Original Article

Stressing a tired host: *Cryptosporidium* species and *Helicobacter pylori* infections in diabetes mellitus patients with gastrointestinal manifestations

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ABSTRACT

Background: *Cryptosporidium* spp. and *Helicobacter pylori* are widespread gastrointestinal infections that appear to resist treatment in many cases. Cryptosporidiosis results in increased intestinal permeability while *H. pylori* causes atrophic changes in stomach, and both are opportunistic pathogens. The outcome of infection depends largely on the degree of the host immune status. Diabetes mellitus (DM) is a growing health problem in Egypt, with detrimental consequences that can affect the immune system, the gastrointestinal tract, and virtually all body systems, exposing diabetic patients to higher susceptibility to infections and intensified morbidity.

Objective: The present study was designed to determine the burden of *Cryptosporidium* spp. and *H. pylori* among diabetic patients compared to non-diabetic patients attending Kasr Al Ainy hospitals.

Subjects and Methods: Stool samples, demographic and clinical data were collected from 80 patients, 40 diabetics and 40 non-diabetics, with gastrointestinal manifestations. Microscopic stool examination and coproimmunoassays for the detection of *Cryptosporidium* spp. and *H. pylori* were performed for all samples.

Results: *Cryptosporidium* spp. infection was detected in 15% of diabetics; with a frequency of 7.4% and 30.8% in patients with controlled DM and uncontrolled DM, respectively, and in 5% of non-diabetics. While *H. pylori* was equally detected at a rate of 60% in non-diabetic and diabetic patients (51.9% and 76.9% in patients with controlled DM and uncontrolled DM, respectively). Microscopic examination of stools revealed *Blastocystis* in 25% of diabetics (22.2% in controlled DM versus 30.7% in uncontrolled DM) and in 5% of non-diabetic patients. Co-infection with *Cryptosporidium* and *H. pylori* occurred in 10% of diabetic cases (3.7% in controlled DM versus 23.1% in uncontrolled DM), and in 5% of non-diabetic patients.

Conclusion: Diabetic patients had a higher infection rate of *Cryptosporidium* as well as *Blastocystis* in comparison to non-diabetics. Screening for intestinal parasites is needed to control the infection and reduce morbidity in diabetics.

Keywords: *Blastocystis*; coproimmunoassays; *Cryptosporidium*; diabetics; gastrointestinal manifestations; *H. pylori*.

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INTRODUCTION

No doubt, DM is a major health issue, with around 451 million individuals affected worldwide[1]. In Egypt, the number of diabetic patients has alarmingly increased over the past decade, placing it among the top 10 countries with highest prevalence of DM[2]. According to the American Diabetes Association, a controlled glycemic state is achieved at a glycated hemoglobin (HbA1c) level of <7%, while higher levels (>7%) are considered uncontrolled DM with high risk of complications[3].

Uncontrolled DM tends to escalate the risk of other diseases by inducing macrovascular and microvascular damage[4]. Moreover, the impaired immune system together with metabolic imbalance intensify the susceptibility to several pathogens[5]. Infections in diabetic patients increase the disease burden by the related comorbidities, in addition to the economic burden caused by the cost of care and the duration of treatment[4]. Diabetic affection has been related to a higher incidence of gastrointestinal symptoms that include gastroparesis[6] and serious small intestinal mucosal injury. Diabetic enteropathy of the large intestine manifests by diarrhea, constipation, and fecal incontinence[7-9]. Studies have shown that association of DM with chronic systemic inflammation presents a risk factor for cancer; including gastric and colorectal carcinomas[10-12].

Cryptosporidium is an obligatory intracellular protozoan that parasitizes gastrointestinal epithelial cells. The parasites develop principally in the jejunum and ileum under the brush border of the epithelial cells[13]. Globally, the number of reported cases of

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cryptosporidiosis is increasing, and the frequency of infection is likely to be one hundred-fold higher than the number of reported cases[14]. Parasite oocysts are transmitted primarily through the fecal oral route[15], and hence *Cryptosporidium* is responsible for several waterborne outbreaks of gastrointestinal disease[16-18]. The oocysts are highly infectious and are resistant to hard environmental conditions[19,20]. The severity, persistence, and outcome of infection depend largely on the host immune status[21]. A self-limited disease usually occurs in the immunocompetent individuals, the most common symptom being a watery diarrhoea, while in immunocompromised patients, prolonged diarrhoea can be life threatening[22]. Cryptosporidiosis pathogenesis may include increased intestinal permeability, chloride loss, altered glucose transport mechanisms in infected enterocytes, malabsorption, and host immune response to infection[22-24]. In patients with immunodeficiencies, the biliary and respiratory tracts may also be involved[25,26]. A link between cryptosporidiosis and colorectal carcinoma was suggested[18]. Cholangiocarcinoma, complicating chronic cryptosporidiosis and cholangitis, was also postulated[27].

Additionally, *H. pylori* is a gram-negative spiral bacterium, found in the stomach of about 50% of the global population. Chronic infection may induce atrophic changes and metaplasia in the stomach[28,29]. Infection by *H. pylori* can spread directly from one person to the other, or indirectly through environmental exposure routes[30]. Concomitant infection of *H. pylori* and intestinal parasites may correlate with fecal exposure. The co-colonization of the gut may be attributed to intestinal parasites affection in millions of individuals globally, thus increasing the odds of coinfection with *H. pylori*[31]. Besides, mutual symbiosis was postulated since *H. pylori* could provide favorable conditions for intestinal parasitosis or *vice versa*[32]. Results of different studies revealed that *H. pylori* infection is a risk factor for type 2-DM[33-35]. The mechanisms involved in the bacterium-type 2-DM interaction may be related to infection induced inflammation, production of inflammatory cytokines, and hormonal imbalance[33].

The co-existence of *Cryptosporidium* spp. and *H. pylori* presents a challenge in diabetics. In addition to the aforementioned possible complications, both pathogens are difficult to treat with increasing reports of *H. pylori* antibiotic resistance[29] and the lack of fully effective medication against *Cryptosporidium*[36]. In this context, it must be noted that detrimental consequences of DM can virtually involve all the body systems[37], rendering diabetic patients compromised hosts. The present study was conducted to determine the burden of *Cryptosporidium* spp. and *H. pylori* among diabetic patients versus non-diabetic patients attending Kasr Al Ainy hospitals.

SUBJECTS AND METHODS

This case-control study was performed in the period from September 2018 to June 2019 on patients attending the Diagnostic and Research Unit of Parasitology (DRUP) and the Diabetes Unit, Kasr Al-Ainy, Faculty of Medicine, Cairo University.

Study population and sample collection: A total of 80 patients presenting with gastrointestinal symptoms and not under immunosuppressive therapy were included in the study; 40 were diabetics and 40 were non-diabetics. Patients in both groups were matched for age and sex. Relevant data were obtained from all participants comprising demographic data, gastrointestinal manifestations, and clinical history of diabetes including a significant HbA1c level. Fecal samples were collected from each patient in labeled, leak-proof, dry, and clean plastic stool containers. From each stool sample, a small part was stored at -20°C for subsequent use in the coproimmunoassays, and the remaining of the sample was preserved in formalin saline fixative for parasitological examination.

Stool examination: The stool samples were examined microscopically using direct wet smear and formalin-ethyl acetate sedimentation methods for routine screening of ova and other parasitic stages[38].

Coproimmunoassays: The frozen fecal specimens were thawed at room temperature before testing. For each sample, two tests were performed using RIDA QUICK *Cryptosporidium* ICT (R-Biopharm AG, Germany - Cat. # N1203) for detection of *Cryptosporidium* coproantigens[39]; and OnSite *H. pylori* Ag Rapid test-Cassette (CTK Biotech, Inc., San Diego, CA, USA - Cat. # R0192C) for detection of *H. pylori* coproantigens[40]. Both tests were done according to the manufacturer's instructions.

Statistical methods: Data were coded and tabulated using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Quantitative variables were summarized using mean, standard deviation (±SD), minimum and maximum, while categorical variables were presented as frequencies (number of cases) and relative frequencies (percentages). Comparisons between groups were by unpaired t-test and chi square (X^2) test. Exact Fisher test was used when the expected frequency was <5. *P*-values <0.05 were considered as statistically significant.

Ethical consideration: All procedures in the present work fulfilled the ethical standards recognized by Helsinki Declaration 1964. An informed consent was obtained from all participants. Infected patients were notified and prescribed the appropriate treatment.

RESULTS

Demographic data: Of the total 80 patients included in the study, 27 (33.8%) were males and 53 (66.2%) females, distributed between the diabetic and non-diabetic groups (Table 1). Their ages ranged between 9 and 80 years with a mean of 46.5±15.3 years. For the diabetic cases, the mean age was 49.7±12.9 years, while the mean age of the non-diabetic group was 43.4±16.9 years.

Clinical and diabetic status: Abdominal pain and diarrhea were the predominant gastrointestinal symptoms, recording 65% and 55% respectively, in the diabetic group; and 60% and 17.5% respectively, in the non-diabetic group. A significant difference in the frequency of diarrhea between both groups (P<0.001) was detected. Other reported gastrointestinal manifestations including constipation, vomiting, distention, and weight loss were insignificant (Table1). Regarding the DM history, the duration of DM ranged from 0.5 to 21 years with a mean of 5.80±5.08 years. The reported HbA1c level ranged from 5% to 11.2% with a mean of 6.85%±1.95 in the diabetic patients; of whom 27 (67.5%) cases had controlled DM, while 13 (32.5%) cases were uncontrolled.

Microscopic examination and coproimmunoassays: The rate of parasitic infections was higher in diabetic patients (27.5%) than in non-diabetic ones (12.5%), with no statistical significance. Direct microscopic examination of the stool samples revealed *Blastocystis* in 10 (25%) diabetic patients (22.2% in controlled DM versus 30.7% in uncontrolled DM), and 2 (5%) non-diabetic patients with statistically significant difference between them (P<0.05). Detection of pathogenic *E. histolytica* complex in the non-diabetic group was insignificant (Table 1).

Using ICT test, *Cryptosporidium* was detected in 6 (15%) and 2 (5%) stool samples of diabetic and non-diabetic groups, respectively, with no statistically significant difference (Table 1). Co-infection of *Cryptosporidium* and *Blastocystis* as detected by coproimmunoassay and fecal microscopic examination respectively, was observed in the diabetic group with statistically significant association (P<0.05) (Table 2). Among the diabetic patients, there was a statistically significant (P<0.05) association between diarrhea and *Cryptosporidium* infection rate. No statistically significant association was observed between *Cryptosporidium* positivity and diabetic patients' age or sex (Figure 1 and Table 2).

Regarding *H. pylori* infection rate, the immunoassay test was positive in 24 (60%) patients in each of the diabetic and non-diabetic groups (Table 1). A significant difference (P <0.05) was found in the mean age between *H. pylori* positive and negative cases in the diabetic group (Figure 1). Chi-square test showed no significant association of *H. pylori* positivity in the diabetic cases with sex and clinical symptoms (Table 3). In the diabetic group, co-infection of *Cryptosporidium* and *H. pylori* was detected in 4 out of 6 *Cryptosporidium* positive cases (3.7% in controlled DM versus 23.1% in uncontrolled DM) (Table 4), without significant association; 3 out of 4 of co-infected cases also had *Blastocystis*.

Table 1. Demographic, clinical and infection characteristics in the study population.

Demographic, clinical and infection characteristics		Diabetic (No.=40) No. (%)	Non-diabetic (No.=40) No. (%)	Statistical analysis (P value)
Sex	**Male**	10 (25.0%)	17 (42.5%)	0.098
	Female	30 (75.0%)	23 (57.5%)	
Age	**Mean±SD**	49.7 ±12.9	43.4 ±16.9	0.062
Clinical				
Diarrhea		22 (55.0%)	7 (17.5%)	<0.001*
Abdominal pain		26 (65.0%)	24 (60%)	0.644
Constipation		6 (15.0%)	1 (2.5%)	0.108
Distension		6 (15.0%)	1 (2.5%)	0.108
Weight loss		1 (2.5.0%)	1 (2.5%)	1
Vomiting		1 (2.5%)	3 (7.5%)	0.615
DM	**Controlled@**	27 (67.5%)	-	-
	Uncontrolled#	13 (32.5%)	-	-
Infection				
Parasites		11 (27.5)	5 (12.5%)	0.093
***Blastocystis* spp.**		10 (25.0%)	2 (5.0%)	0.012*
E. histolytica/E. dispar		0 (0.0%)	1 (2.5%)	1
***Cryptosporidium* spp.**		6 (15.0%)	2 (5.0%)	0.263
H. pylori		24 (60.0%)	24 (60.0%)	1
Total of infections		28 (70%)	26 (65%)	0.633

***:** Statistically significant (P <0.05); **@:** HbA1c <7%; **#:** HbA1c >7%.

Table 2. Association between cryptosporidiosis and demographic, clinical and infection characteristics in the diabetic patients.

Demographic, clinical and infection characteristics		*Cryptosporidium* spp.		Statistical analysis (*P* value)
		Positive (No. = 6) No. (%)	Negative (No. = 34) No. (%)	
Sex	**Male** **Female**	1 (10%) 5 (16.7%)	9 (90%) 25 (83.3%)	1
Age	**Mean±SD**	48.83 ± 19.32	49.88 ± 11.85	0.857
Diarrhea	**Positive** **Negative**	6 (27.3%) 0 (0.0%)	16 (72.7%) 18 (100%)	0.024*
Abdominal pain	**Positive** **Negative**	4 (15.4%) 2 (14.3%)	22 (84.6%) 12(85.7%)	1
Constipation	**Positive** **Negative**	0 (0.0%) 6 (100%)	6 (100%) 28 (82.4%)	0.565
Distension	**Positive** **Negative**	0 (0.0%) 6 (17.6%)	6 (100%) 28 (82.4%)	0.565
Weight loss	**Positive** **Negative**	1(100%) 5 (12.8%)	0 (0.0%) 34 (87.2%)	0.150
Vomiting	**Positive** **Negative**	0 (0.0%) 6 (15.4%)	1(100%) 33 (84.6%)	1
***Blastocystis* spp.**	**Positive** **Negative**	5 (83.3%) 1(16.7%)	5 (14.7%) 29 (85.3%)	0.002*
H. pylori	**Positive** **Negative**	4 (66.7%) 2 (33.3%)	20 (58.8%) 14 (41.2%)	1

***:** Statistically significant (*P* <0.05).

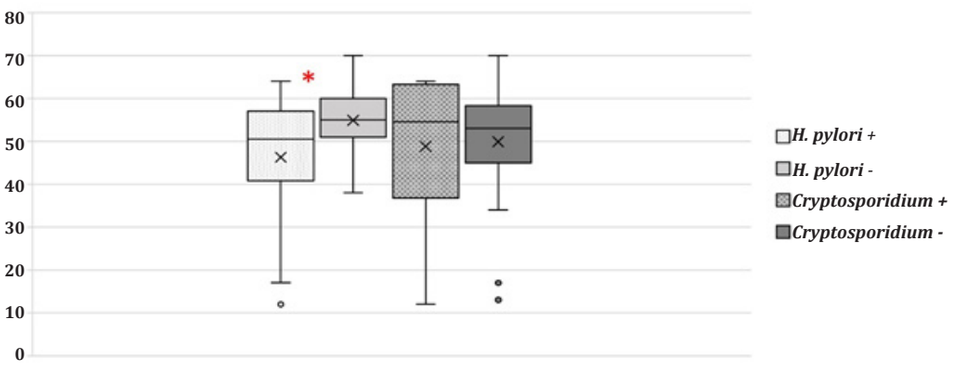

Fig. 1. Age distribution of diabetic cases in relation to positive and negative *H. pylori* and *Cryptosporidium* infection.
***:** Statistically significant difference (*P*<0.05) in the mean age between *H. pylori* positive and negative cases.

Table 3. Association between *H. pylori* positivity and demographic and clinical characteristics in the diabetic patients.

Demographic, clinical and infection characteristics		*H. pylori*		Statistical analysis (*P* value)
		Positive (No. = 24) No. (%)	Negative (No. = 16) No. (%)	
Sex	**Male** **Female**	4 (40%) 20 (66.7%)	6 (60%) 10 (33.3%)	0.159
Age	**Mean±SD**	46.29 ± 14.76	54.88 ± 7.26	0.020*
Diarrhea	**Positive** **Negative**	14 (63.6%) 10 (55.6%)	8 (36.4%) 8 (44.4%)	0.604
Abdominal pain	**Positive** **Negative**	13 (50.0%) 11 (78.6%)	13 (50.0%) 3 (21.4%)	0.079
Constipation	**Positive** **Negative**	4 (66.7%) 20 (58.8%)	2 (33.3%) 14 (41.2%)	1
Distension	**Positive** **Negative**	4 (66.7%) 20 (58.8%)	2 (33.3%) 14 (41.2%)	1
Weight loss	**Positive** **Negative**	1 (100 %) 23 (59.0%)	0 (0.0%) 16 (41.0%)	1
Vomiting	**Positive** **Negative**	0 (0.0%) 24 (61.5%)	1 (100 %) 15 (38.5%)	0.400
***Cryptosporidium* spp.**	**Positive** **Negative**	4 (66.7%) 20 (58.8%)	2 (33.3%) 14 (41.2%)	1

***:** Statistically significant (*P* <0.05).

Table 4. *Cryptosporidium* spp. and *H. pylori* infections in diabetic and non-diabetic participants as diagnosed by copro-immunoassays.

		DM				
		Positive			Negative (No. = 40)	Statistical analysis (*P* value)
		Total (No. = 40)	Controlled@ (No. = 27)	Uncontrolled# (No. = 13)		
		No. (%)	No. (%)	No. (%)	No. (%)	
***Cryptosporidium* spp.**	**Positive**	6 (15.0)	2 (7.4)	4 (30.8)	2 (5.0)	0.075
	Negative	34 (85.0)	25 (92.6)	9 (69.2)	38 (95.0)	
H. pylori	**Positive**	24 (60.0)	14 (51.9)	10 (76.9)	24 (60.0)	0.13
	Negative	16 (40.0)	13 (48.1)	3 (23.1)	16 (40.0)	
Co-infection	**Positive**	4 (10.0)	1 (3.7)	3 (23.1)	2 (5.0)	0.09
	Negative	36 (90.0)	26 (96.3)	23 (76.9)	38 (95.0)	

@: HbA1c <7%; **#:** HbA1c >7%.

DISCUSSION

Impairment of the immune system of diabetics together with the existing metabolic imbalance, may increase the susceptibility to pathogenic agents. A limited number of epidemiological studies have inquired into the association of DM and parasitic infections. Chronic parasitic infections can have an impact on DM risk and enteropathy through converted immune regulation, malnutrition, diarrhea, and gut microbiome alteration[41]. *Cryptosporidium* and *H. pylori* infections can be associated with significant morbidity exerting extra burden on the already stressed diabetic patients. In the present study, using ICT, *Cryptosporidium* spp. infection was detected in 15% of diabetics; with a frequency of 7.4% in patients with controlled DM versus 30.8% in patients with uncontrolled DM, and in 5% of non-diabetics with no significant difference. However, *Cryptosporidium* infection in the diabetic patients showed no significant relation with age, sex, or glycemic control, but had a significant association with diarrhea. Using microscopy, *Blastocystis* was the most frequently detected parasite, with significantly higher rate in diabetics (25%) than in non-diabetics (5%). In a study at Sohag University Hospital, *Cryptosporidium* spp. was detected in 5% of diabetic patients by MZN staining. The most frequent parasite was *G. lamblia* (22%), followed by *E. histolytica* (7%)[42]. Mohtashamipour *et al*.[43] revealed a *Cryptosporidium* infection rate of 1.6% in stained Kinyoun acid-fast samples in a case-control study on diabetics attending Endocrine and Metabolism Research Centre, Isfahan/ Iran. The most reported infections were *Blastocystis* (9.3%), *Endolimax nana* (5.1%) and *G. lamblia* (3.4%). Tangi *et al*.[44] in a similar study on intestinal parasites in DM patients in Limbe and Buca Municipalities, Cameroon, estimated a prevalence of *Cryptosporidium* of 0.67% by MZN, whereas the most frequent parasite was *E. histolytica* (6.7%) followed by *Blastocystis* (2.7%).

However, Alemu *et al*.[45] found that among diabetics at Arba Minch Hospital, Ethiopia, *Cryptosporidium* had the highest frequency (8.4%), then Ascaris (3.7%) and *G. lamblia* (2.8%). Waly *et al*.[46] revealed a prevalence of 44% for intestinal parasites among diabetic patients attending Beni-Suef University Hospital, Egypt. The prevailing parasites were *Blastocystis* (29%), *Cryptosporidium* spp. (12%), *G. lamblia* (7%), and *Microsporidium* spp. (5%).

In our study, infection with *H. pylori* was detected at a rate of 60% in diabetic cases, 51.9% with controlled DM versus 76.9% with uncontrolled DM, as well as in 60% of non-diabetic patients. Unlike *Cryptosporidium* the only significant relation of *H. pylori* positivity was with the mean age of diabetics ($P<0.05$) and not with any of the other parameters. As in DM, *H. pylori* infections are associated with gastrointestinal inflammation that interferes with absorption of glucose and lipids[47]. In hospital based cross sectional studies, *H. pylori* infection was positive in 73% of diabetics versus 51.4% in the non-diabetic group at Liaquat University Hospital/ Pakistan[48]; and 73.11% and 58.05% in diabetics and non-diabetics respectively in Douala, Cameroon[34].

Concurrent infection of *H. pylori* and intestinal parasites was reported in a number of studies[31,32,49-53]. Under normal conditions, gastric juice creates an unfavorable medium for pathogenic microorganisms. *H. pylori* infection causes alteration in gastric acid secretion, moreover the bacterium can compromise the gastric mucosal barrier, depending on the site and duration of the infection; thus allowing a favorable medium for intestinal parasitosis[54,55]. Ghallab and Morsy[56] observed that *H. pylori* was highly prevalent in gastrointestinal symptomatic patients attending Kafrelsheikh University Hospital, and that *Blastocystis* (68%), *Cryptosporidium* spp. (32%), *G. lamblia* (31%) and *E. histolytica* complex (27%) were more frequent in *H. pylori* infected patients.

It is worth noting that polymicrobial interactions in the complex intestinal niche can tremendously influence the course of a disease[57]. In fact the concomitant presence of intestinal protozoa and *H. pylori* can result

in an amplified T helper (Th1) response which fails to clear the infection and exaggerates the mucosal damage[58]. Few studies investigated the dual infection of *Cryptosporidium* and *H. pylori* in diabetic patients[41]. In the present study, coinfection of *Cryptosporidium* and *H. pylori* was demonstrated in 4 (10%) diabetic patients with no statistical significance, but of which 3 patients also had *Blastocystis* infection.

Rady and colleagues[59] investigated the occurrence of intestinal parasites and *H. pylori* infection among diabetic children attending Aboul-Reesh University Hospital, Egypt. The authors reported an infection rate of 45.26% in diabetics, versus 20% in non-diabetics, for intestinal parasites; and a 39.47% infection rate versus 42.3% rate in non-diabetics, for *H. pylori*. *Cryptosporidium* was prevalent in 9.2% of diabetics compared to 0.84% in non-diabetics with statistically significant difference. *H. pylori* positivity was significantly associated with *G. lamblia* infection (10.56%), whereas association with *Cryptosporidium* (5.59%) and *Blastocystis* (9.32 %) were non-significant.

In conclusion, our results revealed a higher infection rate of *Cryptosporidium* among diabetic patients in comparison to non-diabetics, while *H. pylori* infection was evenly detected in both groups. Co-infection with *Cryptosporidium* and *H. pylori* occurred in 10% of diabetic cases, yet its clinical significance needs further investigations. *Blastocystis* was the most frequently detected parasite, showing a significantly higher rate in diabetics. Being stressed hosts, especially in poorly controlled glycemic state, diabetic patients need screening for intestinal parasites to control the infections, reduce morbidity and risk of complications.

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