>Prevalence detected by each method</th>
<th>N</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mansoni</td>
<td>Combined</td>
<td>105</td>
<td>20.2</td>
<td>16.7-23.7</td>
<td></td>
</tr>
<tr>
<td>S. mansoni</td>
<td>FEC</td>
<td>43</td>
<td>8.3</td>
<td>5.9-10.6</td>
<td></td>
</tr>
<tr>
<td>S. mansoni</td>
<td>KK</td>
<td>67</td>
<td>12.9</td>
<td>10-15.8</td>
<td></td>
</tr>
<tr>
<td>S. mansoni</td>
<td>STS</td>
<td>85</td>
<td>16.3</td>
<td>13.2-19.5</td>
<td></td>
</tr>
</tbody>
</table>

N=number positive, CI=Confidence interval.

3.3. Detection and Performance Evaluation of Diagnostic Methods for S. mansoni

The detection rate of the combined methods to S. mansoni was 2.44, 1.57, and 1.24 times more sensitive than FEC, KK and STS methods, respectively. The STS technique had more sensitivity (81.0%) and NPV (95.4%) than KK sensitivity (63.8%) and NPV (91.6%) and FEC sensitivity (41.0%) and NPV (87.0%) in the detection of S. mansoni. The sensitivity of KK technique (63.8%) was higher than FEC technique sensitivity (41%) for the diagnosis of S. mansoni infections. However, specificity and positive predictive values of detecting the S. mansoni was similar (100%) in all the three techniques (Table 2).

<table>
<thead>
<tr>
<th>Method</th>
<th>“Gold” standard</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC</td>
<td>Pos 62 Neg 415</td>
<td>41.0 (32-50.5)</td>
<td>100 (99.1-100)</td>
<td>87.0 (83.7-89.7)</td>
<td>100 (91.8-100)</td>
</tr>
<tr>
<td>KK</td>
<td>Pos 38 Neg 415</td>
<td>63.8 (54.3-72.4)</td>
<td>100 (99.1-100)</td>
<td>91.6 (88.7-93.8)</td>
<td>100 (94.6-100)</td>
</tr>
<tr>
<td>STS</td>
<td>Pos 85 Neg 415</td>
<td>81.0 (72.4-87.3)</td>
<td>100 (99.1-100)</td>
<td>95.4 (93.0-97.0)</td>
<td>100 (95.7-100)</td>
</tr>
</tbody>
</table>

Note: CI- confidence interval, PPV-positive predictive value, NPV-negative predictive value

The detection rate by combination of STS and KK (19.4%) to S. mansoni was better as compared to the other two combined techniques (KK+FEC, and STS+FEC) (Table 3). The STS technique had more sensitivity (81.0%) and NPV (95.4%) than KK sensitivity (63.8%) and NPV (91.6%) and FEC sensitivity (41.0%) and NPV (87.0%) in the detection of S. mansoni. The sensitivity of KK technique (63.8%) was higher than FEC technique sensitivity (41%) for the diagnosis of S. mansoni infections. However, specificity and positive predictive values of detecting the S. mansoni was similar (100%) in all the three techniques (Table 2).
3.4. Agreement of the Diagnostic Methods

The observed and expected agreements between STS method and combined method were 96.15% and 70.06% of the observations, respectively for the identification of *S. mansoni* infection. The agreement of STS technique with the combined results was perfect in detecting *S. mansoni* (κ = 0.872). The KK method agreed substantially in *S. mansoni* (κ = 0.738) detections with combined techniques. The FEC technique agreed moderately in *S. mansoni* (κ = 0.525) detections with gold standard (Table 4).

4. Discussions

Accurate and sensitive diagnostic methods are necessary for the clinical and public health diagnosis of helminth infections, as well as for monitoring treatment and control interventions [23]. In the present study, the prevalence of *S. mansoni* was 20.2% which is comparable with previous study done in Ethiopia (20.2%) [24] and Kenya (21%) [25], but higher than previous study results (3.1%) in Arba Minch Zuria district [26], (4.9%) in Medebay Zana wereda [27], (0.75%) in Homesha district [28] and (10.3%) in Jawi district [29]. On the other hand, higher prevalence of *S. mansoni* was recorded in Lake Hawassa (31%) as compared to the present study [30]. Differences between this result and those previous reports could be the difference in geographical differences, snail distribution and laboratory techniques used.

The STS technique is the simplest, fastest method to perform, requires less equipment and detects many species [11]. In the present study, STS method was 1.26 and 1.98 times more sensitive in *S. mansoni* detection than KK and FEC methods, respectively. Although there is insufficient data which have been conducted on the detection rate of this method before, the result obtained in our study supports STS can be an alternative diagnostic method for *S. mansoni* infections.

In the present study, *S. mansoni* was found in 8.3% of the students using FEC technique and 12.9% by KK method. The difference in their diagnostic sensitivity is statistically significant (P = 0.001). This result agrees with previous studies done in Peusangan Bireuen [31] and Ethiopia [32, 33] which stated that the FEC method was less sensitive than KK method. However, the result of the present study is in contrary with the study done in Coˆ te d’Ivoire [34] and Tanzania [23] which showed that FEC method was more sensitive than KK method. There is also similar reports in Ethiopia which stated as FEC had higher sensitivity than KK to detect *S. mansoni* [24, 35]. These differences in diagnostic performance might be due to stool sample size, number of slides prepared for diagnosis, inter personal skill variations and technical errors of the diagnostic methods used.

Taking the combined results of four techniques as a standard test, STS technique (81%) had higher sensitivity than KK (63.8%) and FEC (41%) techniques in the detection of *S. mansoni* infection. However, lack of previous similar studies made difficulty in making rigorous discussion on this finding. On the other hand, the sensitivity of KK (63.8%) and FEC (41%) were recorded in *S. mansoni* detection. This showed that KK method was 1.55 times more sensitive than FEC method in the diagnosis of *S. mansoni* infection in the current study as previously confirmed by Glinz et al which is 2 times more sensitive than KK method in Coˆ te d’Ivoir [34].

The agreement of STS technique with the combined results was perfect in detecting *S. mansoni* infections (κ = 0.872). On the other hand, the agreement of KK (κ = 0.738) with the gold standard was substantial for the detection of *S. mansoni*. There was difficulty in making rigorous discussion on these findings due to lack of previous reports. The agreement of FEC technique with the “Gold” standard to detect *S. mansoni* was moderate (κ = 0.525) which is comparable with previous study done in Gonder town (κ = 0.58) [32].

5. Conclusions

The present study revealed that STS was the best method.
as compared to the other methods to detect *S. mansoni*. In addition, the KK method showed better performance as compared to FEC technique to detect *S. mansoni*. Therefore, STS technique can be an alternative diagnostic method for *S. mansoni* infections. This result also indicates that there is a need to make evaluation of the detection rate of STS techniques rigorously before using it as a routine diagnostic method.

**Abbreviations and Acronyms**

ANRS: Amhara National Regional State; DALYs - Disability-adjusted life Years; FEC: Formal-ether concentration; KK: Kato Katz; NPV: Negative predictive value; PPV: positive predictive value; STS: Spontaneous tube sedimentation; WHO: World Health Organization.

**Authors’ Contributions**

A. Fenta: Participated in the conception, design, data collection, analysis and interpretation. T. Hailu, M. Alemu, E. Nibret, A. Amor and A. Munshea facilitated the data collection and management, drafted, analyzed and critically reviewed the manuscript. All authors read and approved of the final manuscript.

**Competing Interests**

The authors declare that they have no competing interests.

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**References**


