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The Effect of Treatment on Viability of Eggs among Urinary Schistosomiasis Patients in Al-Shajara Area, Khartoum State-Sudan

TAYSEER ELAMIN MOHAMED ELFAKI[1]
Department of Parasitology and Medical Entomology
College of Medical Laboratory Science
Sudan University of Science and Technology, Khartoum, Sudan
ABU-OBIADA HASSB-ELRASOOL OSMAN
Department of Parasitology, Daweena Health Centre
Health Insurance-Sinnar State, Abohogar Locality, Sudan
MOHIELDIN ELSAYID
Department of Clinical Laboratory Sciences
College of Applied Medical Sciences
King Saud Bin Abdulaziz University for Health Sciences
Riyadh, Saudi Arabia

Abstract:

This study was conducted to evaluate the effect of treatment on the number of viable and dead eggs among urinary schistosomiasis patients in Al-Shajara Area, Khartoum State-Sudan by using methylene blue and trypan blue stain. For this purpose, a cross-sectional study was carried out during the period from December 2012 to April 2013. Participants in this study included one hundred and forty one patients (age between 15-55 years old) all of them were males. Urine samples were collected from all subjects. Parasitological data were obtained and recorded. The urine samples were examined to detect the eggs of Schistosoma haematobium by using wet preparation technique. Out of 141, 50 (35%) were found positive and 91(65%) were found negative. This study showed that there is association between intensity of infection and presence of haematuria and there is no association between age groups and intensity of infection. Sedimentation technique was used to determine the intensity of

[1] Corresponding author: talfaki@yahoo.com

infection. Out of 50, 11 (22%) were presented as light infection and 39 (78%) were presented as heavy infection. The viability of eggs was detected by using methylene blue and trypan blue staining techniques. This study concludes that the mean number of viable eggs in treated patients was lower than the mean number of viable eggs in untreated patients and the mean number of dead eggs in treated patients was higher than the mean number of dead eggs in untreated patients with significant value less than 0.05.

Key words: Methylene blue, *Schistosoma haematobium*, Sedimentation technique, Treatment, Viability test.

INTRODUCTION:

Schistosomiasis is second only to malaria as a parasitic disease of serious public health importance in subtropical and tropical Africa; schistosomiasis is endemic to 76 countries[(1)]. Schistosomiasis is caused by blood dwelling flukes called schistosomes. The worms were first described in 1852 by Theodore Bilharz, hence the illness is also known as bilharzias [(1)]. Schistosomiasis is a global health problem with over 200 million people infected worldwide and an estimated 200,000 deaths per year. There are three main species of *Schistosome* with world distribution largely reflecting the presence of specific snail hosts. Infection in humans results from fresh water exposure and the illness is characterized by both acute and chronic manifestations [(2)]. Reasons for the persistence of the disease in spite of prolonged control and preventive efforts include wide distribution of the intermediate host, migration, the dependency of many poor populations in both rural and urban areas on *Schistosomes*-infested water sources for their domestic, occupational and recreational needs, lack of sanitation, portable water and scarcity of and deficiencies in preventive and curative health services[(2)]. Urinary schistosomiasis results from granulomatous inflammation

resulting from the migration of *S.haematobium* eggs into the ureteric and bladder lumen. Microscopic hematuria is common. However, occasionally gross hematuria is reported, often in the terminal portion of the urine, dysuria [(3)]. Progressive fibrosis and calcification can lead to ureteric obstruction and hydronephrosis. There is also a reported higher incidence of squamous cell bladder carcinoma in individuals with chronic urinary schistosomiasis[(4).] The main objectives of this study were to determine to evaluate the effect of treatment on the number of viable and dead eggs of *S.haematobium* by using methylene and trypan blue in Al-Shajara area, to detect the prevalence of *S.haematobium* in study area, to detect the intensity of infection by using sedimentation techniques, to compare between methylene blue and trypan blue stains, to determine relationship between intensity of infection and hematuria, to determine relationship between intensity of infection and age groups, to determine relationship between intensity of infection, treatment and complete dose of treatment and to evaluate the effect of complete of dose of treatment on the number of viable and dead eggs of S.haematobium by using methylene and trypan blue.

MATERIALS AND METHODS:

Study area:

This study was conducted in Al-Shajara area, Khartoum State, Sudan, during the period from December 2012 to April 2013.

Study subjects:

The studied populations included in this study were males with different residential area (Al-Lamab, Bahar Abiad, Alray Almasry, Eleshlag and Alhamadab) who were come to Al-Shajara to work and swim those participants with different education levels and age ranging from 15 to 55 years with the

mean age was 25 year. Random samples were collected from them after their agreed to participate in this study. The age of study subjects included in the present study was divided into 4 groups 15-25, 25-35, 35-45 and 45-55 year.

Study design:

This was cross-sectional study conducted to determine the effects of treatment on viability of eggs among urinary schistosomiasis patients who attended Al-Shajara Health Centre.

Sample size:

A total of 141patients, who attended Al-Shajara Health Centre during the period from December 2012 to April 2013 were included in the study.

Sampling:

In this study a total of 141 questionnaires were administered. And a total of 141 urine samples were collected, from among those that filled the questionnaire.

Design of questionnaire:

The design of questionnaire include gender, age, observation of blood and mucus in the stool, visit to water bodies (risk factor), history to previous infection and previous treatment, presence of latrines in the houses, source of drinking water and a simple knowledge on the sign and symptoms of the diseases and also employs the activities that put an individual at the risk of infection.

PARASITOLOGICAL INVESTIGATIONS:

Urine examination:

After well mixing, 10ml of urine were transferred into conical tube and centrifuged at medium speed of approximately 5000 RPM for 3 minutes. Supernatant was discharged and the sediment was mixed and then transferred to slide and covered with cover glass, the prepration was examined microscopically using 10x objective lenses for search end 40x for identification, examined all the sediment and the number of egg were counted per 10 ml of urine.

Staining technique:

After well mixing, 10ml of urine were transferred into conical tube and centrifuged at medium speed of approximately 5000 RPM for 3 minutes. Supernatant was discharged and the sediment was mixed and then transferred to slide, drop of methylene blue was added to a preparation and drop of trypan blue was added to another preparation and covered with cover glass, then viable and dead eggs were counted per 10 ml of urine.

DATA ANALYSIS:

Data were analyzed using Statistical Package for Social Sciences (SPSS) under windows, version 15.0. Frequencies, mean, Chi square test statistical analysis were performed and the p values of less than 0.05 were considered statistically significant. Data were presented in tables after analysis using SPSS.

Ethical approval:

The study approved by College of Medical Laboratory Science-Sudan University of Science and Technology and an informed consent was obtained from all subjects included in this study.

RESULTS:

The study was conducted on 141 subjects from Al-Shajara area with an age ranging between 15-55 years old and the mean age of 25 years, all of them were males. The surveyed populations were categorized into four age groups: (15-24), (25-34), (35-44) and (45-55) year. The frequency of each age group was 27 (36%), 32 (42.7%), 7 (9.3%), 3 (4%), 5 (6.7%) and 1 (1.3%) of the total population respectively. For detection of S.haematobium eggs 141 urine samples were collected, within these samples, 50 (35.46%) were found positive by wet preparation technique while 91(64.54%) were negative table (1). The intensity of infection was obtained by counting the number of S.haematobium eggs per 10ml of urine by using sedimentation technique table (2). ≤ 50 eggs per 10ml of urine presented as light infection and > 50 eggs per 10ml of urine as heavy infection table (3). Out of 141studied population, 46 (32.6%) had blood in their urine, among them, 42 (29.97%) were positive and 4 (2.84%) were negative and among 95 (61.4%) had no blood in their urine 8 (5.67%) were found to be positive and 87 (61.7%) were negative table (4). Chi-squire test was used to determine the relationship between intensity and haematuria (p=0.000) table (5) and to determine the relationship between intensity of infection and age groups of patients (p=0.337) table (6). Out of 50 positive cases, 32 (64%) with low level of education, 16 (32%) with medium education level and 2 (4%) with high education level table (7). Out of 50 positive cases, 31 (62%) had previous infection while 19 (38%) were infected for first time previous infection table (8). Chi-squire test was used to determine the

relationship between intensity of infection and treatment (p=0.000) table (9) and to determine the relationship between intensity of infection and complete dose of treatment (p=0.000) table (10). Out of 50 positive cases, 39 (78%) had knowledge about disease while 11(22%) had no knowledge about disease table (11). Independent sample T test were used to determine the relationship between mean of viable and dead eggs and treatment (p=0.013) and (p=0.000) respectively table (12), to determine the relationship between mean of viable and dead eggs and complete of dose of treatment (p=0.003) and (p=0.000) respectively table (13) and to compare between methylene blue and trypan blue as viability stains (p=0.959) and (p=0.927) respectively table (14).

Table (1): Overall prevalence of S.haematobium in study area

Samples	Frequency	Percentage (%)
Positive	50	35.46%
Negative	91	64.54%
Total	141	100%

Table (2): Results of sedimentation technique

Range of eggs	Frequency	Percentage (%)
1-100	19	38%
101-200	21	42%
201-300	6	12%
301-400	2	4%
401-500	1	2%
501-600	0	0%
601-700	1	2%
Total	50	100%

Table (3): Intensity of infection

Intensity of infection	Frequency	Percentage (%)
Light	11	22%
Heavy	39	78%
Total	50	100%

Table (4): Prevalence of *S.haematobium* among studied population with blood in their urine

Result	**Haematuria**				**Total**
	Absent		Present		
	Number	Percent (%)	Number	Percent (%)	
Positive	8	5.67%	42	29.97%	50
Negative	87	61.7%	4	2.84%	91
Total	95	67.4%	46	32.6%	141

Table (5): Relationship between intensity of infection and haematuria

Haematuria	**Intensity of infection**		**Total**
	Heavy	Light	
Present	37	5	42
Percent (%)	74.0%	10.0%	84.0%
Absent	2	6	8
Percent (%)	4.0%	12.0%	16.0%
Total	39	11	50
Percent (%) of total	78.0%	22.0%	100.0%

Table (6): Relationship between intensity of infection and age groups

Intensity	**Age groups**				**Total**	**p value**
	15-25	25-35	35-45	45-55		
Light infection	10	1	0	0	11	0.337
Percent (%)	20.0%	2.0%	.0%	.0%	22.0%	
Heavy infection	25	6	6	2	39	
Percent (%)	50.0%	12.0%	12.0%	4.0%	78.0%	
Total	35	7	6	2	50	
Percent of total	70.0%	14.0%	12.0%	4.0%	100.0%	

Table (7): Overall prevalence of *S.haematobium* according to level of education

Schistosomiasis	**Education level**			**Total**
	Low	medium	high	
Positive	32	16	2	50
Percent (%)	64.0%	32.0%	4.0%	100.0%

Table (8): Overall prevalence of *S.haematobium* according to history of previous infection

Previous infection	Frequency	Percentage (%)
Yes	31	62
No	19	38
Total	50	100

Table (9): Relationship between intensity of infection and treatment

Treatment	Intensity		Total
	Light	Heavy	
Yes	8	3	11
Percentage (%)	16.0%	6.0%	22.0%
No	3	36	39
Percentage (%)	6.0%	72.0%	78.0%
Total	11	39	50
Percentage (%)	22.0%	78.0%	100.0%

Table (10): Relationship between intensity and complete dose of treatment

Complete dose	Intensity		Total
	Light	Heavy	
Yes	5	0	5
Percentage (%)	10.0%	.0%	10.0%
No	6	39	45
Percentage (%)	12.0%	78.0%	90.0%
Total	11	39	50
Percentage (%)	22.0%	78.0%	100.0%

Table (11): Overall prevalence of *S.haematobium* according to knowledge about schistosomiasis

Knowledge	Frequency	Percentage (%)
Yes	39	78%
No	11	22%
Total	50	100%

Table (12): Relationships between mean of viable and dead eggs and treatment

Eggs by viable stain	Treatment	Frequency	Mean
Viable	Yes	11	56.23

	No	39	132.06
Dead	Yes	11	27.58
	No	39	13.42

Table (13): Relationship between mean of viable and dead eggs and complete of dose of treatment

Eggs by viable stain	complete dose	Frequency	Mean
Viable	Yes	5	46.50
	No	45	123.03
Dead	Yes	5	35.40
	No	45	14.58

Table (14): Comparison between methylene blue and trypan blue as viability stains

Method	Eggs	Mean
Methylene blue	Viable	116.04
	Dead	16.82
Trypan blue	Viable	114.72
	Dead	16.50

DISCUSSION:

Schistosomes are parasitic, trematode worms with a complicated life cycle using mammals as the final host in which large amounts of eggs, the cause of almost all symptoms, are produced by the adult female worm. More than a billion people are affected by schistosomiasis, a disease widely spread in the tropical belt of the world where about third of the world's population live. More than 200 million are infected where and up to 800 million daily at risk to become infected (5). Testing viability of eggs is important in determining the stage of infection. In some long standing infections, dead eggs may be found in feces. Viability may be determined by direct examination of the eggs or inducing the eggs to hatch (6). The presence of living miracidia within the eggs indicates an active infection that may require therapy. After patient take

treatment must be followed to evaluate the efficiency of drugs. So, this study was conducted to evaluate the effect of treatment on number of viable and dead eggs of S.haematobium; for this purpose 50 positive samples were examined by viable stain (methylene blue and trypan blue) to show viable and dead eggs. In this study statistical tests were used, the p value is (0.05), less than 0.05 is significant and more than 0.05 is insignificant. So all p value get from results compared with 0.05. The prevalence of S.haematobium infection in Al-Shajara area was 35.46% due direct contact with water infested with snails of S.haematobium so the intensity of infection showed 39 (78%) was heavy while 11 (22%) was light infection this also showed that 31 (62%) of patients have previous infection while 19 (38%) were infected for first time. This study showed that the mean number of viable eggs in treated patients was lower than the mean number of viable eggs in untreated patients (p=0.013) and the mean number of dead eggs in treated patients was higher than the mean number of dead eggs in untreated patients (p=0.000) this result is similar to of result of (Braun and Southgate, 2001) which carried out in South Africa. This study showed that the complete dose of treatment decrease the mean number of viable eggs (p=0.003) and increase the mean number of dead eggs (p=0.000). Also this study showed that no difference between trypan blue and methylene blue in determine viability of eggs (p=0.927) and (p=0.959) respectively. This study showed that there is association between intensity of infection and presence of hematuria (p=0.000) and there is no association between age groups and intensity of infection (p=0.337). This study also showed association between intensity of infection and treatment and complete dose of it (p=0.000) for both. Out of 50 patients 39 (78%) had knowledge about schistosomiasis while 11 (22%) had no knowledge about disease also the majority of them with low education level 32 (64%) and

16 (32%) with medium education level and the rest 2 (4%) with high education level.

CONCLUSION:

This study concluded that the mean number of viable eggs in treated patients was lower than the mean number of viable eggs in untreated patients and the mean number of dead eggs in treated patients was higher than the mean number of dead eggs in untreated patients.

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