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Iranian Society of
Parasitology
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Original Article

Evaluation of *Schistosoma haematobium* Adult and Egg Antigens by ELISA in Diagnosis of Urinary Schistosomiasis

E Elkawaz¹, *F Ghaffarifar²

¹Medical Research Center, College of Medicine, University of Al-Nahrain, Baghdad, Iraq

²Dept. of Parasitology, Medical Sciences Faculty, University of Tarbiat Modarres, Tehran, Iran

(Received 4 Dec 2008; accepted 10 Oct 2009)

Abstract

Background: The aim of this study was using ELISA for detection of anti-*Schistosoma haematobium* antibodies in both sera and urine of patients with urinary schistosomiasis.

Methods: Thirty three sera and urine samples were collected from patients with acute schistosomiasis in Diyala Province, east of Iraq in 2006. Their diseases were confirmed by finding *S. haematobium* ova in urine examination. Sera and urines of 10 healthy individuals as well as 5 patients with hydatidosis and 5 patients with acute toxoplasmosis were examined as well. Samples were examined for antibody detection by ELISA method. The antigens used in this study were egg and adult antigens.

Results: All positive samples (sera and urines) showed positivity by using egg antigen whereas the negative control samples were negative; only two samples with hydatidosis were positive with using serum sample whereas with urine sample only one sample was positive. In this study, the best sensitivity and specificity obtained when using urine and adult antigen.

Conclusion: Antibody detection by using urine is a useful, simple, and sensitive method for diagnosis of schistosomiasis.

Keywords: Schistosomiasis, Iraq, Diagnosis, Sera, Urine, ELISA

* Corresponding Author: Fax ,++98-21-88013030, E-mail ghafarif@modares.ac.ir

Introduction

Schistosomiasis is a clinical term applied to infection with one of a series of related trematode parasites that are endemic in at least 76 tropical and sub-tropical countries (1). Schistosomiasis is a parasitic plathyhelminth infection of the intestinal or urinary system caused by one of several species of *Schistosoma* (1).

Schistosomiasis infects more than 200 million people (2). Of these, 20 million people suffer severe consequences from schistosomiasis, with 800,000 deaths annually. Furthermore, it is estimated that 500-600 million are at risk of infection (2).

Urinary schistosomiasis is a chronic parasitic infection of circulatory system caused by *Schistosoma haematobium*, which affects the bladder and subsequently the urinary tract system of man. The effect of *S. haematobium* infection is due to deposition of eggs in the bladder and urethra, which elicits chronic granulomatous injury. This granulomatous inflammation causes nodules, polypoid lesions, and ulcerations in the lumen of the urethra and bladder which results clinically in urinary frequency, dysuria, and terminal hematuria. The disease may progress chronically and terminates in renal failure and carcinoma of bladders as components of the morbidity and at times mortality. The clinical picture and the disease outcome in persons infected with *S. haematobium* varies dramatically and ranging from mild symptoms to severe damage of the urinary tract especially the kidney and/or bladder (3).

Diagnosis of active schistosomiasis haematobium is made principally by finding of *S. haematobium* eggs in the urine. Parasitic tests are not considered to have high sensitivity because of factors such as irregularity in *Schistosoma* egg shedding (4-5). Several immunological tests using crude or purified egg and adult worm antigens have been de-

veloped in the last decades to detect anti-*S. haematobium* antibodies (4-5). ELISA has shown a high sensitivity and specificity for antibody detection in schistosomiasis, for this, we used this test to detect the antibodies in sera and urines (6-7).

The aim of this study was to use urine of patients with schistosomiasis as an antibody source and then the results compared with the data obtained from the sera.

Materials and Methods

Patients and sera

The samples were collected in Balad Rouz town, Diyala Province, in 2006. This area is considered as one of the bilharziasis foci in Iraq (8).

Thirty three sera were collected from school-age children that were positive for *S. haematobium* ova in urine examination (8).

A group of 10 individuals was chosen from the same area on the basis that they had negative urine sample for *S. haematobium* ova. In addition, they were neither ill nor under any type of therapy at the time of collecting blood samples. On the other hand, a group of 10 apparently healthy individuals living in non-endemic area (Baghdad) was included in the study as negative control. Five sera of Iranian patients with hydatidosis confirmed by surgery and five sera of IgM positive Iranian patients with acute toxoplasmosis were collected as other control group.

The urine samples were placed in a cool-box and brought to the laboratory of the Medical Research Center at the College of Medicine / University of Al-Nahrain, Iraq.

Soluble worm antigen (SWA) and soluble egg antigen (SEA) preparation

Parasite antigens were obtained through personal communication by purchasing both soluble egg antigens (SEA) and soluble worm antigens (SWA) from Theodor Bilharz Institute, Cairo, Egypt.

Urine Samples

The urine samples were collected between 10:00 and 14:00 O'clock in a wide-mouth plastic container with lid. To achieve rapid and accurate results, the Nuclepore Schisto-Kit (7035 Commerce Circle, Pleasanton, California 94566) was used by membrane filtration technique. From the 33 *S. haematobium* ova positive individual's urine samples were concentrated 20 times using Minicon concentrator.

ELISA procedure

ELISA test was conducted as stated earlier (9, 10). Antigen was diluted (10µg/ml) with coating buffer (Carbonate-bicarbonate buffer). Then the plates were blocked with blocking buffer for one hour. The urine samples and sera (1:200 and 1:400 dilutions, respectively) were added to each well. Anti-human IgG already labeled with horseradish peroxidase (HRP) with 1:5000 dilution was added to each well. Then substrate-chromogen solution, Ortho-Phenylenediamine (OPD) with H₂O₂ was added to each well.

After 20 min stopping reagent as 4N H₂SO₄ was added to each well. The OD (optical density) of the ELISA plate was read by ELISA-reader in 492-wave length.

Cut-off value was calculated as Mean OD of negative control + 3 standard deviation (11). All OD readings above cutoff value were considered positive.

Results

The cut-off value for ELISA test using egg antigen with sera at (1/200) & (1/400) dilutions and urine, were evaluated as 0.84, 0.55 and 0.23, respectively.

The cut-off value for ELISA test using adult antigen with sera at (1/200) & (1/400) dilutions and urine, were evaluated as 0.82, 0.69 and 0.26, respectively.

The results for urines and sera samples by ELISA obtained from egg and adult antigens are shown in Table1.

The sensitivity and specificity of ELISA in different conditions are shown in Table 2. The best sensitivity and specificity obtained from using urine and adult antigen.

The most cross-reaction cases occurred when we used the sera with hydatid patients, whereas there was not any cross-reaction for sera with toxoplasmosis. Regarding patients with hydatidosis two sera samples and one urine sample showed positivity with ELISA test.

Table 1: Mean and standard deviation of optical density (OD) of ELISA test obtained from sera with two dilutions (1/200 and 1/400) and concentrated urine

	Study group	Optical Density (OD) of ELISA test		
		Sera(1/200) M±SD	Sera(1/400) M±SD	Urine M±SD
Parasite egg Ag	PG	2.71± 0.18	2.66± 0.085	1.69±0.42
	CG	0.77± 0.09	0.25± 0.14	0.173±0.019
Parasite adult Ag	PG	1.80± 0.46	1.72± 0.656	2.17±0.156
	CG	0.7± 0.4	0.59± 0.33	0.2±0.02

M: Mean
SD: Standard Deviation PG: Patient group CG: Control group

Table 2: Sensitivity and specificity obtained from sera with 1/200 and 1/400 dilutions and urines by using parasite's egg and adult antigens

		Sera(1/200)	Sera(1/400)	Urine
Parasite Egg Antigen	Sensitivity (%)	100	100	100
	Specificity (%)	90	90	95
Parasite Adult Antigen	Sensitivity (%)	82	88	100
	Specificity (%)	95	90	100

Discussion

Schistosomiasis caused by the *S. haematobium* parasite is a problem in some parts of

Iraq, especially in the Tigris and Euphrates delta. Schistosomiasis is common in some

countries of Middle East such as Egypt, parts of Iraq and less common in Saudi Arabia (12). In Iran for the last several years, the country has been free from the incidences of schistosomiasis (13-14).

Schistosomiasis that caused by *S. haematobium* can affect and cause changes in the urinary system. Thus, accurate and early diagnosis is essential to prevent such complications. ELISA is a sensitive method for detection of schistosomiasis (6,7, 15-17). The sensitivity of routine applications of ELISA for schistosomiasis using SEA as antigen reaches over 95% in *S. japonicum* egg-positive individuals. In a non-endemic area, the false positive rate was 1.3–3.3% (15).

Barakat et al. found that diagnosis of schistosomiasis in two groups of schoolchildren, one attending a rural school where schistosomiasis was highly prevalent, the other attending a school in an urban locality where schistosomiasis was absent. The results showed that the ELISA had a sensitivity of 92.2% and 100% in *S. mansoni* and mixed infection, respectively. The specificity of the test increased from 92.1% to 98% when a fourth stool examination was done in cases giving false positive results (19).

ELISA typically is a laboratory-based tool useful for large-scale operations. Its application in the field is attached with considerable difficulty; however, special treatment of the antigens can extend its application (17). ELISA positivity was significantly increased with the increase in egg count until it reached 100% in cases with 160 eggs per gram stools (17).

In the present work, total antibody levels response in sera of schistosomiasis patients against adult worm and egg antigens were determined by ELISA. This response showed a high reactivity against both SWA and SEA, but the antibody response to egg antigen showed a characteristic pattern with higher reactivity than adult worm antigen.

This finding is in agreement with that reported by Hagi et al. (15) who studied antibodies responses in sera of *S. haematobium* infected individuals in Somalia.

In this study, the best sensitivity and specificity obtained when using urine and adult antigen. The present study is in agreement with some studies concerning the detection of specific antibodies in serum of patients by ELISA SEA (6, 7, 18), but the combination of ELISA SEA in urine and serum might offer a better diagnostic improvement.

These good results showed that we could use the urine as a source of antibody for detection and diagnosis of schistosomiasis.

Acknowledgements

This study was supported by Tarbiat Mo-darres University, and the financial source of the Ministry of High Education, Iraq. The authors declare that they have no Conflicts of Interest.

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