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INDIRECT HAEMAGGLUTINATION TEST AND ELISA AS COMPARED TO KATO THICK-SMEAR IN DIAGNOSING *SCHISTOSOMA MANSONI*

By

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Abstract

The risk of schistosomiasis infection is associated to age, sex, and occupations. This study evaluated the reliable method for diagnosis of schistosomiasis in correlation to their sensitivity and specificity. A total of 75 patients (49 males and 27 females) with manifestations suggestive intestinal schistosomiasis, with ages less than 18 years old (46) and 29 above were enrolled. They were subjected to history taking and clinical and stool examinations. Stool examination was done by Kato-Katz technique, and detection of antibodies against *S. mansoni* by ELISA and IHAT. *S. mansoni eggs* were detected among 45 (60%) by microscopic examination (25 of them <18 years and 20 cases >25 years of old), while antibodies were recorded among 38 (50.7%) and 43 (57.3%) by both ELISA and IHA respectively, non-significant differences were recorded between the three applied methods of examination.

On comparing IHA with Kato-Katz technique as gold standard method of diagnosis, it showed 80% sensitivity & 93.3% specificity. On other hand, ELISA revealed high sensitivity and specificity 96.9% & 90.7% respectively. On comparing Kato-Katz technique to IHA it showed low sensitivity and specificity; 78.9% &59.5% respectively.

Key words: *Schistosoma mansoni*; Kato-Katz technique; IHAT; ELISA

Introduction

Schistosomiasis is an endemic parasitic disease in 70 countries and is estimated to infect 200 million worldwide. It is one of the 10 leading causes of morbidity among travelers (Hotez *et al*, 2012). The disease occurs mostly in the tropical regions, particularly in Africa, South America, and the Far East, with most intense in children 5-15 years of age (Wang *et al*, 2009). The imported schistosomiasis are increasing due to changes in travel destinations and habits of travelers while abroad (Steinmann*et al*, 2006)

The identification of eggs in stool or urine is the most practical method for diagnosis. However, eggs can be present in the stool in infections with all *Schistosoma* species (El Ridi *et al*, 2010). From a practical point of view, the egg output can be quantified by using the Kato-Katz technique *(*Katz *et al,* 1972) or Ritchie technique (Sturrock, 2001).

In the light infected patients with low

levels of egg production and excretion, the diagnosis might be inaccurate and other methods were used as CIEP, IHA and ELISA (Khalil *et al*, 1989); IFAT (Oliveira *et al*, 2004); circulating cathodic antigen (CCA) dipsticks (Stothard *et al*, 2006) or even PCR (Liar *et al*, 2008).

Antigen detection assays represent a useful alternative diagnostic tool in, the high sensitivity and allowing diagnosis of active infection (Hanallah *et al*, 2003; Demerdash*et al*, 2004). However, many of these ongoing assays are sophisticated or could not differentiate between recent and past infection and has the problem of cross reactivity among different helminthic parasitic infections (McManus, 2013).

This study aimed to evaluate the efficacy of two methods in diagnosing intestinal schistosomiasis; ELISA and IHA as compared to the golden Kato-Katz smear, regarding sensitivity and specificity.

Subjects, Materials and Methods

This work was conducted on 75 patients from Tropical Medicine Department, King Abdel Aziz University Hospital with intestinal manifestations as chronic diarrhea, blood in stool, abdominal discomfort and pain, abdominal cramps and dysentery. They were 48 males and 27 females with mean age of 28.8±3.2 years. All were subjected to the following investigations: Three slides of stool samples for schistosome eggs count were done by using the Kato-thick smear technique (Katz *et al,* 1972).

Besides, 2ml of venous blood was aseptically drawn to separate sera for anit-schistosomiasis antibodies by the commercial available IHAT (Cellognost Schisto., Behring Marburg GmbH; Germany) and ELISA. All sera were tested in duplicate. The IHA results were evaluated with a cutoff titer of 1:16 and for ELISA optical density (OD) values were read at 450 (Van Gool *et al*, 2002).

Ethical consideration: Data were collected after written informed consent taken from all participants. The results were kept confidential, and the patients were referred to the physician to be given the appropriate treatment.

Statistical analysis: All data were computerized .The chi-square and t-test was used for contrasting sensitivity, specificity, positive and negative predictive values for each test. P-value less than 0.05 were considered significant.

Results

The results are given in tables 1-4.

Table 1: Data of patients' positive cases by microscopy

Patients Data	Patients (75) No.	%	Positive (38) No.	%	Negative (37) No.	%	P value
Age:<18	46	61.3	25	54.3	21	45.7	P>0.1
>25	29	38.7	20	69	9	31	Non Sig.
Total positive	75	100	45	60	30	40	
Males	48	64	24	50	24	50	P>0.1
Females	27	36	21	77.8	6	22.2	Non Sig.
Urban	32	42.7	10	31.3	22	68.7	P < 0.1
Rural	43	57.3	35	81.4	13	18.6	Sig.

Table 2: Kato-Katz smears for 75 patients with suggestive manifestations

Parasites in stool	No. positive	Positive percent
Fasciola spp.	2	2.6
S. mansoni	45	60.0
S. mansoni+Blastocystis hominis	7	9.3
S. mansoni+Entamoeba histolytica	6	8.0
S. mansoni+Entamoeba coli	1	1.3
S. mansoni+ Giardia lamblia	11	13.3
S. mansoni+ Cryptosporidium parvum	5	6.6

3- Serodiagnosis of the 45 pure *S. mansoni* cases by Kato-Katz smears

Diagnostic methods	Positive cases		Negative cases		P value
	No.	%	No.	%	
ELISA	41	91.1	4	8.9	0.398
IHAT (1 : 16)	43	95.6	2	4.4	
IHAT (1 : 32)	30	66.7	15	33.3	

Table 4: IHA and ELISA positive cases in relation to Kato-Katz smears

No. of patients	IHA titer	Patients reactive %	ELISA	Kato-thick smears	P. value
13	16	30.2	1	3 positive	0.676
10	32	23.3	12	13 positive	0.842
7	64	16.3	11	12 positive	0.821
6	128	13.9	8	8 positive	0.943
7	512	16.3	9	9 positive	0.965
Total 43	1: 16 to 1:512	95.6 (1:16), 69.8 (1:32)	41	45 (100%)	

Practically accepted IHAT positive titer= 1:32; Kato vs. ELISA, p. value=0.302; Kato vs. IHA, p. value=0.861, IHA vs. ELISA, p. value=0.493

Discussion

In the present study, the Kato Katz smears showed that only 45 (60%) patients had *S. mansoni* others (30 or 40%) had missed infections with *Blastocystis hominis* (7 patients); *Entamoeba histolytica* (6 patients), *Entamoeba coli* (one patient), *Giardia lamblia* (11 patients), and *Cryptosporidium parvum* (5 patients), Besides, two patients had pure *Fasciola* spp.

Generally, *B. hominis, E. histolytica; G. lamblia* and *C. parvum* were encountered in Saudi Arabia. Awadallah and Morsy (1974) in Riyadh reported giardiasis among preschool aged which exceeded amoebic dysentery. Qadri *et al*. (1989) in Riyadh among 647 *B. hominis*, 132 suffered from enteric complications. Khan and Alkhalife (2005) reported *B. hominis* in 8.5% (17073) among healthy handlers in Dammam. Al-Braiken (2008) in Jeddah stated that intestinal parasites still common public health problem. Al-Megrin (2010) in Riyadh among136 immunocompromised patients (2 to 89 years) reported *C. parvum* 11(8.1%), *G. lamblia* 9 (6.6%), *Cyclospora cayetanensis* 8 (5.9%), *B. hominis* 7 (5.2%), *E. histolytica* 7 (5.2%), *E. coli* 5 (3.7%), *Strongyloides stercoralis* 3 (2.2%), *Ascaris lumbricoides* 1 (0.7%), *Hymenolepis nana* 1 (0.7%), *Dicrocoelium dendriticum* 1 (0.7%) and *hook worm* 1 (0.7%). Also, fascioliasis was reported in man (Bolbol, 1989; El-Mathal and Fouad, 2005) and animals (Magzoub and Kasim, 1978; Sanad and Al-Megrin, 2005). Haseeb *et al*. (2003) reported cross reaction between fascioliasis and schistosomiasis and Hillyer (2005) used *Fas-*

ciola antigens as vaccines for fascioliasis and schistosomiasis. Thus, the fascioliasis cases were excluded.

As to schistosomiasis, human infections were reported in Saudi Arabia by many authors (Tarizzo, 1956; Shoura and Morsy, 1974; Morsy *et al*, 1974; Arfaa, 1976; Morsy and El Dasouqi, 1979; Abdu, 2009; Shalaby *et al*, 2011). Snail intermediate hosts were reported (Kuntz *et al*, 1978; al-Madani, 1988; Bin Dajem, 2009; Al-Daihan, 2010; Al-Daihan *et al*, 2011). Antibodies against schistosomiasis were studied (Allam, 2009). Baharoon *et al.* (2011) reported acute pulmonary schistosomiasis and added that awareness of disease presentation, especially in non-endemic Saudi provinces was lacking.

In the present study, of 45 positive Kato-Katz smears 24 were males and 21 were females with a ratio of 1.14:1. Also, ten were from urban areas and 35 from rural areas with a ratio of 3.5:1.

In the present study, the IHA gave anti-schistosomiasis reactions of 43/45 or 95.6%. These reactions were 1:16 (13 cases), 1:32 (10 cases), 1:64 (7 cases), 1:128 (6 cases) and 1:512 (7 cases). However, there were many discrepancies concerning the positive IHA-titers. Berhanu *et al*. (2009) reported that cut off titer of ≤1: 256 suggested weak titer while a cut off titer of ≥ 1:512 suggested strong titer and that *S. mansoni* as determined by IHAT and single Kato method was (74.6%) and (76.1%) respectively. So, in the present study, only 7 (16.8%) had heavy infection with a cut off titer of ≥ 1: 512.

Hubbard *et al*. (2003) stated that although microscopic examination was the gold standard to diagnose intestinal schistosome infection in feces, yet failure to recover eggs did not rule out the negativity of infection as in early and 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. Tsang and Wilkins (1991) reported that microscopy in diagnosing schistosomiasis are cheap, but are time consuming and have poor sensitivity, although they have excellent specificity. Booth *et al*. (2003) found that Kato-Katz method is widely used for diagnosing helminthes in epidemiological surveys, but the sensitivities of a single Kato-Katz thick smear for detection of *S. mansoni* alone, hookworms alone, or *S. mansoni* plus hookworms were 22.4%, 8.0% and 17.7%, respectively. Therefore, sensitive serological tests have the potential to increase diagnostic yield, especially in those with light infection who excrete few eggs. Yu *et al*. (2007) compared Kato-Katz smears and IHA in detection of human *Schistosoma* infection in two endemic villages in rural China, found that IHA was conventional Chinese diagnostic method in both villages, and Kato-Katz could be used as a reliable gold standard. The IHA had a sensitivity of 80% and a specificity of 48%, sensitivity of Kato-Katz technique was 68%. They added that IHA was unsuitable for individual screening, but it was more effective for the community diagnostic surveys.

Doenhoff *et al*. (1993) stated that high and low specificity with ELISA had previously been reported because

of many variables as using control sera, from patients with parasitic, fungal, bacterial, viral infections, and people with autoimmune antibodies. Its specificity in schistosomiasis was 98.2% with cross-reactivity with early filariasis & hepatitis. Van Gool *et al*. (2002) reported that commercially available IHA test using *S. mansoni* and ELISA were sensitive and specific to diagnose schistosomiasis in travelers from the tropics. However, a combination of both was recommended, because the pooled results gave higher sensitivity than either test alone while maintaining high specificity. In IHA tests the sensitivity ranged from 71% to 80% and specificity from 80 % to 100%, and found that positive ELISA test cases was 43 (57.3%) with high sensitivity and specificity of 86% and 96.9% respectively, while accuracy was 90.7%. Pardo *et al.* (2004) declared that IHAT proved easily applicable and specific and ELISA is also an economic, sensitive, and specific test for detection of schistosomiasis after the onset of egg production, both gave the most reliable outcome because it increased considerably the sensitivity in all stages of infection with maintenance of high specificity. Liar *et al.* (2008) reported that commercially available ELISA proved a very economic, sensitive, and specific test to detect schistosomiasis after the onset of egg production. Artemis *et al*. (2009) reported that schistosomiasis prevalence depends on the use of well-established, but imperfect, diagnostic tests. Appropriate diagnosis becomes increasingly important for urgent clinical diagnosis as lack of specificity and mass treatment might remain cost effective through the use of ineffective diagnostic tools to only target further drug treatment for people actually infected. This is true as Sabah *et al*. (2011) reported correlation HCV & HBV in schistosomiasis patients. Also, Ibrahim *et al*. (2010) reported that development of simple, rapid and sensitive methods for detecting schistosmiasis was indicated as most available assays require laboratory equipment and highly skilled persons, antibody in sandwich ELISA proved very highly sensitivity and specificity.

The present results showed non-significant association between *S. mansoni* and either age or sex. This did not agree with Eridan *et al.* (1997) reported schistosomiasis in age-related prevalence and intensities of infection. Hang and Manderson (1992) males had higher prevalence of *S. mansoni* infection.

Conclusion

Schistosomiasis remains a serious health problem worldwide, mostly in tropical regions, and endemic developing nations. Some of the patients are asymptomatic or have nonspecific biological or clinical signs. Diagnosis is usually based on clinical data associated with the detection of eggs in stool, urine, and/or rectal and bladder biopsy specimens. Kato thick smear is cheap, but time consuming and needs expert technician while IHA is a good diagnostic tool as it is easily applicable and specific, but with many controversies of cut off. ELISA gave the most reliable outcome because it increased considerably sensitivity in all infection stages with maintenance of high specificity.

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