International Journal of Tropical Medicine 13 (4-6): 39-42, 2018
ISSN: 1816-3319

Comparative Validity of ELISA and Indirect Haemagglutination in Diagnosing *Schistosoma haematobium* Infection: An Egyptian Study

[1]Asmaa M. El-kady, [1]Osama H. Abdella, [2]Amal A. Hassan,
[3]Yasser Mokhtar and [4]Mohammed H. Hassan
[1]Department of Medical Parasitology, Faculty of Medicine, University of South Valley, Qena, Egypt
[2]Department of Zoology, Faculty of Science, University of Damanhur, Damanhur, Egypt
[3]Department of Medical Parasitology, Faculty of Medicine, University of Assiut, Assiut, Egypt
[4]Department of Medical Biochemistry, Faculty of Medicine, University of South Valley, Qena, Egypt

Abstract: Schistosomiasis is one of the most devastating neglected tropical diseases. Several serological methods have been developed recent decades to diagnosis *Schistosoma* infections. The present study aimed to evaluate the sensitivity and specificity of ELISA and IHA in the diagnosis of both acute and chronic *S. haematobium* infections. The present study was conducted on 120 patients (100 were microscopically diagnosed as urinary schistosomiasis and 20 healthy controls. Serum samples from each patient were examined using ELISA and IHA. ELISA with *S. mansoni* soluble egg antigen was used to detect anti-*Schistosoma* IgG (SEA/ELISA) and IHA was performed using Worm Antigen (WA/IHA). With regard to ELISA, the sensitivity of the SEA/ELISA is 97%. Using IHA, the sensitivity of the WA/IHA with a cutoff of 1:80 (WA/IHA80) is 86%. After the combined use of ELISA and IHA, the sensitivity of the combined use of SEA/ELISA and WA/IHA80 is 100%. Analysis of the negative control cases using IHA showed that the specificity of the test was 85%. Using ELISA, the specificity of the test was 95%. Therefore, the specificity of the combined use of WA/IHA80 and SEA/ELISA is 95%. Our findings suggest that WA/IHA and SEA/ELISA are both sensitive and specific serological tests that are easy to use for the diagnosis of schistosomiasis. The combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of sensitivity and specificity.

Key words: SEA/ELISA, WA/IHA, *S. haematobium*, validity, Egypt, schistosomiasis

INTRODUCTION

Schistosomiasis is one of the most devastating neglected tropical diseases. It is considered a major cause of morbidity and mortality in Africa, South America, the Caribbean, the Middle East and Asia (Ortu *et al.* 2017). Almost 732 million people are vulnerable to infection worldwide (Anonymous, 2014) and more than 200 million people have already been infected (Colley, 2014). Despite a steady decrease in the incidence of *Schistosoma haematobium* in Middle and Upper Egypt, infection still persists in most Southern governorates, at an average rate of 7.8% of the population (Khoby *et al.* 2000). *Schistosoma mansoni* is another species that is endemic in the Egyptian Nile River Delta due to the presence of the snail *Biomphalaria alexandrina* (Nour, 2010). According to Barakat (2013), the average rate of infection has reached 36.45% among the villagers in the Nile Delta governorates.

Untreated patients are susceptible to several complications of schistosomiasis such as obstructive uropathies, hepatic fibrosis and granulomatous cerebral lesions which can be prevented by early diagnosis and proper treatment. Additionally, accurate diagnosis of schistosomiasis is of critical importance for epidemiologists and disease control managers for all aspects of disease prevention, control and surveillance. At the population level, the estimation of the burden of disease, the evaluation of drug efficacy and the monitoring of control programmes depend on accurate diagnostic tests (Bergquist and Colley. 2006).

Microscopic examination of urine or stool is the gold standard for the diagnosis of schistosomiasis (Feldmeier *et al.*, 1999) as the eggs are easy to detect and identify (De Vlas and Gryseels, 1992). However, up to 50% of newly infected patients remain asymptomatic (Nicolls *et al.*, 2008).

Several serological methods have been developed in recent decades to detect antibodies against *Schistosoma*

Corresponding Author: Mohammed H. Hassan, Department of Medical Biochemistry, Faculty of Medicine, South Valley University, Qena, Egypt

antigens including Indirect Haemagglutination Assays (IHAs) and Enzyme-Linked Immunosorbent Assays (ELISAs) using different antigens such as crude or purified Adult Worm Antigen (AWA) Soluble Egg Antigen (SEA) and Cercarial Antigen (CA) preparations (Doenhoff *et al*., 2003; Bierman *et al*., 2005; Chand *et al*., 2010).

The present study aimed to evaluate the sensitivity and specificity of ELISA and IHA in the diagnosis of both acute and chronic *S. haematobium* infections.

MATERIALS AND METHODS

The present study was conducted on 120 patients who visited the urology outpatient clinic at Qena University Hospital and were complaining of terminal haematuria and 20 healthy controls. Our study was approved by the institutional review board of the Qena Faculty of Medicine at South Valley University and informed written consent was obtained from each adult participant and from the parents of children.

Each participant was asked to provide 10 mL of urine (10 am-2 pm) which was examined microscopically after concentration by sedimentation or centrifugation.

The 2 stool samples from each participant were examined macroscopically and microscopically by different techniques for the presence of eggs of *Schistosoma mansoni* and other parasites that may show cross reactivity with *Schistosoma* species (Garcia, 2001).

Serum samples from each patient were examined using ELISA and IHA. ELISA with *S.mansoni* soluble egg antigen was used to detect anti-*Schistosoma* IgG (SEA/ELISA) (IVD Inc.; Carlsbad, USA). Serum samples were diluted 1:40 in sample diluent and transferred to the microtiter wells of the Assay Kit. All detection steps were carried out according to the manufacturer's instructions. The reaction was considered positive if the reading was equal to or greater than the cut-off value which was 0.222.

IHA was performed using Worm Antigen (WA/IHA) according to the manufacturer's instructions (Bilharziose Fumouze IHA, Fumouze Diagnostics, Levallois-Perret, cat. No. 5140, France). Serum samples were diluted 1:80. The reaction was considered positive in the presence of a reddish-brown film in the bottom of the well.

The sensitivity of WA/IHA, SEA/ELISA and the combination of both tests was defined as the number of patients with a positive test result as a proportion of the total number of patients who had parasitologically proven schistosomiasis or the probability of being test positive when disease was present, according to Parikh *et al*. (2008). Sensitivity is calculated by the following equation:

$$\text{Sensitivity} = (\text{True positive})/(\text{True postitve}+\text{False negative})$$

The specificity of the individual tests and the combination of the two tests in detecting schistosomiasis was defined as the number of patients with a negative test result as a proportion of the total number of control patients, or the probability of being test negative. Specificity is calculated by the following equation:

$$\text{Specificity}=(\text{True negative})/(\text{True negative}+\text{False postitve})$$

RESULTS AND DISCUSSION

The number of participants involved in the present study was 120. A total of 77.5% (93/120) of the participants were males and 93.3% (112/120) were from rural areas. The age ranged from 13-93 years (mean 34±7 years).

After microscopic examination of urine, 100 patients were diagnosed parasitologically as *S. haematobium* infection while stool examination of all participants was negative for *S. mansoni* and other intestinal parasites with may cross react with *S. haematobium*. With regard to ELISA there were 97 positive patients. Based on these results, the sensitivity of the SEA/ELISA is 97% (Table 1).

Using IHA, the total number of positive cases was 86. Therefore, the sensitivity of the WA/IHA with a cutoff of 1:80 (WA/IHA80) is 86% (Table 2).

After the combined use of ELISA and IHA to analyse sera from 100 patients with urinary schistosomiasis, we found that all patients were diagnosed positive for *S. haematobium* infection as there were no patients who were shown to be negative by the combination of the two tests. Therefore, the sensitivity of the combined use of SEA/ELISA and WA/IHA80 is 100%.

Analysis of the negative control cases using IHA showed that 17cases were negative which means that the specificity of the test was 85%. Using ELISA,

Table 1: Results of the analysis of serum of participants using ELISA

	Urine analysis		
ELISA	+Ve	-Ve	Total
+Ve	97	1	98
-Ve	3	19	22
Total	100	20	120

Positive predictive value of ELISA is: 97/ 98 = 98.9%; Negative predictive value of ELISA is: 19/22 = 86.3%

Table 2: Results of the analysis of participants using IHA

	Urine analysis		
IHA	+Ve	-Ve	Total
+Ve	86	3	89
-Ve	14	17	31
Total	100	20	120

Positive predictive value of ELISA is: 86/89 = 96.6%; Negative predictive value of ELISA is: 17/31 = 54.8%

only one case was falsely diagnosed as positive for *S. haematobium* which means that the specificity of the test was 95%. Therefore, the specificity of the combined use of WA/IHA80 and SEA/ELISA is 95%.

Immune diagnosis is based on the detection of parasite antigens or antibodies. Although, immune diagnosis usually requires better-equipped laboratories than direct techniques using microscopy, immunological methods may have higher sensitivities, especially for antibody detection. In the present study, we attempted to evaluate the sensitivity and specificity of ELISA and IHA for the serodiagnosis of acute and chronic *S. haematobium* infection. SEA/ELISA showed high sensitivity and specificity in the detection of anti-*Schistosoma haematobium* antibodies (97.0 and 95.0%, respectively). WA/IHA80 with a cutoff of 1:80 yielded a sensitivity and specificity of 86 and 85%, respectively. The combination of ELISA and IHA tests had 100% sensitivity and 95.0%, specificity. Our results were in accordance with those of several studies worldwide. Zhang *et al.* (2009) found that the sensitivity and specificity for the combined IHA and ELISA tests in the diagnosis of schistosomiasis were 100 and 93%, respectively.Another study was performed at the Hospital for Tropical Diseases in London (HTD) by Tosswill and Ridley (1986), who found that the sensitivity and specificity of ELISA for the detection of *S. haematobium* infection were 92 and 97%, respectively. Additionally, our findings were in accordance with results obtained by a Dutch study conducted by Van Gool *et al.* (2002) who evaluated the IHA together with ELISA, using a panel of serum samples from 100 patients with schistosomiasis. In that study, IHA with a cutoff of 1:80 and SEA/ELISA had sensitivity values of 92.0% for the detection of *S. Haematobium* and specificity values of 94.7 and 98.2%, respectively. The combination of ELISA and IHA had a sensitivity of 96.0% for the detection of *S. haematobium* while the calculated specificity of this combination for detecting *S. haematobium* was 97.2%. On the other hand these values are higher than those reported by Kinkel *et al.* (2012) who found that the sensitivity of IHA for *S. haematobium* was 71.4% while the specificity was 99.0%; in that study, ELISA showed a sensitivity of 57.1% and a specificity of 97.1%.

Hassan (1987) investigated 86 bilharzial patients. ELISA, Indirect Fluorescent Antibody (IFA) and IHA tests were performed for all patients. ELISA gave the most sensitive results (82.6%), followed by IFA (79.1% positivity rate) and IHA (77.9% positivity rate). According to Xue *et al.* (1993) who used 187 human serum samples collected from the *S. haematobium* endemic area of Pemba Island, Tanzania and 30 normal serum samples from blood donors in Europe, the sensitivity of ELISA was 95.56% but the specificity was poor (31.90%). Azab *et al.* (1993) evaluated ELISA in relation to an IHA test in schistosomiasis patients who were classified by clinical, sonographic and direct diagnostic methods. The sensitivities of ELISA and IHA proved to be 100 and 69.23%, respectively in patients with acute *S. mansoni* infection, 95.5 and 90.4%, respectively in chronic active schistosomiasis patients; and 86.06% and 67.41%, respectively, in patients with a past history of exposure.

CONCLUSION

Our findings suggest that WA/IHA and SEA/ELISA are both sensitive and specific serological tests that are easy to use for the diagnosis of schistosomiasis. The combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of sensitivity and specificity. SEA/ELISA is a good serological screening test for schistosomiasis but gives no indication of the infecting species of schistosome as *S. haematobium* antigens are not easily obtained because the life cycle of *S. haematobium* is difficult to maintain in the laboratory and egg yields are low.

REFERENCES

Anonymous, 2014. Schistosomiasis. World Health Organization, Geneva, Switzerland. http://www.who.int/en/news-room/fact-sheets/detail/schistosomiasis

Azab, M.E., S. Zakaria, H.M. Hussein, M.Y. Abdel-Hamid and A.M. Kamel *et al*., 1993. Evaluation of an ELISA test in past and present Schistosomiasis. J. Egypt. Soc. Parasitol., 23: 437-443.

Barakat, R.M.R., 2013. Epidemiology of Schistosomiasis in Egypt: travel through time. J. Adv. Res., 4: 425-432.

Bergquist, R.N. and D.G. Colley, 2006. Schistosomiasis vaccine: Research to biological features at presentation and during treatment. J. Infect., 52: 339-345.

Bierman, W.F.W., J.C. Wetsteyn and T. van Gool, 2005. Presentation and diagnosis of imported Schistosomiasis: Relevance of Eosinophilia, Microscopy for ova and Serology. J. Travel Med., 12: 9-13.

Chand, M.A., P.L. Chiodini and M.J. Doenhoff, 2010. Development of a new assay for the diagnosis of schistosomiasis, using cercarial antigens. Trans. R. Soc. Trop. Med. Hyg., 104: 255-258.

Colley, D.G., 2014. Morbidity control of Schistosomiasis by mass drug administration: How can we do it best and what will it take to move on to elimination?. Trop. Med. Health, 42: 25-32.

De Vlas, S.J. and B. Gryseels, 1992. Underestimation of *Schistosoma mansoni* prevalences. Parasitol Today, 8: 274-277.

Doenhoff, M.J., J.G. Wheeler, K. Tricker, J.V. Hamilton and R.F. Sturrock *et al*., 2003. The detection of antibodies against *Schistosoma mansoni* Soluble Egg Antigens (SEA) and CEF6 in ELISA, before and after Chemotherapy. Ann. Trop. Med. Parasitol., 97: 697-709.

Feldmeier, H., P. Leutscher, G. Poggensee and G. Harms, 1999. Male genital Schistosomiasis and Haemospermia. Trop. Med. Intl. Health, 4: 791-793.

Garcia, L.S., 2001. Diagnostic Medical Parasitology. 4th Edn., ASM Press, Washington, USA., ISBN:9781555812003, Pages: 1092.

Hassan, I.M., 1987. Studies on the role played by some snails in transmitting parasites to animals and man in Qena province. Ph.D. Thesis, Faculty of Science, Assiut University. Egypt.

Khoby, T.E., N. Galal, A. Fenwick, R. Barakat and A.E. Hawey *et al*., 2000. The epidemiology of schistosomiasis in Egypt summary findings in nine Governorates. Am. J. Trop. Med. Hyg., 62: 88-99.

Kinkel, H.F., S. Dittrich, B. Baumer and T. Weitzel, 2012. Evaluation of eight serological tests for diagnosis of imported Schistosomiasis. Clin. Vaccine Immunol., 19: 948-953.

Nicolls, D.J., L.H. Weld, E. Schwartz, C. Reed and F. von Sonnenburg *et al*., 2008. Characteristics of Schistosomiasis in travelers reported to the GeoSentinel Surveillance Network 1997-2008. Am. J. Trop. Med. Hyg., 79: 729-734.

Nour, N.M., 2010. Schistosomiasis: Health effects on women. Rev. Obstet. Gynecol., 3: 28-32.

Ortu, G., O. Ndayishimiye, M. Clements, D. Kayugi and C. Campbell Jr. *et al*., 2017. Countrywide reassessment of *Schistosoma mansoni* infection in Burundi using a urine-circulating cathodic antigen rapid test: Informing the national control program. Am. J. Trop. Med. Hyg., 96: 664-673.

Parikh, R., A. Mathai, S. Parikh, G.C. Sekhar and R. Thomas, 2008. Understanding and using sensitivity, specificity and predictive values. Indian J. Ophthalmol., 56: 45-50.

Tosswill, J.H. and D.S. Ridley, 1986. An evaluation of the ELISA for schistosomiasis in a hospital population. Trans. R. Soc. Trop. Med. Hyg., 80: 435-438.

Van Gool, T., H. Vetter, T. Vervoort, M.J. Doenhoff, J. Wetsteyn and D. Overbosch, 2002. Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with *Schistosoma mansoni* adult worm antigens and an enzyme-linked immunosorbent assay with S. mansoni egg antigens. J. Clin. Microbiol., 40: 3432-3437.

Xue, C.G., M.G. Taylor, Q.D. Bickle, L. Savioli and E.A. Renganathan, 1993. Diagnosis of *Schistosoma haematobium* infection: Evaluation of ELISA using keyhole limpet haemocyanin or soluble egg antigen in comparison with detection of eggs or haematuria. Trans. Roy. Soc. Trop. Med. Hyg., 87: 654-658.

Zhang, Y.Y., J.P. Luo, Y.M. Liu, Q.Z. Wang and J.H. Chen *et al*., 2009. Evaluation of Kato-Katz examination method in three areas with low-level endemicity of *Schistosomiasis japonica* in China: A Bayesian modeling approach. Acta Trop., 112: 16-22.