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Original Article

Evaluation of the role of ELISA in confirmation of Schistosoma haematobium infection in bilharzial bladder cystitis and bilharzial bladder cancer patients

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Abstract

Background: The Human Schistosoma haematobium (SH) antibody is an IgG that can be used in the diagnosis of SH infection complicated by bladder cancer (BC)

Aim: To evaluate the role of ELISA in the confirmation of *Schistosoma haematobium* infection in bilharzial bladder cystitis and bilharzial bladder cancer patients.

Patients and Methods: This case-control study enrolled 60 cases. 17 to 70 years old, males and females, had urinary manifestations, were grouped into three equal groups. Group I: Twenty patients with bilharzial BC. Group II: Twenty patients with chronic bilharzial cystitis. Group III: control: Twenty Healthy cases, microscopic urine examination, and an ELISA test for blood samples were done for all groups.

Results: Age ranged from 17 to 74 years. The Ages were higher in the bilharzial BC group than in the chronic bilharziasis group (P=0.048). Sex was insignificant among groups. Rural residence was found in all groups. By microscopy, the presence of ova in the urine sample was positive in 14 (70%) in the chronic bilharziasis group, 5 (25%) in the bilharzial bladder cancer group, and was negative in the control group (P value<0.001). Anti-bilharzial AB was positive in 15 (75%) in the chronic bilharziasis group, 17 (85%) in the BC group, and was negative in the control group (P value<0.001). Anti-bilharzial AB can predict bilharzial disease (P value<0.001) with 84.2% sensitivity, 100% specificity, 100% PPV, 76.9% NPV, and 89.7% Accuracy.

Keywords: ELISA; Schistosoma haematobium; Urine; Bladder Cancer

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Introduction:

Schistosoma haematobium (SH) is a parasite that affects millions of people all over the world. This infection spreads mainly in underdeveloped countries, where low socioeconomic status, decreased clean water supply, and inadequate health care are common. (1) This adult trematode inhabits the veins of the genitourinary system, and its eggs can be lodged in its organs. These eggs cause mucosal irritation, inflammatory changes, and granuloma formation. This granuloma is responsible for the chronic manifestations of the disease. Bladder cancer (BC) can occur as a result of the previously mentioned SH egg pathology. [(2)] These egg changes may change the proliferation of the urogenital system epithelial cells and their malignant transformation. *SH* infection is a risk factor for BC disease in endemic countries, especially in Africa and the Middle East. [(3)]

Urine examination for *S. haematobium* egg detection was mainly used in the diagnosis of SH infection. This test is easy and cheap, but it lacks good accuracy and good sensitivity, especially in cases that have light infections. [(4)]

The use of urine microscopy to detect *Schistosoma* eggs in chronic bilharziasis and bilharzial BC has many difficulties. This is because the infection complicates and develops to an advanced stage. This may lead to fibrosis or cancer formation, and this can impair egg shedding. Lastly, this decreases the number of eggs in urine samples. [(5)]

ELISA has been used for antibody or antigen detection of SH infection because this test is simple, specific, and cheap. [(6)]

Patients and Methods:

This research was conducted over 2 years from November 2022 to November 2024, and was carried out in Oncology and Urology Polyclinics, Sohag University Hospital, Biology Molecular Research and Studies Institute, Assiut University.

Study Design: This case-control study was made on 60 patients, who visited Sohag Urology Clinic. their ages were from 17 to 70 years old, males and females, with urinary symptoms (dysuria, haematuria, urinary retention, etc.) who were suspected to had bilharzial bladder cancer or bilharzial bladder cystitis and were planned to do cystoscopy plus twenty healthy cases, were used as controls.

Patients were divided into three equal groups: **Group I:** Twenty Patients with bilharzial BC were selected after cystoscopy and histopathologic examination. **Group II:** Twenty cases with chronic bilharzial cystitis were also chosen after cystoscopy and histopathologic examination. **Group III (control group):** Twenty healthy cases.

The full history was obtained from all cases. Clinical examinations were performed for all participants in the study.

Exclusion criteria: The cases were selected after exclusion of patients who had been diagnosed previously with any cancer within the previous 5 years and who had received chemotherapy.

Urine examination: five or 10 mL of FreSH urine samples were centrifuged at 4000 rpm for 5 minutes. Then, 9 parts of 10 of the supernatant were removed carefully. The sediments were microscopically examined to detect bilharzial eggs.

Blood samples: 3 ml were withdrawn from patients using sterile, labeled tubes.

ELISA: The blood samples were prepared and tested as described in the Human *Schistosoma haematobium* antibody manual kit, IgG **(Cat ED0176Hu)**.

Semi-automated ELISA system Absorbance Reader (**TECAN, Switzerland**), and **SHaker Incubator (N-Biotek (NB205), Korea) were used.** The steps were done according to the manufacturer's technique.

Statistical analysis: SPSS v27 (IBM©, Chicago, IL, USA) was used. Mean, median, and standard deviation (SD) were used to analyse data. The Shapiro-Wilk test and histograms were used in the data evaluation. The T-test was used in data analysis. The chi-square and ANOVA tests were used in data analysis. Positive predictive value (PPV) and negative predictive value (NPV) were used to examine the accuracy of the data. A P value < 0.05 was considered statistically significant.

Ethical consideration: The research was conducted after agreement of the Ethical Committee of the

Faculty of Medicine, Sohag University, Sohag, Egypt, with IBR Registration number (Soh-med-22-11-12). Informed written consent was obtained from the cases or their relatives.

Results:

Age ranged from 17 to 74 with a mean value (± SD) of 49.7 (±17.76) years in the chronic bilharziasis group, ranged from 48 to 80 with a mean value (± SD) of 61.1 (±9.01) years in bilharzial bladder cancer group and ranged from 35 to 70 with a mean value (± SD) of 53.7 (±10.95) years in the control group. The Ages were higher in the bilharzial BC group than in the chronic bilharziasis group (P= 0.048). These ages were insignificantly different between the bilharzial BC group, the chronic bilharziasis group, and the control group. Sex was insignificantly different among the three groups. Rural residence was found in all three groups (Table 1).

Table 1: Demographic data, age distribution, gender, and residence of the studied groups

Variable	**Chronic bilharziasis group (N=20)**		**Bilharzial BC group (N=20)**		**Control group (N=20)**	
	49.7±17.76		61.1±9.01		53.7±10.95	
	P1=0.048*, P2=0.494, P3=0.408					
	Number	**Percentage %**	**Number**	**Percentage %**	**Number**	**Percentage %**
Gender						
Male	16	80	17	85	16	80
Female	4	20	3	15	4	20
Residence						
Urban	0	0	0	0	0	0
Rural	20	100	20	100	20	100

P1: P value of age distribution between chronic bilharziasis group and bilharzial BC group, **P2**: P value of age distribution between chronic bilharziasis group and control group, **P3**: P value of age distribution between bilharzial BC group and control group.

The presence of ova in the urine sample was positive in 14 (70%) in the chronic bilharziasis group, 5 (25%) in the bilharzial bladder cancer group, and was negative in all patients in the control group. The presence of ova in the urine sample was significantly higher in the chronic bilharziasis group than in the bilharzial bladder cancer group and control group (P value<0.001) (Table 2).

The presence of ova in the urine sample was significantly higher in the chronic bilharziasis group (75%) than in the bilharzial BC group and the control group (P <0.001) (Table 2).

Table 2: Detection of Schistosoma haematobium eggs in the urine samples of the studied groups:

Variable	Chronic bilharziasis group (N=20)		Bilharzial BC group (N=20)		Control group (N=20)	
	Number	Percentage %	Number	Percentage %	Number	Percentage %
Positive Anti-bilharzial AB	14	70	5	25	0	0
Negative Anti-bilharzial AB	6	30	15	75	20	100
P-value	**(P value<0.001)**					

Anti-bilharzial AB was positive in 15 (75%) in the chronic bilharziasis group, 17 (85%) in BC group in the chronic bilharziasis group, and was negative in all patients in the control group. Anti-bilharzial AB was significantly higher in the bilharzial bladder cancer group than in the chronic bilharziasis group and control group. (P value<0.001). (Table 3).

Table 3: Anti-bilharzial antibodies of the studied groups

Variable	Chronic bilharziasis group (N=20)		Bilharzial BC group (N=20)		Control group (N=20)	
	Number	Percentage %	Number	Percentage %	Number	Percentage %
Positive Anti-bilharzial AB	15	75	17	85	0	0
Negative Anti-bilharzial AB	5	25	3	15	20	100
P-value	**(P value<0.001)**					

Anti-bilharzial AB can predict bilharzial disease (P value<0.001) with 84.2% sensitivity, 100% specificity, 100% PPV, 76.9% NPV, and 89.7% Accuracy (Table 4).

Table 4: Accuracy of anti-bilharzial AB in the prediction of diagnosis of bilharzial disease

Sensitivity	Specificity	PPV	NPV	Accuracy
84.2%	**100%**	**100%**	**76.9%**	**89.7%**
(P-value<0.001)				

*Significant as P value ≤0.05, PPV: Positive predictive value, NPV: Negative predictive value.

Discussion

Urine microscopy (UM) is the first-line diagnostic method of urogenital schistosomiasis. Enzyme-linked immunosorbent assay (ELISA) is a common method for screening many parasitic infections, primarily or alternatively. The ELISA system can supplement the conventional diagnosis by UM. [6]

In our study, age was significantly lower in the chronic bilharziasis group than the bilharzial

bladder cancer group, and was insignificant between the chronic bilharziasis group and the bilharzial bladder cancer group. And the control group. Sex was insignificantly different among the three groups. Residence was rural in all patients of the three groups, which could be attributed to the fact that bilharzial infestation requires an agricultural environment. [7]

Chronic infection by *S. haematobium* must be present for the development of carcinogenesis, which leads to serious complications such as bladder cancer, which may explain why age was significantly lower in chronic bilharziasis than bilharzial bladder cancer. [8]

Our results agreed with Eissa et al., 2015 [9], who reported that sex was insignificantly different among the groups of their study on bilharzial bladder cancer.

This work agreed with Mursi et al., 2013,[10] who conducted that age was higher in the bladder cancer group than in the chronic cystitis group.

This study disagreed with Gaber et al., 2020. [11] who showed that age was insignificantly different between cases with bladder cancer and cases with bilharzial cystitis. They reported also that sex was significantly different, with male predominance in both groups. This study disagreed with them, they reported that the residence was rural in all cases with bilharzial cystitis and urban in cases with bladder cancer. This difference may be attributed to different sample sizes, demographic areas and populations.

In the current study, the presence of egg in urine samples was higher significantly in the chronic bilharziasis group than in the bilharzial BC group and control group. This work agreed with Gaber et al. [11] who reported that the presence of ova in urine samples was significantly higher in bilharzial cystitis cases compared to bilharzial BC cases and controls. Supporting our findings, Abd Ellah and Mohammed, 2024, [12] showed that *S. haematobium* eggs embedded in cancer tissue may explain why the eggs in the urine sample of cases with bilharzial BC were low.

The results in our research may be explained by the immunological and inflammatory reaction to egg deposition, resulting in granuloma formation. In chronic bilharziasis, eggs travel from the lumen of blood vessels to surrounding tissues, where many pass through the bladder mucosa and are shed in the urine. This host's response to egg deposition may lead to calcification of the urinary bladder, chronic infection, and stone formation, and these changes may cause bladder cancer. In chronic bilharziasis, SH eggs are deposited in the urinary bladder mucosa and submucosa. This chronic stage leads to continuous SHedding of eggs into the urine. But in the case of bilharzial BC, the infection progresses to an advanced stage accompanied by fibrosis or tumor formation; these changes impair the shedding of eggs. This reduces the number of eggs found in the urine samples Schwartz and Fallon, 2018a. [13]

Also, Barsoum et al., 2013 [14]) reported that the host's cell-mediated response to the trapped SH eggs initiates a granuloma formation, fibrotic changes, and tissue calcification, that may explain the presence of a few eggs in the urine samples.

In this study, Anti-bilharzial AB against soluble egg antigen by ELISA was significantly higher in the bilharzial bladder cancer group than in the chronic bilharziasis group and control group. Anti-bilharzial AB can diagnose bilharzial disease with 84.2% sensitivity, 100% specificity, 100% PPV, 76.9% NPV, and 89.7% Accuracy.

Fasogbon et al., [15] showed that the ELISA test was better than the indirect haemagglutination (IHA) test in bilharzial BC because it can detect active infections despite BC pathology, has higher specificity and sensitivity, provides quantitative results which are useful for monitoring disease progression, and is very effective in early-stage assessment.

This work agreed with Gaber et al., 2020, [11] who reported that anti-Schistosoma antibodies by the IHA test were significantly higher in the bladder cancer group than in the bilharzial cystitis group.

In agreement with our findings, El-SHall et al. [16] showed that ELISA is a good alternative diagnostic procedure in SH infection diagnosis due to its high sensitivity, leading to the diagnosis of active SH infection, and antigen assessment, which allows the direct determination of SH worm burden.

In the same line with our findings, Mahmoud et al., 2021, [17] in their study to evaluate an immunomagnetic-bead (IMB)-based-ELISA in the diagnosis of urinary SH infection in areas at risk of SH infection in Upper Egypt, They reported that the evaluated ELISA assay's sensitivity, specificity, and diagnostic accuracy using urine examination by microscopy, were 94.87%, 95.22%, and 94.48%, respectively.

Supporting our findings Song et al., [6] conducted a study on 149 cases for the diagnosis of SH Infection. They found that ELISA for detecting serum immunoglobulin (Ig) G antibodies by using soluble egg antigen of SH for diagnosis of urogenital schistosomiasis had a 94.8% sensitivity and 29.7% specificity.

Moreover, Hinz et al.[18] mentioned in their review the serological methods for the diagnosis of schistosomiasis. They detected antibodies against SH by using ELISA with 100% specificity and 94% sensitivity.

Additionally, Elhag et al., [19] on their study on 66 urine and 66 serum-paired samples obtained from patients who confirmed parasitological negative and positive with SH infection, They showed that by ELISA the percentage of positive IgG cases in urine was 92.40%, whereas 96.97% in serum compared to negative IgG cases, which were 7.60% and 3.03% in urine and serum. The ELISA is a useful, sensitive diagnostic method for diagnosis, especially in detecting endemic foci.

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