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Relationship between intestinal Schistosomiasis and enteric fever among Sudanese patients, New Halfa Town, Kassala State, Sudan

Abstract

The present study was conducted in New Halfa Agricultural Scheme, Kassala state during the period from March to April 2011. The study aimed to determine the association between intestinal schistosomiasis and typhoid fever. Seventy-five (n=75) schistosomiasis patients were included in this study, they were 26 males (38.7%) and 49 females (61.3%) with different ages ranging from 1 to 60 years. The stool and blood samples were collected. The stool samples were examined to detect the eggs of Schistosoma mansoni by using wet preparation and calculating the number of eggs by using Kato Katz technique. The stool samples were cultured in MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar to isolate Salmonella species, and the serum samples tested for antibody titer by using Widal test (slide method). The results showed that 16 out of 75 (21.3%) exhibited positive growth for the genus salmonella, eleven (11) were Salmonella paratyphi B (14.7%), while four (4) were Salmonella typhi (5.3%). Widal test revealed that 43 out of 75 (57.3%) were insignificant (1/20, 1/40), 15 out of 75 were suggestive (20%) and 17 out of 75 were significant (22.7%). Furthermore, there was no significant association between typhoid fever and intensity of intestinal schistosomiasis (P> 0.05) and no significant variation between antibody titers and intestinal schistosomiasis (P> 0.05).

Keywords: intestinal schistosomiasis, Typhoid fever, schistosoma mansoni, salmonella species

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Background

Salmonellosis continues to be a major health problem in developing countries, Salmonella enterica serotype Typhi and Salmonella enterica serotype Para-typhi A are common causative organisms for typhoid and paratyphoid fever, respectively.[1] These bacteria are commonly found in the tegument or the intestinal tract of Schistosoma mansoni adult worms. The role of schistosomes as a source and vehicle of Salmonella infection has been well established.[2] Sera from patients with hepatosplenic schistosomiasis were found to have reduced antibody activity against *S .typhi* and *S. cholerasuis* compared with normal controls. Further, chronic persistent Salmonella bacteremia has been described in association with S. mansoni infection.[2] The association of Gram-negative bacteria and helminthes parasites has been reported previously. The above mentioned clinical evidence largely suggests that the bacteria-parasite association may provide the persistence of *Salmonella* infection.[1]

Rationale

Salmonella continues to be a major health problem in developing countries. The association of Salmonella and S. mansoni has been reported previously[2] and the role of schistosomes as a vehicle of Salmonella infection has been suggested.[2] Therefore, the detection of persistent Salmonella infection should be carried out in the cases of intestinal schistosomiasis by cultural techniques of stool specimens and serologically using the Widal test.

Objectives

General objective

This study was carried out to determine the possible relationship between typhoid and intestinal schistosomiasis in New Halfa.

Specific objectives

a. To detect S.mansoni eggs and their intensity in stool specimens collected from patients suspected with intestinal schistosomiasis.

b. To isolate and characterize *Salmonella* species from patients infected with *S. mansoni*.

c. To perform a Widal test among patients co-infected with intestinal schistosomiasis and salmonellosis to confirm this parasite-bacteria co-infection.

Materials and methods

Study design

It is a descriptive cross-sectional study.

Study duration

This study carried out during the period from March to April 2011.

Study area

New Halfa Agricultural Scheme, Kassala State, Sudan. This is an irrigation scheme with many irrigation canals heavily infected with snails that host S.mansoni.

Study population

Males and females with different ages and occupations resident in different localities in New Halfa city, suffering from intestinal schistosomiasis were selected for this study. Most, if not all, of the selected persons, are either peasants or agricultural workers continually exposed to contaminated canals.

Sample size

Seventy-five (n=75) patients were included in this study. Fresh stool and blood specimens were collected from each patient, properly packed, well preserved and immediately transported to Khartoum.

Sample collection:

Types of specimens:

Stool

Fresh stool specimens were obtained from the patients and examined for the presence of *S.mansoni* eggs, and cultured in Selenite-F broth for primary isolation of bacteria.

Blood

Five mL venous bloods were collected in a plain container, and then the serum was separated by centrifugation to the subsequent examination of antibody titer by Widal test.

Data collection

Data was collected through a well designed questionnaire, contained all of the necessary variables.

Statistical analysis

The data were analyzed using the SPSS computer program, frequencies, cross-tabulation, and P values were calculated.

Laboratory analysis

Stool examination

Schistosoma mansoni was detected in stool samples using wet preparation and Kato Katz technique as described by Cheesbrough.[3]

Culture

This was done by inoculation of stool specimen in enrichment media (Selenite F broth) and sub-cultured after overnight incubation in MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar. The identification of bacteria was performed by Gram staining and biochemical tests.

Primary Isolation

Using a wood stick, all specimens were inoculated under aseptic conditions into the Selenite F Broth and the inoculated media were aerobically inoculated at 37°C overnight.[3]

Subculture

Using sterile loops, all specimens were inoculated under aseptic conditions onto MacConkey agar and XLD agar. The inoculated media were incubated at 37°C, aerobically, for overnight.[3]

Identification

The organism was identified by Gram staining and biochemical tests.

Gram staining

Fixed and dried smear of the bacteria were covered with crystal violet stain for 30 seconds. After washing with water, the smear was covered with Lugol's iodine for 30 seconds, washed and decolorized rapidly by alcohol for few seconds, washed immediately and covered with safranin for 2 minutes, washed and examined microscopically (×100).[3]

Biochemical tests

Indole test

This test based on the breakdown of tryptophan to produce the indole which then detected by the addition of Kovac's reagent.[3] The organism cultured using a wire loop in about 3 mL of sterile peptone water and incubated aerobically at 37°C overnight. The Kovac's reagent was added and the red ring indicated the presence of indole and yellow or brown ring indicated no indole.[3]

Citrate utilization test

This test is based on the ability of an organism to use citrate as the only source of carbon.[4] Koser's citrate broth was inoculated by using wire loops and incubated aerobically at 37°C overnight. Detection of bright blue color indicated positive result and no change indicated negative result.[3,4]

Urease test

The test is based on the ability of bacteria to produce urease enzyme to give ammonia and carbon dioxide.[4] The organism was cultured using a straight loop in agar medium containing urea and phenol red. If bacteria were urease producer it breakdown the urea and changed the color into pink.[3,4]

Kligler Iron Agar

The organism was inoculated using a straight loop. The butt was stabbed and the slope was streaked. Then incubated aerobically at 37°C overnight to observe the yellow butt (glucose fermentation), yellow slope (lactose fermentation), air bubbles or cracks (formation of gas) and black color (formation of H_2S).[3]

Widal test

The bacterial antigens are used to detect anti-*Salmonella* antibodies in human serum. The reagents standardized suspension of killed and stained bacteria (antigens) agglutinate when mixed with samples containing the homologous antibody (Edward, 1995). 50µL of the reagents were added to each sample. By using disposable stirrer the sample and reagents were thoroughly mixed. After one minute incubation, the agglutination was read macroscopically.[3]

Results

A total of 75 subjects were included in this study collected from different parts of the New Halfa Irrigation Scheme from March to April 2011. The study was carried out to detect the relationship between typhoid and paratyphoid with intestinal schistosomiasis.

Distribution of schistosomiasis patients according to gender

Figure 1 shows that out of 75 schistosomiasis patients, 46 were females (61.33%) and 29 were males (38.7%).

Distribution of schistosomiasis patients according to age group

Out of 75 schistosomiasis patients, 32 were within age group 11–20 (42.67%), 27 were within 1–10 (36%), 7 were within 21–30 (9.33%), 5 were within 41–50 (6.67%). 3 were within 31–40 (4%), and 1 was 51–60 years old (1.33%) (Figure 2).

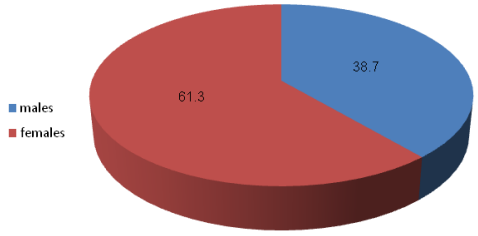

Figure 1 Distribution of schistosomiasis patients according to gender.

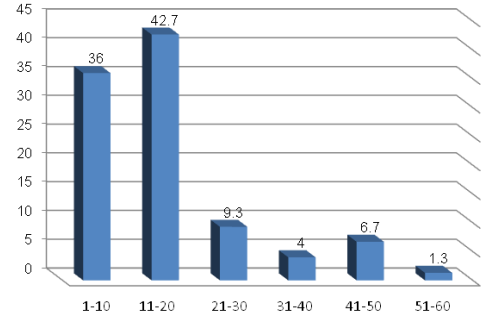

Figure 2 Distribution of schistosomiasis patients according to age.

Detection of anti-Salmonella antibodies among schistosomiasis patients by using Widal test

The results of Figure 3 demonstrate that out of 75 schistosomiasis patients examined using Widal test, 17 were shown positive for anti-Salmonella antibodies (22.7%), however, 15 subjects were suggestive (20%) and 43 schistosomiasis patients (57.3%) did not reveal any anti-Salmonella antibodies in their sera.

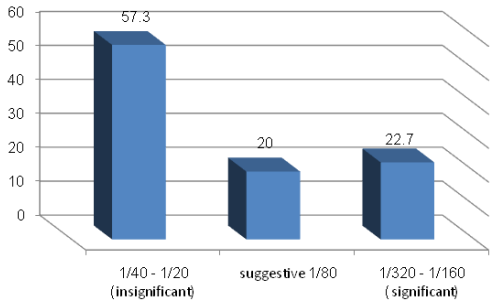

Figure 3 Isolation of anti-Salmonella antibodies among schistosomiasis patients using Widal test.

Isolation of Salmonella species from stool specimens of the schistosomiasis patients

Figure 4 illustrates that while Salmonella species where isolated from stool specimens collected from 16 schistosomiasis patients (21.3%), 59 stool specimens did not show any Salmonella growth (78.7%).

Intensity of S. mansoni infection among schistosomiasis patients

According to the schistosomes intensity measured by counting the number of eggs in one gram of stool, 54 revealed mild infections (72%) with S. mansoni 24–100 egg /g), 20 exhibited moderate infection (26.7%) (101–399 eggs/g), and one had a severe infection with S.mansoni (>399 eggs/g) (Figure 5).

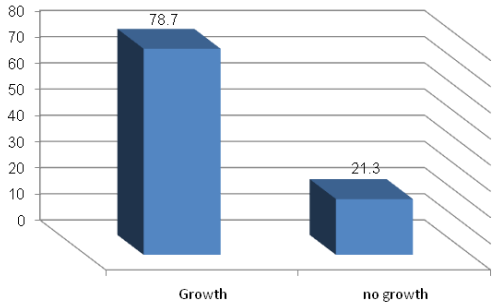

Figure 4 Isolation of Salmonella species from stool specimens of schistosomiasis patients.

The effect of S. mansoni intensity on infection with Salmonella among schistosomiasis patients

The results of table 1 reveal that out of 54 mild intensity schistosomiasis patients, 4 showed S. typhi from stool specimens (8%), 6 recovered S. paratyphi B (11%). Out of the moderate schistosomiasis patients, 5 revealed S. paratyphi B (25%), and only one exhibited S. typhi (5%). These findings showed no significant variation between S. mansoni intensity and infection with Salmonella (P> 0.05).

The effect of age of schistosomiasis patients on Salmonella infection

Table 2 exhibits that three schistosomiasis patients were infected with S.typhi (9.4%) among the 11–20 years age group, and 6 recovered *S.paratyphi* B from their stool specimens. Two schistosomiasis patients were infected with S.typhi among the age group 21–30 (28.6%). However, 5 infected with S.paratyphi B among the 1–10 years age group (18.5%). These results demonstrated no significant variation of *Salmonella* infection among age groups (P>0.05(.

The relationship of anti-Salmonella antibodies with the isolation of Salmonella species among schistosomiasis patients

Out of 43 schistosomiasis patients with an insignificant titer of anti-Salmonella antibodies, 8 revealed isolation of Salmonella (18.6%). While 15 patients with suggestive titers, only 3 exhibited anti-Salmonella antibodies from their sera (20%). However, 5 patients infected with Salmonella had significant titers from anti-Salmonella antibodies (29.4%). These findings showed no significant variation of anti-Salmonella antibodies with the isolation of Salmonella species (P> 0.05) (table 3).

The effects of S. mansoni intensity on anti-Salmonella antibodies among schistosomiasis patients

The results in table 4 demonstrated that out of 75 schistosomiasis patients examined by Widal test, 43 showed insignificant titer, out of whom 32 were of mild intensity for S.mansoni (74.4%), 11 were of moderate-intensity (25.6%), and no severe cases observed. However, 15 showed suggestive titer, out of whom 11 were of mild intensity (73.3%), and 4 revealed moderate intensity. Seventeen schistosomiasis patients had significant titer, out of whom 11 were exhibited mild intensity (64.7%), 5 revealed moderate intensity (29.4%) and only one of severe intensity (> 399 eggs/g). These results revealed no significant variation of *S.mansoni* intensity on anti-Salmonella antibodies (P> 0.05).

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Table 1 The effect of S. mansoni intensity on infection with Salmonella among schistosomiasis patients

Intensity of Schistosoma infection	Result of Salmonella culture					
	S. typhi		S. paratyphi B		No growth	
	No.	percentage	No.	percentage	No.	percentage
Mild (54)	4	8%	6	11%	44	81%
Moderate (20)	1	5%	5	25%	14	70%
Severe (1)	0	0%	0	0%	1	100%

P. value: 0.920

Table 2 The effect of age of schistosomiasis patients on Salmonella infection

Age groups	Salmonella infection					
	S.typhi		S. paratyphi B		No growth	
	No.	percentage	No.	percentage	No.	percentage
1 – 10 (27)	0	0%	5	18.50%	22	81.50%
11 – 20 (32)	3	9.40%	6	18.70%	23	71.90%
21-30 (7)	2	28.60%	0	0%	5	71.40%
31 – 40 (3)	0	0%	0	0%	3	100%
41 – 50 (5)	0	0%	0	0%	5	100%
51 – 60 (1)	0	0%	0	0%	1	100%

P. value: 0.296

Table 3 The relationship of anti-Salmonella antibodies with the isolation of Salmonella species among schistosomiasis patient

Widal test results	Isolation of Salmonella			
	Growth		No Growth	
	No.	percentage	No.	percentage
Significant (43)	8	18.60%	35	81.40%
Suggestive (15)	3	20%	12	80%
Insignificant (17)	5	29.40%	12	70.60%

P. value: 0.843

Table 4 The effect of S. mansoni intensity on anti-Salmonella antibodies among schistosomiasis patients

Widal test results	Schistosoma mansoni intensity					
	Mild		Moderate		Severe	
	No.	percentage	No.	percentage	No.	Percentage
Significant (43)	32	74.40%	11	25.60%	0	0%
Suggestive (15)	11	73.30%	4	26.70%	0	0%
Insignificant (17)	11	64.70%	5	29.40%	1	5.90%

P. value: 0.456

Discussion

Studies on the relationship between schistosomiasis and salmonellosis revealed some sort of correlation between each other as the Schistosoma acts as a vehicle for Salmonella infection (Lindquist et al, 1987; Lambertucci et al, 1998). This study involved 29 males and 46 females (36.7% and 61.3% respectively), the age groups ranged from 1–60 years. The most prevalent infection was in the age group of 11–20 years. This was partially explained by the increase in water contact with this age group. Many investigators believe that this epidemiologic pattern also results from the slow development of acquired immunity to re-infection over time.[5]

Out of 75 schistosomiasis patients, 16 patients showed positive culture for Salmonella (21.3%). These findings were higher than those

reported by Al Shazly et al.,[6] in Egypt (9%). These differences could, possibly, be because the previous investigators used a large sample size (161 subjects) in the study, as well as other variable factors in each study.

Out of 16 patients examined, 68.8% were positive for S.paratyphi B and 31.2% for S.typhi. These results were not in agreement with those of Owais et al.,[7] in southern coastal Pakistan. These variations could be attributed to the socio-economic differences, ethnic background and the ages of target populations.

Although New Halfa city is considered as an endemic area of S.mansoni infection due to the presence of many irrigation canals heavily infested with the snails that host S.mansoni, the intensity of S.mansoni infection showed only one sever case (1.3%), while 20 were moderate (26.7%), and 54 were mild (72%). This could, possibly, be due to the fact that prior immunologic experience with the parasitic induced some degrees of immunity to newly invading cercariae.[5] Furthermore, the regular treatment with praziquantel had remarkably reduced the incidence of schistosomiasis in the area.

Statistically, there was no significant relationship between intestinal schistosomiasis and typhoid (P> 0.05). These results were not in agreement with the previous study of Junqueria et al.,[8] in Brazil. Once again, the relationship between the Widal test and intensity of S.mansoni infection showed no significant relationship (P> 0.05). The possibility exists that the sample size was fairly small (75), and the use of the screening method for Ab detection (Widal test) which had low specificity and sensitivity.[9-14]

Conclusion

a. The study showed no relationship between Salmonella spp. and Schistosoma mansoni.

b. The intensity of S.mansoni infection was shown to be fairly high among the small-aged individuals.

c. The prevalence of Salmonella paratyphi B among schistosomiasis patients was fairly high in comparison with *S. typhi*.

Recommendations

a. Use of Large sample with an extended period and place.

b. Use of advanced molecular techniques in the diagnosis of Salmonella (e.g. Real-time- Polymerase Chain Reaction "RT-PCR").

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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