

Magnitude of Clostridium Difficile Infection in Hospitalized Egyptian Patients with Liver Cirrhosis

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Abstract

Background and Aim: Watery diarrhea is the cardinal clinical symptom of Clostridium Difficile infection that causes a spectrum of manifestations ranging from asymptomatic carrier state to severe fulminant disease with toxic megacolon. The basis for symptomatic responses range is related to host and pathogen factors. We studied the prevalence of Clostridium Difficile infection in hospitalized cirrhotic patients and its possible risk factors.

Patients and methods: This study was carried out on 200 cirrhotic patients admitted to National Liver Institute hospital divided into 2 groups: 100 asymptomatic patients and 100 patients with diarrhea. Direct stool toxin detection was done using an assay named RIDASCREEN® Clostridium Difficile Toxin A/B test; an enzyme immunoassay (EIA) for the qualitative determination of toxins A and B of Clostridium difficile in stool.

Results: Among 200 patients with mean age of (51.81 ± 12.94), EIA revealed 16 and 32 positive cases in both groups. In group I, there was statistical significance in ALT, serum creatinine (p<0.05) and bilirubin level (p<0.01) as regard EIA test result. Also, statistical significance was found (p<0.05) as regard duration of antibiotic and PPI intake. Logistic regression analysis revealed that gender and duration of PPIs use were independent factors in group I while the patient's age, duration of antibiotic use and γ-GT serum level were independent factors in group II for clostridium difficile infection development.

Conclusion: Clostridium difficile infection is not an uncommon infection in hospitalized cirrhotic patients. Risk factors were antibiotic intake, gastric acid suppression, old age, long hospital stay and/or history of bilharziasis.

Keywords: *Clostridium difficile*; Diarrhea; Bilharziasis; Gastrointestinal surgery; Cancer chemotherapy; Diarrhea

Spontaneous Bacterial Peritonitis; AUROC: Area under the Receiver Operating characteristic Curve; SD: Standard Deviation.

Keypoints

Clostridium Difficile is an anaerobic gram-positive, spore-forming, toxin-producing bacillus first described in 1935, watery diarrhea is the cardinal clinical symptom of *C. difficile* infection but may be asymptomatic.

Clostridium difficile infection is not an uncommon infection in hospitalized cirrhotic patients.

Risk factors were antibiotic intake, gastric acid suppression, old age, long hospital stay and/or history of bilharziasis.

Antibiotic use is the most widely recognized risk factor for Clostridium Difficile-Associated Diarrhea (CDAD).

List of Abbreviations: CDAD: Clostridium Difficile-Associated Diarrhea; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; γGT: Gamma-Glutamyl Transpeptidase; *C. difficile*: *Clostridium Difficile*; EIA: Enzyme Immuno-Assay; IRB: Institutional Review Board; NLI: National Liver Institute; CCFA: Cycloserine-Cefoxitin-Fructose Agar; CTP: Child-Turcotte-Pugh; MELD: Model for End-stage Liver Disease; PPIs: Proton Pump Inhibitors; SBP:

Introduction

Clostridium Difficile (*C. Difficile*) is an anaerobic gram-positive, spore-forming, toxin-producing bacillus first described in 1935 [1], and it is the causative organism of antibiotic-associated colitis. Colonization of the intestinal tract occurs via the feco-oral route and is facilitated by disruption of normal intestinal flora due to antimicrobial therapy used in treatment and/or prophylaxis of many infections specially during repeated short hospitalization periods which is very common in cirrhotic patients [2].

Antibiotic use is the most widely recognized risk factor for *Clostridium Difficile*-Associated Diarrhea (CDAD) [3]. Other established risk factors include hospitalization, advanced age, and severe illness. Possible additional risk factors include gastric acid suppression, enteral feeding, gastrointestinal surgery, cancer chemotherapy and hematopoietic stem cell transplantation [4]. However, CDAD can occur in the absence of any risk factor.

Watery diarrhea is the cardinal clinical symptom of *C. difficile* infection although it can cause a spectrum of manifestations ranging from the asymptomatic carrier state to severe fulminant disease with

toxic megacolon [5]. The basis for this range of symptomatic responses is not fully understood but may be related to various host and pathogen factors.

Some studies showed that *C. difficile* infection is common among hospitalized patients with liver disease and that these patients may have specific risk factors for this infection, such as the previous intake of antibiotics [6].

The aim of the study

The present study was aimed to determine the prevalence of *C. Difficile* infection in cirrhotic patients admitted to the National Liver Institute hospital and its possible risk factors.

Patients and methods

This study was approved by Institutional Review Board (IRB), Ethical Committee of National Liver Institute (NLI), Menoufia University. It was carried out on 200 patients with liver cirrhosis admitted to Hepato-Gastroenterology inpatients ward of NLI hospital from May 2013 to December 2014. All patients gave informed consent.

Inclusion criteria

Cirrhotic patients of Child-Turcotte-Pugh (CTP) class B or C. Previous history of medications that includes Antibiotics and/or Proton Pump Inhibitors (PPIs) before admission.

Exclusion criteria

We excluded from our study patients who had hepatocellular carcinoma, patients who presented by inflammatory bowel diseases or patients who had history of using laxative drugs or any specific treatment for clostridium difficile (as Metronidazole or Vancomycin) in the last three months.

Regarding present history of diarrhea, the selected patients were classified into two groups

Group I: This included 100 asymptomatic cirrhotic patients.

Group II: This included 100 cirrhotic patients complaining of diarrhea.

All patients were subjected to thorough history taking with particular attention to drug history including antibiotics and PPIs, full clinical examination and investigations in the form of complete blood picture, liver function tests, renal function tests and abdominal ultrasound was done using the real-time ultrasound equipment TOSHIBA Xario and TOSHIBA Nemio XG with a 3.5 MHz convex array transducer which was made by TOSHIBA Corporation, Japan.

Diagnosis of Clostridium Difficile infection

We used stool samples for:

Anaerobic stool culture by separating *C. difficile* from the other normal colonic flora was done by selective media. We used a selective agar medium named Cycloserine-Cefoxitin-Fructose Agar (CCFA), but this wasn't of value because of the high rate of negative results which were due to the difficult technique as it must be done under completely anaerobic conditions.

Culture by itself did not differentiate toxigenic from non-toxigenic strains which can both colonize the colon. It soon became apparent that the detection of toxin in the stool was required to establish that a patient had active *C. Difficile* [7].

Direct stool toxin detection using an assay named RIDASCREEN® Clostridium Difficile Toxin A/B test which is an enzyme immunoassay for the qualitative determination of toxins A and B of *C. Difficile* in stool samples by the following procedure

Specimen collection and storage: Stool samples must be stored at 2-8°C until it was used in the test. If the material was not to be used in the test within 3 days, it was recommended that it be stored at -20°C or colder. Multiple freezing and thawing of the sample must be avoided. Stool samples was not collected in transport containers which contained transport media with preservatives, animal sera, metal ions, oxidizing agents or detergents since these may interfere with the RIDASCREEN® *C. Difficile* Toxin A/B test.

Preparing the samples: We placed 1 ml RIDASCREEN® sample dilution buffer in a labeled test tube. Suck up the liquid stool in a disposable pipette until it passed the second thickening (approx. 100 µl) and suspending it in the sample dilution buffer. With solid stools, we took an equivalent amount (100 mg) with a spatula or a disposable inoculation loop and suspended it in solution. The stool suspension must be homogenized by suction and ejection from a disposable pipette.

After leaving for a short time for the coarse stool particles to settle, the clarified supernatant of the stool suspension used directly in the test.

III. First incubation and washing: After inserting a sufficient number of wells in the frame, 2 drops (100 µl) of the positive control, of the sample-dilution buffer (=negative control) or the fecal sample suspension were added to the wells. Subsequently followed for 60 minutes and incubated at room temperature (20-25°C).

The incubated substance in the wells must be emptied into a waste container containing hypochlorite for disinfection. After this, the plate was knocked out on absorbent paper in order to remove the residual moisture. Then the plate was washed 5 times using 300 µl wash buffer each time.

IV. Second incubation and washing: 1 drop (50 µl) of the conjugate was added to the wells and incubated at room temperature (20-25°C) for 30 minutes. The same washing procedure was repeated.

V. Third incubation: 2 drops (100 µl) of the substrate were added to each well. Then, the plate was incubated at room temperature (20-25°C) for 15 min in the dark. After this, stop the reaction by adding 1 drop (50 µl) of stop reagent to each well. After careful mixing (slight tipping on the plate frame), the absorbance was measured at 450 nm (optional: reference wave length ≥ 600 nm), then calibrating the zero against air, that means without microtiter plate.

Statistical Procedure

The data were collected and statistically analyzed using SPSS computer program version 21. The data were expressed as mean ± SD and differences between 2 groups were analyzed by student t-test for parametric variable distribution Pearson's correlation coefficient was used to test the relationship between various variables. P value was considered significant if <0.05. Stepwise logistic regression analysis was

performed to detect the factors that were independently associated with the presence of the factor under the study.

Results:

By studying baseline clinical, biological and biochemical characteristics of the participants, different parameters, and variables among 200 cirrhotic patients who agreed, met the inclusion and exclusion criteria and were randomly selected [Group I:100 patients without diarrhea (asymptomatic) and group II:100 patients with diarrhea] showed the following results

Groups	Frequency	Percent %
I-Cases with no diarrhea		
Positive		
Negative	16	16
Total	84	84
	100	100
II-Cases with diarrhea		
Positive	32	32
Negative	68	68
Total	100	100

Table 1: Direct stool anti-toxin detection test (EIA) in stool samples of studied patients.

The Age of the patients ranged from 19 to 81 years in both groups with a mean of (51.81 ± 12.94) with a male majority (80 patients in group I and 88 patients in group II) and it had a statistical significance in the group of patients with diarrhea (i.e. group II) (p<0.01).

Qualitative determination of toxins A and B of C. Difficile in stool samples by enzyme immunoassay (EIA) revealed, 16 and 32 positive cases in group I and group II respectively (Table 1).

Association between EIA test result and studied quantitative variables in group I showed statistical significance in alanine transaminase (ALT), serum creatinine and direct bilirubin levels (p<0.05). Also, statistical significance was detected in serum total bilirubin level, hemoglobin level (p<0.01). Statistical significance was found (p<0.05) as regard duration of antibiotic and PPI intake (Table 2).

Variables	EIA	N	Mean ± SD	t- test	P-value
MELD score	Negative	84	19.76 ± 9.99	0.96*	>0.05
	Positive	16	23.56 ± 14.02		
AGE (years)	Negative	84	43.21 ± 10.71	1.43	>0.05
	Positive	16	51.81 ± 12.94		
Duration of antibiotic use	Negative	72	4.86 ± 1.93	0.69*	>0.05
	Positive	12	3.83 ± 1.75		

Duration of proton pump inhibitors	Negative	64	7.22 ± 3.42	0.99*	>0.05
	Positive	12	7.83 ± 4.06		
AST (u/ml)	Negative	84	51.06 ± 0.24	2.29	<0.05
	Positive	16	49.13 ± 0.07		
ALT (u/ml)	Negative	84	39.48 ± 0.50	1.09	>0.05
	Positive	16	41.63 ± 0.50		
Albumin (gm/dl)	Negative	84	3.00 ± 0.18	---	---
	Positive	16	1.00 ± 0.00		
Total Bilirubin (mg/dl)	Negative	84	1.19 ± 0.39	4.42	<0.01
	Positive	16	1.00 ± 0.00		
Direct Bilirubin (mg/dl)	Negative	84	1.07 ± 0.2	2.53	<0.05
	Positive	16	1.00 ± 0.00		
Alkaline Phosphatase u/ml	Negative	84	1.56 ± 0.49	0.48	>0.05
	Positive	16	1.63 ± 0.50		
GGT (u/ml)	Negative	84	39.29 ± 12.24	0.44	>0.05
	Positive	16	56.38 ± 13.74		
INR	Negative	84	1.17 ± 0.37	0.41	>0.05
	Positive	16	1.13 ± 0.34		
UREA (mg/dl)	Negative	84	1.48 ± 0.50	1.82	>0.05
	Positive	16	1.25 ± 0.45		
CREATININE (mg/dl)	Negative	84	1.65 ± 0.48	2.62	<0.05
	Positive	16	1.31 ± 0.48		
Haemoglobin (gm/dl)	Negative	84	1.21 ± 0.41	4.76	<0.01
	Positive	16	1.00 ± 0.00		
WBCs (Cell/mm ³)	Negative	84	8254.76 ± 4566.94	0.32*	>0.05
	Positive	16	7137.50 ± 2572.65		
Platelets (Cell/mm ³)	Negative	84	134.26 ± 61.44	1.39	>0.05
	Positive	16	129.13 ± 72.34		

Table 2: Association between Enzyme Immuno Assay (EIA) test result and studied quantitative variables in Group I (cases with no diarrhea).

On the other hand, association of diabetes mellitus, ascites and CTP classification score have no statistical significance with Clostridium Difficile infection in group I cirrhotic patients (P>0.05).

History of bilharziasis and gender of the patients showed statistical significance (p<0.05), (p<0.01) respectively with Clostridium Difficile infection in group I. (Table 3)

Variables	EIA				χ ² test	p-value
	Negative (N=84)		Positive (N=16)			
	Number	%	Number	%		
Gender						
Male	72	85.7	8	50	10.71	<0.01
Female	12	14.3	8	50		
Child class						
Child B	6	7.1	0	0	1.22	>0.05
Child C	78	92.9	16	100		
Diabetes Mellitus						
Negative	72	85.7	14	87.5	0.04	>0.05
Positive	12	14.3	2	12.5		
Bilharziasis						
Negative	56	66.7	6	37.5	4.85	<0.05
Positive	28	33.3	10	62.5		
Ascites						
Negative	27	32.1	6	37.5	0.17	<0.05
Positive	57	67.9	10	62.5		
Endoscopy						
Negative	56	66.7	12	75	0.43	<0.05
Positive	28	33.3	4	25		
Antibiotic intake						
Negative	12	14.3	4	25	1.15	<0.05
Positive	72	85.7	12	75		
Duration of antibiotic intake						
3-10 days	68	97.1	10	100	0.29	<0.05
>10 days	2	2.9	0	0		
Proton pump inhibitors intake						
Negative	20	23.8	3	18.8	0.19	<0.05
Positive	64	76.2	13	81.2		
Duration of Proton pump inhibitors intake						
≤ 7 days	44	68.8	4	33.3	5.45	<0.05
>7 days	20	31.2	8	66.7		

Table 3: Association between Enzyme Immuno Assay test result and different studied variables in Group I (cases with no diarrhea).

Association between EIA test result and studied quantitative variables in group II revealed a statistical significance of model for end-stage liver disease (MELD) score, the age of the patient and serum

gamma-Glutamyl Transpeptidase (GGT) level (p<0.01). Duration of antibiotic use, indirect serum bilirubin and alkaline phosphatase showed statistical significance (p<0.05) with C. Difficile infection. Also,

was detected in serum total bilirubin level, hemoglobin level and red blood cell count ($p < 0.01$). Statistical significance was found ($p < 0.05$) as regard duration of antibiotic and PPI intake (Table 4).

Variables	EIA	N	Mean \pm SD	t- test	p- value
MELD score	Negative	68	20.09 \pm 9.32	2.74*	<0.01
	Positive	32	15.25 \pm 10.86		
Age (years)	Negative	68	50.67 \pm 8.91	4.44	<0.01
	Positive	32	43.83 \pm 7.64		
Duration of antibiotic use	Negative	68	5.82 \pm 1.84	2.07	<0.05
	Positive	29	5.03 \pm 1.40		
Duration of proton pump inhibitors	Negative	60	7.97 \pm 3.88	0.11*	>0.05
	Positive	22	7.64 \pm 3.24		
AST u/ml	Negative	68	49.9 \pm 16.1	0.07	>0.05
	Positive	32	55.8 \pm 29.2		
ALT u/ml	Negative	68	31.46 \pm 0.50	1.57*	>0.05
	Positive	32	32.63 \pm 0.49		
Albumin gm/dl	Negative	68	3.00 \pm 0.14	1.44	>0.05
	Positive	32	3.06 \pm 0.25		
Total Bilirubin mg/dl	Negative	68	1.12 \pm 0.32	0.1	>0.05
	Positive	32	1.13 \pm 0.34		
Direct Bilirubin mg/dl	Negative	68	1.09 \pm 0.29	0.44	>0.05
	Positive	32	1.06 \pm 0.25		
Indirect Bilirubin mg/dl	Negative	68	1.16 \pm 0.37	2.18	<0.05
	Positive	32	1.38 \pm 0.49		
Alkaline Phosphatase u/ml	Negative	68	111.62 \pm 0.49	2.62	<0.05
	Positive	32	109.34 \pm 0.48		
GGT u/ml	Negative	68	61.60 \pm 0.49	3.59	<0.01
	Positive	32	51.25 \pm 0.44		
INR	Negative	68	1.15 \pm 0.36	0.51	>0.05
	Positive	32	1.19 \pm 0.39		
Creatinine mg/dl	Negative	68	1.69 \pm 0.47	1.35	>0.05
	Positive	32	1.81 \pm 0.39		
Hemoglobin gm/dl	Negative	68	10.12 \pm 0.33	0.1	>0.05
	Positive	32	10.13 \pm 0.34		
WBCs Cell/mm ³	Negative	68	8226.47 \pm 5157.54	0.65*	> 0.05
	Positive	32	9650.00 \pm 7126.15		
Platelets	Negative	68	157.4 \pm 49.1	0.32	>0.05
	Positive	32	149.2 \pm 56.6		

Cell/mm ³					
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Table 4: Association between Enzyme Immuno Assay test result and quantitative variables in Group II (cases with diarrhea).

Regarding MELD score, it ranged from 9 to 44 (19.455 ± 10.385) and it had a statistical significance in group II (p.value <0.01).

Various baseline factors such as AST, total bilirubin, direct bilirubin, creatinine, hemoglobin, gender, bilharziasis and duration of PPIs use were analyzed using univariate linear regression analysis in group I and MELD score, age, Duration of antibiotic use, indirect bilirubin, alkaline phosphatase, GGT, ascites and PPI use in group II to detect possible correlation with development of *C. Difficile* infection. Gender and duration of PPIs use were independent factors for development of *C. Difficile* infection in group I while AST and total serum bilirubin level were dependent factors.

Univariate analysis in group II showed that age of the patient, duration of antibiotic use and GGT serum level were independent factors for the development of *C. Difficile* infection.

According to the area under the receiver operating characteristic curve (AUROC); serum AST level of 50 U/ml or more, serum total bilirubin level of 1 mg/dl or more or MELD score of 10.5 or more were a specific but not a sensitive nor an accurate predictor for the presence of *C. Difficile* infection (p>0.05).

On the other hand, antibiotic use for a duration of 3.5 days or more was a sensitive but not a specific nor an accurate predictor for the presence of *Clostridium difficile* infection (p>0.05) and white blood cells count of 5550 days or more was not a sensitive or a specific or an accurate predictor for the presence of *C. Difficile* (p>0.05).

Discussion

Cirrhosis is an immuno-compromised state which predisposes the patient to a variety of infections [8]. Despite the advancement in medical care for patients with advanced liver disease in the past decades, bacterial infections remain very common and account for significant morbidity and mortality in those patients [9].

C. Difficile is an increasingly prevalent hospital-acquired infection that affects patients with cirrhosis. A study of over 80,000 patients with cirrhosis found patients with *C. difficile*-associated disease to have higher mortality and longer length of stay in higher care units than those without infection [2].

Antibiotics and proton pump inhibitors were independently associated with *C. difficile* infection. Thus it is recommended that antibiotic prophylaxis be limited to patients at highest risk of developing spontaneous bacterial peritonitis (SBP) and that proton pump inhibitors be used selectively [10].

C. Difficile is a prevalent cause of pseudomembranous colitis in hospitalized patients and those in the community [11] approximately 20% of individuals who are hospitalized acquire *C. difficile* during hospitalization, and more than 30% of these patients develop diarrhea. Thus, *C. difficile colitis* is currently one of the most common nosocomial infections [11].

Analysis of the results revealed that the prevalence of *C. Difficile* infection in the whole 200 studied patients was 24% and in hospitalized cirrhotic patients who complained of diarrhea (i.e. group

II), it was 32% while in those who didn't complain of diarrhea (i.e. group I), it was 16% and this is much higher than Bajaj who found that the prevalence of *C. Difficile* was 2% and Vanjak who stated that *C. Difficile* infection rate was 0.9% among Hepato-Gastroenterology ward inpatients [2,6].

Evaluation and interpretation of the test results:

In order to establish the cut-off, 0.15 extinction units were added to the measured extinction for the negative control (Cut-off=Extinction for the negative control+0.15).

We considered samples to be positive if their extinction was more than 10% above the calculated cut-off. Samples were considered equivocal and must be repeated if their extinction was within 10% of the cut-off. If repeating the test with a fresh stool sample again yields a value in the gray range, the sample must be considered negative. Samples with extinctions more than 10% below the calculated cut-off must be considered negative.

Ninety-two percent (184 patients-92%) of the studied cases had CTP class B and eight percent (16 patients-8%) of them had CTP class C, and the CTP class didn't have a statistical impact which is also in accordance with Bajaj who noted that there is no significant relation between the CTP class and the presence or absence of *C. Difficile* infection and also Vanjak found that the CTP class of the patient is not affected by the presence or absence of *C. Difficile* infection [2,6].

Regarding MELD score, it had a statistical significance in group II (p value <0.01) and this was against the results of the study done by Bajaj who stated that MELD score was insignificant regarding its effect on the presence or absence of *C. Difficile* infection (p=0.52) [2]. According to AUROC, we noticed that MELD score of 10.5 or more was a specific but not a sensitive nor an accurate predictor for the presence of *C. Difficile* infection (p>0.05).

Comorbid conditions as (Diabetes mellitus, Hypertension, Thyroid dysfunction and Tuberculosis) gave no statistical significance except for Bilharziasis which was statistically significant in the group of patients with no diarrhea (i.e. group I) (p<0.05) and this point wasn't clarified in the studies done by both Vanjak and Bajaj [2,6], this may be attributed to the low incidence of bilharziasis in industrialized countries as the United States and France, where no formal national or international statistics exist because schistosomiasis in these countries is not a notifiable disease [12].

Clinical manifestations of decompensated cirrhosis in the studied population (i.e. both groups) had no statistical impact except for the presence of ascites which had a statistical significance in group II (p<0.01) and this agreed with the results of the study done by Bajaj who found that presence of ascites in the studied patients was positively related to the presence of *C. Difficile* infection [2].

Some laboratory investigations had statistical impact as Kidney function tests as serum creatinine level was statistically significant in group I (p.value <0.05) and this point was against the results of the studies done by Vanjak who noted that renal insufficiency is not a risk factor for developing *C. Difficile* infection in patients with cirrhosis

and Bajaj who found that elevated serum creatinine level is not significant in acquiring *C. Difficile* infection ($p=0.1$) [2,6].

Past history of drug intake concerning antibiotic intake (with stress on the duration and the type of antibiotic used) and PPIs intake (with stress on the type and the duration of usage of PPIs) had the following statistical significance: First, Antibiotic intake ($p<0.05$) and its duration ($p<0.05$) were statistically significant in group II and this agreed with the results of the studies done by both Vanjak who stated that case-patients were more likely than control patients to have received antibiotics, especially amoxicillin/clavulanate and other b-lactams during the 2 weeks before the onset of symptoms (96% vs. 58%; $p<0.01$) and Bajaj who stated that the rate of antibiotic use for spontaneous bacterial peritonitis prophylaxis was significantly higher in the cirrhosis and *C. Difficile* group compared to cirrhosis alone group ($p<0.01$). Second, duration of PPIs ($p<0.05$) intake was statistically significant in group I while in group II, PPIs intake ($p<0.05$) -regardless of its duration- had a statistical significance and this was in accordance with the results of the studies done by Bajaj who mentioned that there was history of previous outpatient PPIs use with a higher significance in the cirrhotic group with *C. Difficile* infection (40/54, 74%) compared to cirrhotic patients without *C. Difficile* infection (38/108, 35%; $p<0.0001$) [2].

Patients with cirrhosis may be at particular risk of developing *C. Difficile* infection, for three reasons: First, antibiotic use in cirrhosis is common [13]. Second, cirrhotics also commonly receive PPI, both for established indications such as symptomatic gastro-esophageal reflux, prior peptic ulcer disease as well as for unproven indications such as healing of esophageal ulcers after endoscopic band ligation [14] and these drugs have been associated with enteric infections, namely *C. Difficile* infection [15]

Third, frequent need for hospitalization to treat complications of cirrhosis, such as variceal bleeding, ascites or encephalopathy, places patients in an environment in which there is high likelihood of exposure to *C. Difficile* infection [16].

The most striking point in the results of this study is the noted effect of bilharziasis on the rate of *C. Difficile* infection in hospitalized cirrhotic patients in the NLI hospital and the positive impact of bilharzial infection on *C. Difficile* infection in our study, it may be attributed to high prevalence of bilharziasis in Egyptian population where studies from 2006 indicated approximately 7 million cases of schistosomiasis [17].

By using tartar emetic drugs in the 1960s, Egypt started governmental treatment projects to control bilharziasis infection in Egyptian patients, then it was completed by praziquantel mass drug administration [18]. But the widespread use of improperly sterilized needles, which led to schistosomiasis and hepatitis C co-infections, with elevated viral loads and more rapid progression to cirrhosis and hepatocellular carcinoma was an unfortunate consequence of the early phases of schistosomiasis control with parenteral tartar emetic in Egypt [19].

IL-13 and IL-4 were dispensable to the suppression of dextran sulfate sodium-induced colitis (Th2 cytokine dominant and macrophage-mediated colitis) by *S. mansoni* male worms. Schistosome eggs have immunomodulatory potential inducing the alternative activation of macrophages and regulatory T-cell expansion [20]. Especially in schistosome infections, egg deposition in the host tissues was the major stimulus of Th2 responses [21] and egg proteins (e.g.,

omega-1 and peroxiredoxin) are involved in the Th2-biasing activity [22].

Conclusion

C. Difficile infection is not an uncommon infection in hospitalized cirrhotic patients and it may influence the prognosis of these patients and the length of their hospital stay.

The most common risk factors for this infection are antibiotic intake, gastric acid suppression, old age, long hospital stay.

History of bilharziasis was noted to be a risk factor for developing *C. Difficile* infection in hospitalized cirrhotic patients.

Compliance with Ethical Standards
Ethical approval: This study was approved by Institutional Review Board (IRB), Ethical Committee of National Liver Institute (NLI), Menoufia University.

Informed consent: A written informed consent was obtained from all subjects participated in the study.

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