Detection of Schistosomia Haematobium Among Patients with Urinary Tract Manifestations by ELISA, IHT and Western Blot Techniques

Samah S. Abdel Gawad[1], Enas Yahia Abu Sarea[1], Medhat Abdel-Fattah Abdel-Mohsen[2], Sara A Khedr[2], Ibraheem B. M. Ibraheem[2]

[1]Department of Medical Parasitology, Faculty of Medicine, Beni-Suief University, Beni-Suef, 62514, Egypt
[2]Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni-Suef, 62514, Egypt

ABSTRACT

Schistosomiasis is a chronic, debilitating water born disease. About 600 million people are at a high risk with more than 200 million infected in 74 countries mainly in intropic and subtropic areas. This study aimed to compare serological (IHT and ELISA) and immunological (Western blot technique) methods for diagnosis of schistosomiasis haematobium. The study was conducted on 110 patients with urinary tract manifestations as loin pain, dysuria, frequency, haematuria, incontinence, nocturia, othersand with negative urine samples for Schistosoma haematobium eggs. These patients were from different sex and age groups. They were selected from the attendances of two hospitals (Beni-Suef General Hospital and the Hospital of Health Insurance). Urine samples were subjected to microscopic examination of urine sediments after centrifugation to exclude the presence of S. haematobium eggs. Blood samples were centrifuged to obtain serum for indirect haemagglutination, ELISA, and western blot technique. The study revealed that young age group (30-39) was the most prevailing age group (33.6%), while old age group (60-70) was the least one (8.2%). Regarding residence, the study revealed that the prevalence rate in rural areas was (83.6%), and (16.4%) in urban areas. These urinary tract manifestations were dysuria (32%), loin pain (29%), frequency (8%), haematuria (9%), incontinence (7%), nocturia (3%) and others (12%). ELISA and IHT detected S. haematobium antibody in 42 cases (38.18%) and 11 cases (10%), respectively. W.B. detected S. haematobium proteins in 4 cases (3.63%). The performance characteristics of the used methods showed that the western blot technique comparing to ELISA method had a sensitivity of 100%, specificity of 64%, while the western blot comparing to IHT method had a sensitivity of 100%, specificity of 93%, WB profiles allowed the identification of four well-defined bands that were characteristic of Schistosoma haematobium infection. These bands were 65, 70, 80 and 120 kDa. Four positive cases with western blot were also positive by both ELISA and IHT. Western blot technique is a good positive test for detection of schistosomia haematobium.

Key Words: Schistosoma haematobium, IHT(Indirect-Haemagglutination Test), ELISA (Enzyme-Linked Immune-Sorbent Assay), WB(Western Blot)

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INTRODUCTION

Schistosomiasis is a parasitic disease that affects more than 200 million people worldwide [1]. The symptomatology and severity of disease depend on the Schistosome species, the total number of blood flukes harbored in a host, length of infection, level of host immunity, age and gender [2]. Schistosomiasis is a health problem in rural Egypt, with more than six million people infected [3]. The presence of Aswan Dam has led to elimination of S. heamatobium from the Nile Delta, but

Corresponding author: Ibraheem B. M. Ibraheem
Address: Department of Medical Parasitology, Faculty of Medicine, Beni-Suief University, Beni-Suef, 62514, Egypt.
E-mail: ✉ ibraheemborie@science.bsu.edu.eg
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led to the establishment of S. mansoni in Upper Egypt [4]. The early diagnosis of schistosomiasis is necessary for treatment of the acute infection, as the early symptoms and signs are not pathogonomonic and may be neglected leading to chronic diseases [5]. Comparative studies of serological and parasitological methods approved higher sensitivity of the latter, mainly in areas with low endemicity. The presence of cross reactivity with other helminthic infections, and its low specificity after treatment due to slow reduction of specific antibody titer constitutes great disadvantage of the immunodiagnostic techniques [6].The western blot technique is an analytical technique which is used to determine specific protein in a sample of extract or tissue homogenate. These methods are more specific and sensitive than serological methods [7].

MATERIALS AND METHODS

This study was conducted on 110 patients with urinary tract manifestations as loin pain, dysuria, frequency, haematuria, incontinence, nocturia, othersand with negative urine samples for S. haematobium eggs. These patients were from all age groups and from both genders. They were selected from attendances of two hospitals (Beni-Suef General Hospital and the Hospital of Health Insurance). These patients were from rural and urban areas. The samples were collected from January 2015 to August, 2016.

We determined the prevalence of S.haematobium infection. Also, we evaluated different techniques (indirect-haemagglutination, enzyme-linked immune-sorbent assay and Western blot technique). ELISA and IHT may give cross reaction with other parasites so we used another technique called western blot to compare it with ELISA and IHT.

Urine samples were subjected to microscopic examination of urine sediments after centrifugation to exclude the presence of S. haematobium eggs. Blood samples were centrifuged to obtain serum for indirect haemagglutination, ELISA and western blot test. Blood samples were stored at -20 C. A commercial IHT kit (SchistosomiasisFumouze kit) manufactured in Paris, France, was purchased and the test was performed following procedures given by the manufacturer. Samples with a titre below 1:60 were non-significant as it may represent treated patients or old infection, but samples with a titre above 1:60 were positive. ELISA kit (DRG schistosomiasisIgG) manufactured by Dade Behring Marburg GmbH, Germany, was purchased and the test was performed following procedures given by the manufacturer. A spectrophotometer reads all wells using a bichromatic reading with filters at 450 nm and 620-650 nm. Positive - Absorbance reading is equal or more than 0.2 OD units.

Negative - Absorbance reading is less than 0.2 OD units.

Western blot technique detected anti S. haematobium Ab in human serum against specific protein bands of S. haematobium, and S. haematobium adults (crude antigens) were obtained from Schistosome Biological Supply Program Unit, Theodor Bilharz Research Institute. S. haematobium adults were washed for several times and homogenized with phosphate buffered saline (pH 7.4), in a homogenizer (6000r.p.m) for 20 minutes in ice bath and, then sonicated. Sonicated sample was exposed to high speed centrifugation (20.000 r.p.m) for one hour at 4°C. The protein was measured and then stored at -20°C [8]. The molecular weight of S. haematobium antigens was detected by SDS-PAGE in which gel was soaked overnight in coomassiae stain, de-stained with the de-staining solution with several changes till bands became clear. Protein bands were scanned to detect molecular weights of S. haematobium antigen. Then electrophoretic transfer of proteins from Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis to a nitrocellulose sheet was performed. Nitrocellulose strips of fractionated S. haematobium adults (crude antigens) were used against the specific anti-S. haematobium sera at dilution 1:100 by EITB technique to determine immuneresponse to protein bands. Reaction was read by Gel pro-analyzer 3.1.

RESULTS

The study involved both genders, 30.9% of the studied population were males and 69.1% females. As regarding age groups, the study revealed that young group (30-39) was the most prevailing age group (33.6%) while old age group (60-70) was the least group (8.2%). As regarding residence, the study revealed that the prevalence rate in rural areas was (83.6%), and (16.4%) in urban areas. ELISA detected S. haematobium antibody in 42 cases (38.18%),while IHT detected S. haematobium antibody in 11cases (10%). W.B. detected S. haematobium proteins in 4 cases (3.63%).

These urinary tract manifestations were loin pain (29%), dysuria (32%), frequency (8%), haematuria (9%), incontinence (7%), nocturia (3%) and others (12%).The performance characteristics of the used method showed that the western blot comparing to ELISA method had a sensitivity of 100%, specificity of 64%, while the western blot comparing to IHT method had a sensitivity of 100%, and specificity of 93%, WB profiles allowed the identification of four well-defined bands that were characteristic of S. haematobium infection. These bands were 65, 70, 80 and 120 kDa. Four positive cases with western blot were also positive by both ELISA and IHT. We found that bands < 65 kDa were not specific for S. Haematobium, and could not be used for diagnosis.

Statistical Analysis

Data was processed and analyzed using Statistical Package for Social Science, (SPSS) software version 20. Description of qualitative data by frequency distribution with its quantitative and percentage statistics were presented by mean and standard deviation (mean±SD). Comparing seronegative and seropositive patients was performed using t-test and Chi-square. P values less than 0.05 were significant.

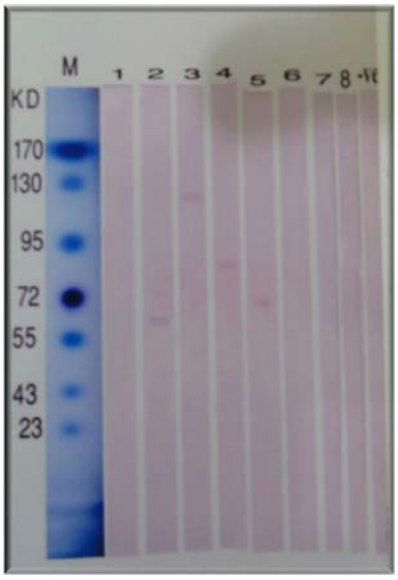

Fig. 1. Showing Western Blot Technique characterization of crude antigen of S. haematobium

Table 1. Results of used diagnostic methods for detection of Schistosoma haematobium among study individuals.

		WB(n=)		
		Positive (n=4)	**Negative (n=106)**	**Total**
ELISA	Positive	4(100)	38(35.8)	42
	Negative	0(0)	68(64.2)	68
IHT	Positive	4(100)	7(6.6)	11
	Negative	0 (0)	99(93.4)	99
Total		4	106	110

DISCUSSION

Schistosomiasis is chronic infections with significant morbidity and of considerable public health importance. The early diagnosis of schistosomiasis is essential for treatment of acute infection, as the initial symptoms and signs are not pathognomonic and may be neglected leading to chronic diseases**.**

The study was performed on 110 patients with urinary tract manifestations as loin pain (29%), dysuria (13%), frequency (8%), haematuria (9%), incontinence (0.5%) and nocturia (3%), and others (37.5%) with negative urine samples for S. haematobium eggs. We determined the prevalence of S. haematobium by different methods and comparative study between serological (IHT, ELISA), and immunological (western blot technique) methods were performed.

We found that ELISA detected S. haematobium antibody in 42 cases (38.18%), while IHT detected S. haematobium antibody in 11cases (10%). WB technique detected S. haematobium proteins in 4 cases (3.63%). The performance characteristics of the used method showed that western blot technique comparing to ELISA method had a sensitivity of 100%, specificity of 64%, while western blot comparing to IHT method had a sensitivity of 100%, specificity of 93%, WB profiles allowed the identification of four well-defined bands that were characteristic of S. haematobium infection. These bands were 65, 70, 80 and 120 kDa. Four positive cases with western blot were also positive by both ELISA and IHT. We found that bands < 65 kDa were not specific for S. Haematobium, and could not be used for diagnosis.

We found that the prevalence of S. haematobium was higher by IHT and ELISA than by WB technique. Because of the high prevalence by IHT and ELISA due to the presence of cross reactivity with other helminthic infections or the presence of antibodies in serum even after treatment [9, 10], we used western blot technique in which we used specific protein for S. haematobium (crude antigen from adult worms) to compare sensitivity and specificity of WB to these serological methods.

Other studies reported that IHT and ELISA are important in diagnosis of schistosomiasis in cases with negative parasitological examination as [11,12], while [13] reported that infected cases with shistosomiasis may be missed due to low sensitivity and specificity of diagnostic methods (ELISA, IHT), so there is no accurate golden test for diagnosis of schistosomiasis.

Regarding WB technique, our study comes in agreement with finding of other studies, as [14] who reported that the sensitivity of WB was 97.3%, and bands of S.haematobium were detected at 65,70,80,95 and 110 kDa. On the other hand, this study is in contrast to the findings of [15] who used homologous adult microsomal antigens reporting that 23-kDa band was specific for infection with S. haematobium by western blot**.** These discrepancies might be due to the use of different antigen.

CONCLUSION

From these results, we found that negative microscopic examination for urine samples do not exclude schistosomiasis haematobium especially in light and old infections. WB showed high sensitivities comparing to ELISA and IHT, and this means it is a good positive test for schistosomiasis without cross reaction with other parasites. So, a combination of clinical manifestation, serological methods and western blot technique increase the chance for detection of schistosomia haematobium.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts.

Author contribution

All manuscript authors contributed to every activity of it; idea of paper, study design, collection of materials, methodology, writing the paper and revising it.

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