Comparison of the Kato-Katz Technique and Direct Wet Mount for Diagnosis of Intestinal Schistosomiasis in New Halfa City, Eastern Sudan

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Abstract: The main aim of this study was to compare between Kato-Katz technique and direct wet mount for diagnosis of intestinal schistosomiasis in New Halfa City, Eastern Sudan. A cross- sectional study was carried out during the period between March to June 2011. A total number of 519 inhabitants from different villages in New Halfa city were included in this study. Stool samples were taken from all the study subjects. Epidemiological and parasitological data were obtained and recorded. Out of 519 stool samples, 112 (21.5%) were positive for S.mansoni by using direct wet mount by examining 3 slides preparation for each sample and Kato-Katz technique respectively. The study reflected that the two methods direct wet mount by examining 3 slides preparation and Kato technique showed highly significant differences (p=0.000). Regarding the gender and age groups, the current study showed that the difference in prevalence rate was found to be statistically significant at p. value=0.000. This study indicated that the study area is highly endemic for intestinal schistosomiasis and the prevalence rate of S.mansoni infection is reflecting high.

Keywords- Comparison; S.mansoni; Kato-Katz technique; Direct wet mount

1. INTRODUCTION

Kato and Miura introduced a thick-smear technique for fecal examination in 1954 [1]. Soon thereafter, Katz modified and adapted this technique for use in field studies [2]. The Kato-Katz thick smear technique is the standard technique recommended by World Health Organisation (WHO) for the quantitative and qualitative diagnosis of Schistosoma mansoni and other intestinal helminth infections such as Ascaris lumbricoides, Trichuris trichiura and hookworm [3], and has also been used in laboratory diagnosis. Although the Kato-Katz method is widely used for the diagnosis of intestinal helminths infections, its reported effectiveness varies. The Kato-Katz method was more sensitive than the thick smear technique for diagnosing helminthes [4]. The Kato thick-smear technique is reliable and practical for the quantitative diagnosis of hookworm, Trichuris Trichiura and Schistosome infections [5]. Previous study showed that the Kato-Katz method is not suitable for hookworm, protozoa and filariform larvae detection [6]; while another study concluded that the Kato method has a low sensitivity for identifying hookworm eggs, and observed that hookworm eggs collapsed and disappeared shortly after the thick smear had cleared [7]. Many publications have noted that the Kato technique is a suitable method in view of its sensitivity, simplicity and minimal cost, especially in epidemiological surveys [8, 9, 10]. The objective of the current study was to compare between Kato-Katz technique and direct wet mount for diagnosis of intestinal schistosomiasis in New Halfa City, Eastern Sudan.

2. Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area and study period:

This study was carried out in different villages (Tiba, Tabark allah, AlQadesia, AlGamhoria and AlWehda) in New Halfa city in the eastern Sudan (figure 1), during the period between March to June 2011.

2.3 Study population:

The study populations included in this study were the inhabitants of Al Qadesia, Tiba, Tabark allah, AlGamhoria and Alwehda villages in New Halfa, males and females with different ages, suffering from schistosomiasis were recruited to the study.

2.4 Sample size:

A total of 519 stool samples were examined.

2.5 Data collection:

The primary data were collected by using self-administrated per-coded questionnaire which was specifically designed to obtain information that helped in the study.

3. Methods

3.1 Collection of stool samples:

Because of the fragile nature of many intestinal parasites, and need to maintain their morphology for accurate identification, reliable microscopic diagnosis cannot be made unless the stool is collected properly. In this study, 519 stool samples were collected to detect *S. mansoni* eggs. The stool specimen was large enough for satisfactory examination and egg count (about 2 grams). The containers with the

specimens were labeled clearly with patient's name or number, date of collection and time the patient's passed the stool.

3.2 Direct wet mount:

Stool specimens were collected in clean and dry containers. A drop of normal saline was placed in the centre of the left half of the slide. A small piece of stool was added by using applicator stick. Mixed well and then covered with a cover glass. Slides were examined under microscope (×10 and ×40 lenses objectives) with a reduced condenser aperture [11]. Three slides were prepared for each stool specimen.

3.3 Kato-Katz technique:

The stool specimen was forced through the screen (sieve) by a spatula to separate stool material from large debris. Then the sieved stool material was transferred into the hole of a template (approximately 41.7mg) that previously placed on a clean and dry microscope slide. The template hole was completely filled with screened stool material and leveled to the surface of the template and the template was removed gently leaving the sample to take its form. A cellophane strips (25x 35mm) were soaked in 50% glycerol-malachite green solution for at least 24 hours before use (as a clearing agent) was laid on top of the sample, and gently pressed to print a thin film on the cellophane lower surface. The preparation then kept for an hour, before it will be examined microscopically; using the 10x objective lens for search and the 40x will be used for identification. The number of eggs observed was multiplied by 24 to obtain the number of eggs per gram of stool [12].

3.4 Data analysis:

All information and data were entered in Computer. Data were analyzed and tested by Chi Square Test and descriptive statistics using statistical package of social science (SPSS) version 11.5 for windows.

3.5 Ethical consideration:

Approval of the study was taken from the College of Medical Laboratory Science-Sudan University of Science and Technology. Permission for the samples collection was taken from study subjects or their gardeners after explaining the study purpose. Ethical clearance will also be taken from Ministry of Health-Kassala State, Department of Preventive Medicine Office of the anti-bilharzia and intestinal worms New Halfa City.

4. Results

For detection of S. mansoni eggs, 519 from inhabitants of Al Qadesia, Tiba, Tabark Allah, Al Gamhoria and Al Wehda village, in New Halfa city, stool samples were collected, within these samples, 112 (21.5%) were found positive by direct wet mount and these positive results obtained by examining 3 slides preparation for each sample while 407 (78.5%) samples were negative (table 1). The prevalence of infection in each village/city were as follow: from Tiba 88 stool samples were collected, 11(2.1%) were found positive, from Al Gamhoria 58 were collected, 11(2.1%) were positive, from Al Wehda 161were collected, 41 (7.9%) were positive, from Tabark Allah 152 were collected, 41 (7.9%) were positive, from Al Qadesia 60 were collected, 8 (1.5%) were positive (table 2). In this study, 519 stool samples were examined by Kato-Katz technique to detect S. mansoni infection. Results showed 112 stool samples (21.5%) were positive (table 3) and worm load was found to be between (24-960) eggs per gram of stool (table 4). The two methods direct wet mount by examining 3 slides preparation and Kato technique showed highly significant differences (p value was less than 0.05, p=0.000). The intensity of infection was obtained by counting the number of S. mansoni eggs per gram (epg) of stool, (≤ 50 epg) presented as mild infection, (51-200 epg) as moderate infection, (201-300 epg) as severe infection and (≥ 400 epg) as hyper infection. Results showed that 112 stool samples (21.5%) were positive by Kato technique, within these, 43 (38.4%) were mild, 56 (50.0%) were moderate, 10 (8.9%) were severe and 3 (2.7%) were hyper infection (table 5). From 112 positive cases, 46 (41.1 %) were males and 66 (58.9%) were females (figure 2). The differences in prevalence according to gender was found to be significant (p value was less than 0.05, p=0.000). The surveyed populations were categorized into five age groups: (4-12), (13-19), (20-45), (46-60) and (61-85) years old. The frequency of each age group was 81 (15.6%), 145 (27.9%), 183 (35.0%), 60 (11.5%) and 50 (10.0%) of the total population respectively. The positive cases within each age group were 69 (13.3%), 21 (4.0%), 18 (3.5%), 3 (0.5%) and 1 (0.2) respectively (table 6). The differences in prevalence according to age groups was highly significant (p value was less than 0.05, p=0.000).



Figure 1: A map of villages in New Halfa City (Remote Sensing Authority, 2012)

Number examined	Number positive	Prevalence rate (%)
519	112	21.5

Table 1: Overall prevalence of S. mansoni in the study area by direct wet mount technique

Table 2: Overall prevalence of S. mansoni according to different villages by direct wet mount technique

Village	Frequency	Positive	Prevalence rate (%)
Tiba	88	11	2.1
Al Gamhoria	58	11	2.1
Al Wehda	161	41	7.9
Tabark Allah	152	41	7.9
Al Qadesia	60	8	1.5
Total	519	112	21.5

Table 3: Overall prevalence of S. mansoni in the study area by Kato-Katz technique

Number examined	Number positive	Prevalence rate (%)	
519	112	21.5	
Table 4: Frequency of Kato technique (eggs/1gram of stool)			
Number of eggs/1gram of stool	Frequency	Percentage (%)	
24	6	1.15	
48	37	7.12	
72	15	2.89	

96	21	4.04	
120	8	1.54	
144	8	1.54	
168	1	0.19	
192	3	0.57	
216	2	0.38	
240	4	0.77	
336	1	0.19	
360	1	0.19	
384	2	0.38	
480	1	0.19	
720	1	0.19	
960	1	0.19	
Total	519	21.5	

Intensity of infection	Frequency	Percentage (%)
Mild infection	43	38.4
Moderate infection	56	50.0
Severe infection	10	8.9
Hyper infection	3	2.7
Total	112	100.0



Figure 2: Prevalence of S. mansoni according to gender by direct wet mount technique

Age groups (years)	Frequency	Percentage (%)	Positive	Prevalence (%)
4-12	81	15.6	69	13.33.5
13-19	145	27.9	21	4.0
20-45	183	35.0	18	3.5
46-60	60	11.5	3	0.5
61-85	50	10.0	1	0.2
Total	519	100	112	21.5

Table 6: Prevalence of S. mansoni according to age groups by direct wet mount technique

5. Discussion

The results from the present study in New Halfa scheme (for S. mansoni) showed that from 519 collected stool samples. the overall prevalence was 21.5%. This reveals that several factors could be responsible for the transmission of the disease in these areas. Among these factors: the social behavior, non hygienic community practices, improper disposal of human waste and lack of health care and education. The current study was carried out to determine the sensitivity and compare between direct wet mount technique and Kato-Katz concentration technique that can be used for diagnosis of intestinal schistosomiasis (S. mansoni). For detection of S. mansoni, each stool sample was examined using 2 different methods. The present study compared the Kato-Katz technique with the results indicated by the routine direct wet mount method obtained by examining 3 slides preparation for each sample, the two methods were showed highly significant differences (p=0.000). The direct wet mount is not considered as quantitative method for egg count. Although it is distinguishable by being a quick and simple method and requires small amounts of sample but it is not efficient in detecting mild and chronic infections. Examination of stool samples by Kato-Katz smears is the standard method recommended by the World Health Organization (WHO) for the field diagnosis of intestinal schistosomiasis (WHO, 1993) [13]. The results of direct wet mount by examining 3 slides preparation for each sample were similar to results obtained by Kato-Katz technique, these findings were in agreement with findings of Elfaki and Goreish (2013) [14] and these findings were in disagreement with the findings of Elfaki and Elhassan (2015) [15] who reported the prevalence were 14% and 16% when using direct wet mount and Kato-Katz technique respectively. The current study showed that the differences in prevalence according to gender and age groups was highly significant, these findings were in agreement with the findings of Elfaki and Elhassan (2015) [15] and findings of Elfaki and Goreish (2013) [14].

6. Conclusion

The present study concluded that, the prevalence rate of *S.mansoni* in the study area was 21.5% by using direct wet mount by examining 3 slides preparation for each sample and Kato-Katz technique respectively. The difference in prevalence according to gender and age groups was highly significant

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