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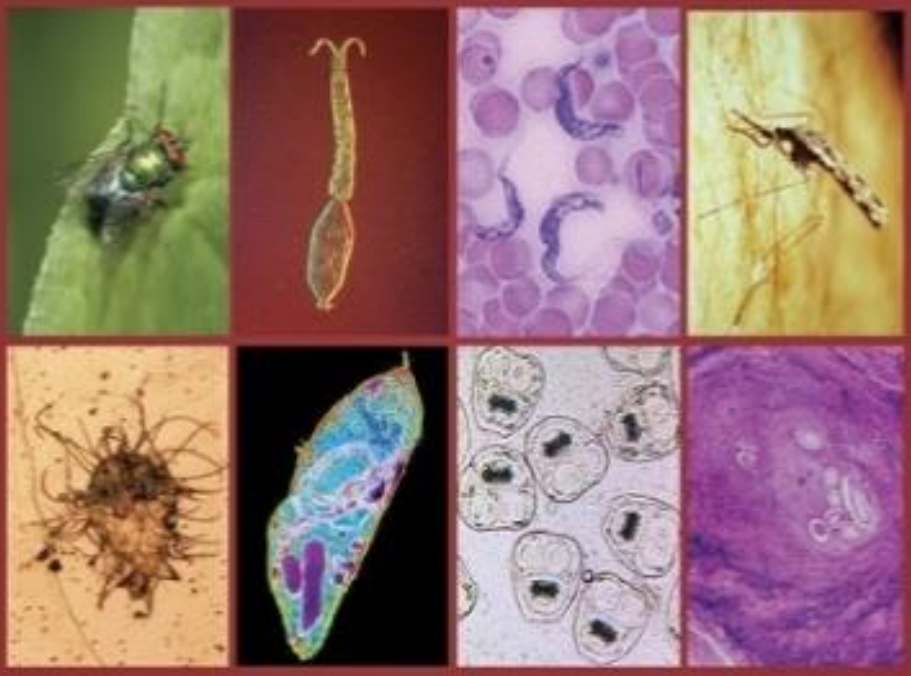

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Prevalence of Opportunistic Parasites Among Liver Cirrhosis Patients in Beni-Suef

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Liver cirrhosis, Immunocompromised patients, Opportunistic parasites, *B. hominis*, *Cryptosporidium spp*.

ABSTRACT

Liver cirrhosis patients are susceptible to many opportunistic parasitic infections such as *Cryptosporidium spp., Isospora belli* (*I. belli*) and *Blastocystis hominis* (*B. hominis*) as there are variable degrees of immune alteration accompanying cirrhosis, that affect the innate and adaptive immunity, according to the extent of liver damage.

A total of 135 stool samples were collected from patients who attended the outpatient clinics of Tropical Medicine Departments, Faculty of Medicine, and Beni-Suef University Hospitals. Patients were classified into two groups: the cases group which contained 90 patients suffering from liver cirrhosis with gastrointestinal (GIT) symptoms (mainly abdominal pain and diarrhea) and the control group formed of 45 patients complaining of gastrointestinal symptoms without liver cirrhosis. The samples were microscopically examined directly by wet saline and Lugol's iodine mounts. Negative specimens were reexamined after the formal-ether concentration method. Samples were then stained with modified Ziehl-Neelsen (MZN) stain for the suspected presence of *Cryptosporidium spp.*

The most prevalent protozoan was *B. hominis* in both cases and control groups, followed by *Cryptosporidium spp.* with a higher percentage in the cases group (26.7% vs 11.1% and 21.1% vs 4.4% respectively), and both were statistically significant (*P*=0.038 and 0.012 respectively). *Capillaria* was the most frequent helminth found in both cases and control groups (6.7% vs 4.4%). However, the correlation to liver cirrhosis was insignificant (*p*= 0.718).

INTRODUCTION

Chronic hepatic illness is one of the most serious health issues as reported by the recent GIT studies (Kirmaz *et al.*, 2004). Liver cirrhosis as the latest phase of a chronic liver disease causes a disorder called cirrhosis-associated immune dysfunction syndrome (CAIDS) (Bonnel *et al.*, 2011). Cirrhosis comes to be a systemic disease, with several organ disorders (Bernardi *et al.*, 2015). At this phase, patients become highly vulnerable to various infections because of CAIDS, which comprises both innate and adaptive immunity (Jalan *et al.*, 2013). Patients with hepatic cirrhosis and ascites are more prone to other complications of liver disease, including hyponatremia, refractory ascites, or hepatorenal syndrome (HRS) (Moore *et al.*, 2003). Liver cirrhotic patients are thought-out as immunosuppressed and are vulnerable to a large scale of entero-parasites (Hegab *et al.*, 2003).

The liver is critically affected in numerous parasitic diseases, as it is the initial solid organ facing parasitic stages after skin or mucosal penetration, whether through systemic or portal circulation (schistosomal larvae). Parasites can modulate the host's immune response in order to be able to mature and spread in liver tissue (Deslyper *et al.*, 2019). Cell-mediated immunity was found to be depressed in various liver diseases where the immunological changes are equivalent to the degree of liver affection (Unger *et al.*, 1986).

Intestinal parasitic diseases have been also reported in association with diabetes mellitus (DM) which is considered a predisposing factor (Sisu *et al.*, 2021). Immunosuppression among DM cases is due to increased blood glucose levels, which modifies several immune responses and this renders the body susceptible to various opportunistic infections comprising parasitic infections (Bora *et al*., 2016)

Intestinal parasites such as *B. hominis, Cryptosporidium spp., I. belli, Cyclospora cayetanensis,* and *Microsporidia* have appeared as significant opportunistic parasites that are responsible for severe illness in immunocompromised patients (Nazeer *et al*., 2013), subsequently, patients suffer from severe morbidity and high mortality (Arvaniti *et al.,* 2010; Jalan *et al.,* 2013).

Opportunistic parasites in hepatic patients may cause severe diarrhea, electrolyte imbalance, and dehydration (Tuli *et al.*, 2010). These parasites are mostly transmitted by eating and drinking contaminated food and water, or through unhygienic habits such as feco-oral transmission (El Shazly *et al.,* 2015).

This current work was performed to estimate the prevalence of opportunistic parasites in liver cirrhosis patients in Beni-Suef governorate.

MATERIALS AND METHODS

In this case-control study, 135 stool samples were collected from patients who attended the outpatient clinics of Tropical Medicine Departments, Faculty of Medicine, Beni-Suef University Hospitals. The study was done in the period from May 2019 to April 2021.

Participants were classified into 90 patients known to suffer from liver cirrhosis, with GIT complaints (cases group), and 45 individuals having GIT complaints and proved to be negative for liver cirrhosis (control group). Patients were of both sexes (Females: 93, Males: 42) and aged between 28 and 63 years old, all patients suffer from gastrointestinal complaints and/or diabetes or hypertension. Exclusion criteria were pregnant women and patients with hepatocellular carcinoma. Only patients who offered not less than three stool samples were enrolled in this research.

Cases were subjected to investigations including complete blood picture with differential, liver enzymes: AST and ALT "with a normal reference range of 0-45 IU/L for ALT and 0-35 IU/L for AST" (Vagvala and O'Connor, 2018), liver function test (albumin and bilirubin) and abdominal ultrasound. Sheets were filled out including personal and medical history data as well as clinical examination findings.

Parasitological diagnosis: Three stool specimens were obtained from each participant in labeled carton boxes to be examined for helminths' ova, protozoal trophozoites, and/or cysts. The stool samples were inspected macroscopically for consistency, gravid segments…etc, then microscopically examined (low and high-power magnifications) directly by wet saline and Lugol's iodine mounts. Negative samples were then reexamined following the formal-ether concentration method (WHO, 1991). In addition, samples were stained with modified Ziehl-Neelsen (MZN) stain for the suspected presence of *Cryptosporidium spp.* (El Naggar *et al.,* 2006).

Statistical analysis: Data were coded and entered using the Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was outlined using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Groups were compared to each other using an unpaired t-test (Chan, 2003a). Chi-square ($\chi 2$) test was performed to compare categorical data. The exact test was applied instead when the expected frequency is less than 5 (Chan, 2003b). *P*-values less than 0.05 were considered statistically significant**.**

RESULTS

In this study, the age, sex, residence and occupation did not show significant differences when related to cirrhosis (*P*>0.05). Clinical symptoms such as nausea, anorexia, and diarrhea were higher among the cases group as compared to the control group without significant differences. However, abdominal pain showed statistical significance in the cases group (*P*<0.001). Clinical symptoms such as hematemesis and melena related to liver cirrhosis showed no significant difference. As regards chronic diseases; diabetes mellitus was more among the cases group (31.1%) compared to the control group (13.3%), showing statistical significance with cirrhotic patients (*P*=0.025), while hypertension was (18.9%) in the case group compared to control group (2.2%) and was statistically significant (*P*=0.007). (Table 1).

Table 1: Socio-demographic data, clinical features, and chronic diseases among participants

		Cases		Control		*P* value
		Count	%	Count	%	
Gender	**male**	29	32.2%	13	28.9%	0.693
	female	61	67.8%	32	71.1%	
Occupation	**worker**	30	33.3%	15	33.3%	1
	not worker	60	66.7%	30	66.7%	
Residence	**rural**	38	42.2%	25	55.6%	0.143
	urban	52	57.8%	20	44.4%	
Melena	**yes**	7	7.8%	0	0.0%	-----
	no	83	92.2%	0	0.0%	
Hematemesis	**yes**	41	45.6%	0	0.0%	-----
	no	49	54.4%	0	0.0%	
Bowel habits	**Diarrhea**	47	52.2%	14	31.1%	0.057
	Constipation	25	27.8%	20	44.4%	
	Normal	18	20.0%	11	24.4%	
Abdominal pain	**yes**	66	73.3%	14	31.1%	< 0.001*
	no	24	26.7%	31	68.9%	
Anorexia	**yes**	18	20.0%	10	22.2%	0.764
	no	72	80.0%	35	77.8%	
Nausea	**yes**	20	22.2%	5	11.1%	0.117
	no	70	77.8%	40	88.9%	
Hypertension	**yes**	17	18.9%	1	2.2%	0.007*
	no	73	81.1%	44	97.8%	
Diabetes	**yes**	28	31.1%	6	13.3%	0.025*
	no	62	68.9%	39	86.7%	

*Statistically significant (*P*<0.05).

The most prevalent protozoan in both cases and control groups, in the present study, was *B. hominis* (26.7%), followed by *Cryptosporidium spp.* (21.1%), where both were significant (*P*=0.038 and 0.012 respectively). Additionally, *Capillaria* was the most frequent helminth found in both cases and control groups (6.7%) but was not

significant (*P*= 0.718). This study detected the presence of *Entamoeba histolytica* (*E. histolytica*)*/ dispar* and *Giardia lamblia* (*G. lamblia*) at a higher percentage in cases than in the control group (5.6% vs 2.2% and 4.4% vs 2.2% respectively) and was statistically insignificant (*P*= 0.663 and 0.665 respectively). Also, non-opportunistic helminths such as *Ascaris lumbricoides*, *Hymenolepis nana*, *Schistosoma mansoni* and *Ancylostoma duodenale* were detected, with no significant difference observed (Table 2 and Figs. 1,2,3 and 4).

Table 2: Prevalence of parasitic infections among studied participants.

Parasitic infections	Cases		Control		*P* value
	Count	%	Count	%	
B. hominis	24	26.7%	5	11.1%	0.038*
***Cryptosporidium spp.* (MZN stain)**	19	21.1%	2	4.4%	0.012*
E histolytica/ dispar	5	5.6%	1	2.2%	0.663
G. lamblia	4	4.4%	1	2.2%	0.665
I. belli	1	1.1%	0	0.0%	1
Capillaria	6	6.7%	2	4.4%	0.718
Ascaris	2	2.2%	1	2.2%	1
S. mansoni	1	1.1%	0	0.0%	1
H nana	1	1.1%	0	0.0%	1
Ancylostoma	1	1.1%	0	0.0%	1

*Statistically significant (*P*<0.05).

The association of *B. hominis* with abdominal pain and/ or diarrhea in this study, was the most frequent in both cases and control groups, followed by *Cryptosporidium spp*. and *Isospora* but all of them showed no significant difference, compared to the control group (Tables 3 and 4).

A higher number of positive cases of *B. hominis* (15/24) and *Cryptosporidium spp*. (17/19) were detected in the cases group with diabetes when compared to the control group with or without diabetes. Interestingly, the single positive case of *I. belli* in this study had liver cirrhosis combined with diabetes (Table 5).

Positive cases of *B. hominis* were accompanied by elevated AST (84.87 IU) compared to negative cases (63.18 IU) and also HGB level was higher among positive cases (8.60 g/dl) than negative cases (7.88 g/dl) showing statistical significance (*P*< 0.001 and *P*=0.014 respectively). Similarly, AST was elevated in *Cryptosporidium spp.* positive cases (80.79 IU) than negative cases (65.84 IU) which was statistically significant (*P*=0.002) (Table 6).

Table 3: Associated abdominal pain with opportunistic parasites

Abdominal pain					
Parasitic infections	Cases		Control		*P* value
	Count	%	Count	%	
B. hominis	15	62.5%	1	20.0%	0.969
***Cryptosporidium spp.* (MZN stain)**	14	73.7%	1	50.0%	0.161
I. belli	1	100%	0	0.0%	1

Statistically significant (*P*<0.05).

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Table 4: Associated diarrhea with opportunistic parasites.

Parasitic infections	Diarrhea				
	Cases		Control		*P* value
	Count	%	Count	%	
B. hominis	14	58.3%	2	40.0%	0.484
***Cryptosporidium spp.* (MZN stain)**	12	63.2%	1	50.0%	0.283
I. belli	0	0.0%	0	0.0%	-----

Statistically significant ($P<0.05$).

Table 5: Associated DM with opportunistic parasites.

Parasitic infections	Cases		Control	
	+ve DM	-ve DM	+ve DM	-ve DM
B. hominis	15	9	3	2
***Cryptosporidium spp.* (MZN stain)**	17	2	2	0
I. belli	1	0	0	0

Table 6: Comparison of laboratory findings among positive cases of *B. hominis* and *Cryptosporidium spp.*

	B. hominis				
	+ve		-ve		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Albumin(g/dl)	2.00	0.50	2.00	0.52	0.970
total bilirubin(mg/dl)	8.04	5.06	7.34	4.37	0.519
HGB (g/dl)	8.60	1.13	7.88	1.21	0.014*
ALT(IU)	31.08	2.95	31.51	3.04	0.557
AST(IU)	84.87	11.47	63.18	20.24	< 0.001*
	Cryptosporidium spp.				
	+ve		-ve		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Albumin (g/dl)	2.14	0.55	1.97	0.50	0.201
total bilirubin (mg/dl)	7.91	5.05	7.42	4.44	0.684
HGB (g/dl)	8.44	1.35	7.98	1.18	0.150
ALT (IU)	31.16	3.30	31.46	2.94	0.702
AST (IU)	80.79	15.74	65.84	20.74	0.002*

*Statistically significant ($P<0.05$).

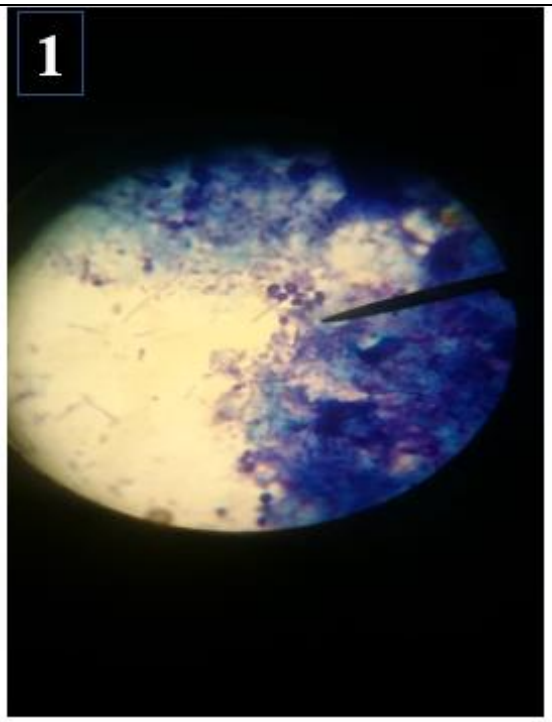

Fig. 1: *Cryptosporidium spp.* oocysts by MZN stain (X 100)

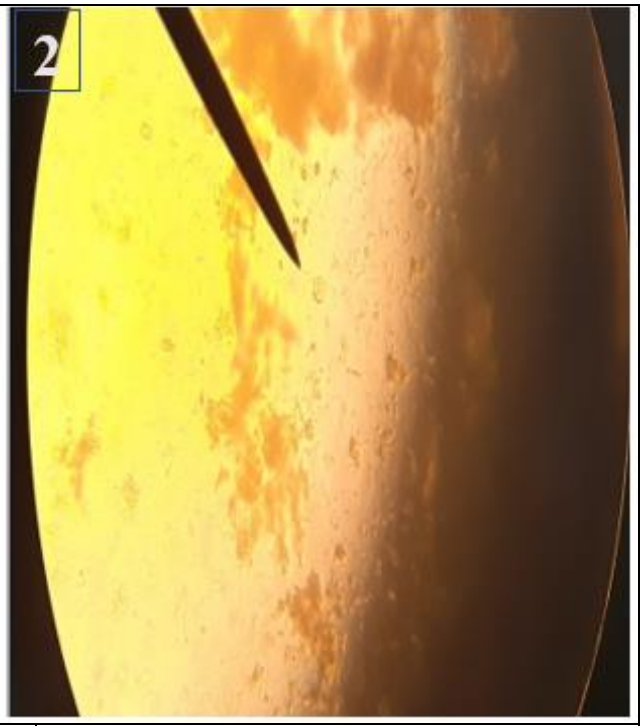

Fig. 2: *Blastocystis hominis* by Lugol's iodine mount (X 100)

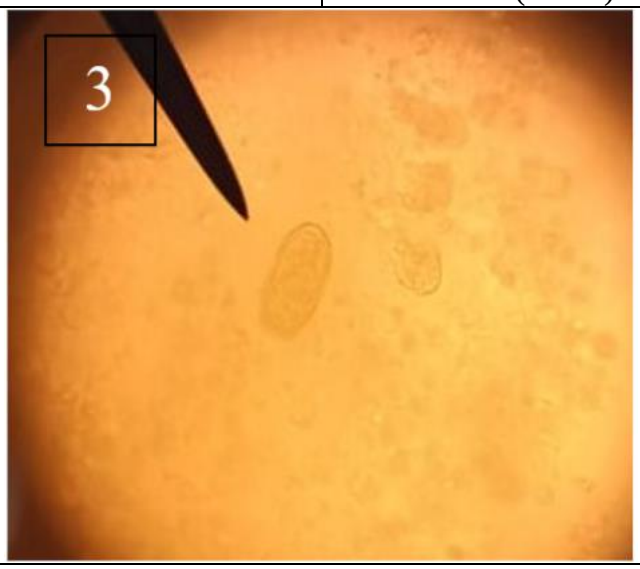

Fig.3: *Capillaria* egg by wet mount (X 10)

DISCUSSION

Cirrhosis-associated immune dysfunction causes alterations in the innate and adaptive immunity, which prone patients with cirrhosis to various infections (Noor and Manoria, 2017). Hegab *et al.* (2003) also found that patients with liver cirrhosis are considered immunosuppressed and are exposed to various parasitic infections such as *Cryptosporidium spp*., *B. hominis*, and *I. belli*. These opportunistic protozoa were constantly reported in immunocompromised cases suffering from protracted diarrhea (Mariam *et al.*, 2008), however, their presence was rare in immunocompetent ones (Mohandas *et al.*, 2002). This was in agreement with this current work, where the incidence of these protozoa was remarkably higher in liver cirrhosis cases (considered immunosuppressed) compared to the control group. This could be due to the

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decrease of local and cell-mediated immune responses to entero-parasites in immunocompromised patients which favors their establishment (Lindo *et al.*, 1998). On the contrary, Fontanet *et al.* (2000) reported that the incidence of these parasites in immunosuppressed patients was similar to that in immunocompetent ones.

Immune responses are suppressed in DM, resulting in a more aggressive and chronic form of morbidity among diabetic patients harboring opportunistic parasites (Mohtashamipour *et al.*, 2015). This agreed with the results of the current study where a higher number of positive cases of *Cryptosporidium spp*. (17/19) and *B. hominis* (15/24) were detected in the cases group with DM when compared to cirrhotic cases without DM and in the control group with or without DM. This could suggest that diabetes intensifies the state of immune suppression accompanying liver cirrhosis and this will favor the establishment of opportunistic parasites on a larger scale. This disagrees with Nazligul *et al.* **(**2001), who stated that non-diabetic individuals had a significantly higher prevalence of intestinal parasitic infections.

This work found that *B. hominis* was the commonest protozoan in both liver cirrhotic cases and the─control group, however, the prevalence was higher in liver cirrhosis (26.7% and 11.1% respectively) and this was statistically significant (*P*=0.038). Similarly, Abdel-Hafeez *et al.* (2012) reported that *B. hominis* was diagnosed in 12.1% of immunocompromised patients and in 7.2% of immunocompetent participants.

Cryptosporidium spp. was higher in cirrhosis cases than in the control group with a percentage of 21.1% and 4.4% respectively (statistically significant: *P*=0.012). This agrees with Ghoshal *et al.* (2018) who detected *Cryptosporidium spp.* in renal transplant recipients with a prevalence percentage of 8.4% and this was statistically significant when compared to the healthy control group (*P* < 0.001). Also, Mousa *et al.* (2014), found that the incidence of *Cryptosporidium* in patients with Chronic Liver Disease (CLD) was 30%, while Shrestha *et al.* (1993) diagnosed *Cryptosporidium* in 20% of CLD cases.

The presence of *I. belli* was insignificant in this study (1.1% of cases) which was similar to the results of Salehi *et al.* (2016) who detected *Isospora* oocysts in 4 (1.1%) samples out of 350 immunocompromised patients (HIV/AIDS, malignancy or congenital/acquired immunodeficiency disorders). However, Kumar *et al.* (2002) stated that *I. belli was* the most common parasite among AIDS patients with chronic diarrhea at 18.6% (11/59).

Detection of *E. histolytica/ dispar* and *G. lamblia* in the current study was more prevalent in cirrhotic patients compared to the control group (of 5.6% vs 2.2% and 4.4% vs 2.2% respectively). This agreed with Botero *et al.* (2003) who stated that *E. histolytica/ dispar* (10.0%) and *G. lamblia* (7.2%) were frequently met in immunocompromised patients. On the contrary, Abdel-Hafeez *et al.* (2012) found that these protozoa were more predominant in immunocompetent individuals than in immunosuppressed patients (24.6% vs 6% and 17.6% vs 4.8%, respectively). They clarified the former findings by some alterations in the gut structure caused by the infection in immunosuppressed patients which might be inappropriate for both *E. histolytica* and *G. lamblia*.

Adults *S. mansoni* inhabit the venous plexus of the intestine but the main disease sequelae are caused by eggs trapped in the liver and subsequently liver fibrosis and cirrhosis (Orihel and ash, 1995). The present study detected *S. mansoni* at a percentage of 1.1% and was found to be statistically insignificant (*P*= 1).

Detection of *Cryptosporidium spp.* in infected cirrhotic patients was accompanied by elevated liver enzymes (AST). These results disagreed with Mousa *et al.* (2014), who did not find any significant difference between serum liver

enzymes in CLD patients with *Cryptosporidium spp.* infection.

Conclusion:

Blastocystis and *Cryptosporidium spp.* are the most prevalent intestinal parasites that infect immunosuppressed patients. The etiology of this state of immune suppression could be a wide range of diseases including liver cirrhosis and diabetes being accompanied by innate and cell-mediated immune depression throughout the chronic course of this disease.

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