Opportunistic intestinal parasites and *Helicobacter pylori*: Co-infection and associated risk factors among HIV patients

Original Article

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ABSTRACT

Background: In immunocompromised human immunodeficiency virus (HIV) infected patients, opportunistic parasites and *H. pylori* are a major public health concern. In Egypt, few data regarding the problem magnitude and risk variables emerge from settings with scarce resources.
Objective: The goal of this study was to determine detection rates of intestinal parasitic co-infections with *H. pylori* in HIV patients, as well as to record co-infection risk and predictors.
Subjects and Methods: From Suez Fever Hospital, single fecal samples were collected from 70 HIV patients to detect intestinal parasitic infections. Stool samples were examined microscopically, fresh with iodine and acid-fast stain. Molecular diagnosis for *H. pylori* and *Cryptosporidium* spp. was performed using nested-PCR technique. Risk factors for *H. pylori* and parasites co-infection among HIV patients were assessed by a comprehensive behavioral questionnaire that included demographics data and clinical history.
Results: Intestinal parasites (IP) were detected in 74.3% of the total study population. Pathogenic parasites identified were *Cryptosporidium* spp., *E. histolytica/E. dispar* complex, and *G. lamblia* in 28.5%, 27.1%, and 22.8%, respectively. Molecularly, *H. pylori* was detected in 15/70 (21.4%) of HIV patients of which *H. pylori* co-existed with *Cryptosporidium* spp. in seven patients (46.66%), and with *I. belli* in two patients (13.33%). Statistically, there was a strong positive correlation between cryptosporidiosis and diarrhea, as well as between infection with *I. belli* and diarrhea. Co-infection of *H. pylori* with *Cryptosporidium* spp., or *I. belli* was statistically linked to abdominal pain and diarrhea. Gender showed a statistically significant association with both *Cryptosporidium* and *I. belli* infections.
Conclusion: It was concluded that HIV-positive patients with diarrhea were more likely to additionally suffer from *Cryptosporidium* spp. and *H. pylori*. The co-occurrence of *Cryptosporidium* spp. and *H. pylori* supports the theory of co-infection.

Keywords: co-infection; *H. pylori*; HIV patients; n-PCR; opportunistic pathogens.

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INTRODUCTION

One of the major problems in public health is HIV infection especially in low- and middle-income countries. At the end of 2021 an additional 1.5 million patients had acquired HIV; 650 000 had died from HIV-related causes. The total estimate of HIV-positive patients worldwide in 2022 was 33.9-43.8 million[1]. Due to dread of stigma, HIV cases in Egypt are underreported. According to the Ministry of Health and Population in 2022, over 22000 Egyptians are estimated to be surviving with AIDS[2]. It is worth mentioning that HIV infection is frequently accompanied by low $CD4^+$ cell counts (<200 cells/mm^3), with inability to access highly active antiretroviral therapy (HAART), and poor hygiene[3].

Opportunistic intestinal parasites (OIPs), such as *Cryptosporidium* spp., *I. belli*, and *C. cayetanensis,* are the most frequent intestinal parasites to infect immunocompromised individuals[4]. Patients with acquired HIV/AIDS are frequently infected with opportunistic parasites which can lead to complications and death[5]. In a recent meta-analysis, Utami *et al*.[6] revealed that cryptosporidiosis in HIV patients predisposes to the development of chronic diarrhea that is aggravated by the associated low $CD4^+$.

On the other hand, *H. pylori* is among the highly prevalent infections in the world. Epidemiologically, 30–50% of populations in developed countries and 85–95% of those in underdeveloped countries, have *H. pylori* infections[7]. Although the infection is typically asymptomatic, it can cause dyspeptic illness, non-malignant consequences such as iron deficiency anemia[8], and malignant complications including gastric and esophageal cancer[9]. In different geographical areas, between 10% and 80% of HIV patients have *H. pylori* infection[10,11]. Low-income

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countries frequently suffer from colonization of the intestinal tract by *H. pylori* and other parasites. Because of this, minimal data is available to define the exact prevalence of *H. pylori*, its co-infection with another infection or with OIPs, and its relation with the related risk factors in HIV patients. The purpose of our study was to evaluate the prevalence of OIPs and *H. pylori*, and their coinfection among HIV/AIDS patients in Egypt. Risk variables that could increase the prevalence of this co-infection were also investigated.

SUBJECTS AND METHODS

This hospital-based cross-sectional study on collected stool samples was conducted in the Laboratory of Molecular Medical Parasitology (LMMP) and Diagnostic and Research Unit of Parasitic Diseases (DRUP), Medical Parasitology Department, Faculty of Medicine, Cairo University, during the period from December 2021 to May 2022.

Study design: Single samples were collected from HIV patients, with or without diarrhea, attending the Fever Hospital in Suez governorate. Samples were examined microscopically for detection of intestinal parasites, and molecularly to diagnose *Cryptosporidium* spp. and *H. pylori* using nested-PCR technique. With the aid of a structured questionnaire, study participants were interrogated about their demographics. Risk factors linked to co-infection of intestinal parasites and *H. pylori* were statistically analyzed.

Target population and sample size calculation: The HIV patients receiving anti-parasitic medications in the two weeks before test collection, as well as those who used antacids in those same two weeks, were excluded. Antacid medication can alter the morphology of a protozoan, making it difficult to identify. Patients with additional immunocompromising diseases such as cancer patients receiving chemotherapy, organ transplant recipients, or hemodialysis patients were disqualified. For the calculation of the minimum sample size required, Epi InfoTM 7 software (CDC, Atlanta) was used. According to the available number of HIV patients matching the inclusion and exclusion criteria, 70 positive cases aged 16 years and above were enrolled in the study.

Samples collection and copro-parasitological examination: The stool sample (~20–30 gm) was collected from each patient in a clean dry container with tight cover. Using a direct wet mount with Lugol's iodine staining, all samples were microscopically examined for opportunistic parasites and presence of pus, RBCs, and Charcot–Leyden crystals. Microscopic examination of samples after formalin-ether treatment was also done. For coccidian protozoa detection, fecal smears were stained with Kinyoun-modified acid-fast stain[12].

Molecular analysis

Genomic DNA extraction: Thermal shock, i.e., 10 cycles of freezing at liquid nitrogen and thawing at 95°C, was performed to break oocysts walls. Each sample's genomic copro-DNA was isolated using the Favor Stool DNA Spin Columns Isolation Kit (cat. no. FAST1; Favorgen Biotech Corporation, Taiwan) according to the manufacturer's instructions with modification in the form of prolongation of incubation at 95°C for one hour, after which the purified DNA was measured for concentration and purity[13].

H. pylori **nested polymerase chain reaction (nPCR) assay:** Using nested PCR (n-PCR) targeting the *H. pylori* gene, two successive PCR reactions were used to amplify the DNA from the *H. pylori* strain. According to Sasaki *et al*.[14], amplification occurred in both reactions. After electrophoresis on a 1.5% agarose gel in TAE buffer, the ethidium bromide-stained nested PCR products were analyzed using a UV transilluminator.

***Cryptosporidium* spp. nPCR assay:** In two successive PCR reactions, n-PCR was used to target the gene encoding *Cryptosporidium* oocyst wall protein (COWP) and amplify DNA from *Cryptosporidium* spp. In each reaction, amplification was carried out[15,16]. The amplified products were stained with ethidium bromide, electrophoresed on a 1.5% agarose gel in TAE buffer, and visualized with a UV transilluminator. According to the manufacturer's recommendations, *Cryptosporidium* PCR products were fragmented using RsaI to analyze restriction-fragment length polymorphism (RFLP) (product no. ER1121; Thermo Scientific). Fragmented PCR products were electrophoresed in Metaphor agarose gel (3%) and inspected by UV transillumination after being stained with ethidium bromide.

Statistical analysis: The IBM SPSS statistics version 21(Chicago, IL, USA) was used for data entry, editing, and analysis. The chi-square test or Fisher's exact test was used to compare categorical variables that were provided as frequencies and percentages. Statistical significance was considered at $P<0.05$.

Ethical considerations: Ethical approval was obtained from the regional ethics committee of the National Hepatology and Tropical Medicine Research Institute (NHTMRI) (Reference number 26122). All participants provided verbal informed agreement, and were advised that they could stop the study at any point, and that all information would be kept private, secure, and anonymous.

RESULTS

Socio-demographic characteristics: Of the seventy HIV-positive patients enrolled over the study period, the gender split was 21 females (30%), and 49 males (70%). Their ages range was 16-60 years with a mean

± SD of 36.11±12.07 years, and a predominance of age groups 46-60 and 31-45 (42.8 % and 41.4 %, respectively). According to stool consistency, the results were recorded as follows: 35 (50%) patients were diarrheic and 35 (50%) were non-diarrheic. Overall, 42 (60%) patients were literate and 36 (51.43%) reported farm animal contact.

Microscopic examination of stool samples from HIV patients: Intestinal parasites were recorded in 52 (74.3%) patients of which *E. histolytica/E. dispar* was recorded in 8 (11.4%), *G. lamblia* in 6 (8.6%), *H. nana* in 4 (5.7%), *S. mansoni* in 2, and *T. saginata* in 2 (2.9%). In terms of OIPs, *I. belli* was found in 10 (14.3%) and *Cryptosporidium* spp. in 20 (28.5%). Polyparasitism was found in 18 (34.6%) of the infected cases. A single parasitic agent was found in the remaining cases 34 (65.4%) as summarized in Table (1).

Relation between OIPs, and *H. pylori* infection and risk factors: Table (2) describes the relation of detection rates of *Cryptosporidium* spp., *I. belli* and *H. pylori* to age, sex, education level and farm animal contact; in addition to the association with diarrhea and abdominal pain as main symptoms of infections. Statistically, there was a strong positive correlation between cryptosporidiosis and diarrhea (*P*=0.000, OR= 4.22, 95% CI= 1.91-9.68), as well as between infection with *I. belli* and diarrhea (*P*=0.006, OR= 4.20, 95% CI= 2.11-8.45).

Table 1. Frequency of single parasitic infections.

Single parasites	Frequency (%)
***E. histolytica* complex**	5 (9.6)
Giardia lamblia	2 (3.8)
***Cryptosporidium* spp.**	14 (26.9)
I. belli	7 (13.5)
H. nana	2 (3.8)
S. mansoni	2 (3.8)
T. saginata	2 (3.8)
Total	**34 (65.4)**

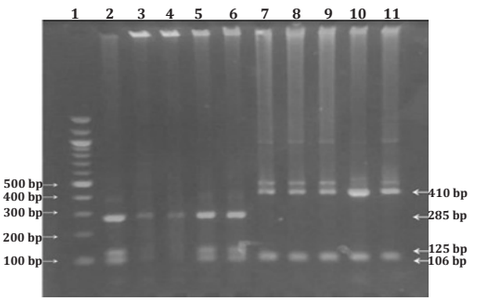

Fig. 2. After digestion by RsaI, the nPCR targeting *cowp* gene of *Cryptosporidium* spp. was separated using agarose gel electrophoresis. **Lane 1:** 100 bp DNA molecular weight marker "ladder". **Lanes 2–6:** Positive *C. hominis* samples (285, 125, 106). **Lanes 7–11:** Positive *C. parvum* samples (410 and 106).

Molecular identification and genotyping of *Cryptosporidium* spp.: The nPCR amplification targeting cowp gene resulted in detection of *Cryptosporidium* spp. at 553 bp (Figure 1). On conducting RFLP, results revealed a C. *parvum* detection rate in 13 cases (65%) (Figure 2).

Detection rate of *H. pylori* in HIV-positive patients: The *H. pylori* DNA was found in 15 (21.4 %) of the study population (Figure 3), with a significant detection rate in the 46-60 age group (8; 53%) and the 31-45 age group (5; 33%). Table (2) shows the insignificant changes in *H. pylori* detection rates according to socio-demographic conditions.

Concomitant infection of OIPs with *H. pylori* in HIV-positive patients: The presence of *H. pylori* and IPs was recorded in 13/52 (25%) of the total participants. *Cryptosporidium* spp. was the most common co-infected OIP, accounting for 7/13 (53.85%) cases, *E. histolytica/E. dispar* complex (3/13, 23.07%), *G. lamblia* (2/13, 15.38%), and *I. belli* (1/13, 7.7%). Table (2) summarizes the co-infection rate and socio-demographic data association. Statistically, there was a considerable association between *H. pylori* and the presence of OIPs (OR=3.90, 95% CI=1.72-8.24). Several symptoms were noted, with diarrhea being the most common gastrointestinal infection symptom and abdominal discomfort being the most common parasite infection symptom (Table 2).

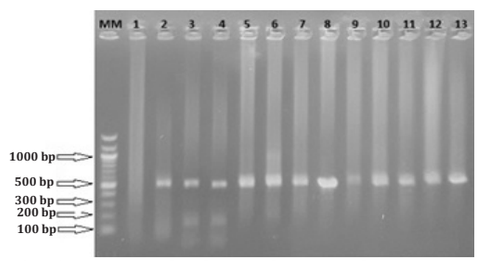

Fig. 1. Agarose gel electrophoresis for *cowp* gene of *Cryptosporidium* spp. at 553 bp-targeted nPCR products. **MM:** 100 bp DNA molecular weight marker "ladder". **Lane 1:** Negative control. **Lane 2:** Positive control at 553 bp. **Lanes 3-13:** Positive samples.

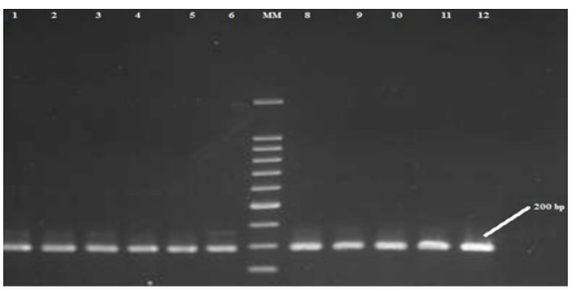

Fig. 3. Agarose gel electrophoresis for the products of the nPCR targeting UreA gene of *H. pylori* at 200 bp. **Lane 7:** 100 bp DNA molecular weight marker. **Lanes 1-6 and 8-12:** Positive samples.

Table 2. Socio-demographic data and infection rates of *Cryptosporidium* spp., *I. belli*, and *H. pylori* in HIV patients.

	Positive samples (No. 45/70: 64.3%)					
	***Cryptosporidium* spp.**		***I. belli***		***H. pylori***	
	No. (%)	***P* value**	**No. (%)**	***P* value**	**No. (%)**	***P* value**
Total number	**20 (28.6%)**		**10 (14.3%)**		**15 (21.4%)**	
Age						
16-30	6 (30)	**0.000***	0 (0)	**0.000***	2 (13)	0.569
31-45	0 (0)		9 (90)		5 (33)	
46-60	14 (70)		1 (10)		8 (53)	
Sex						
Male	9 (45)	**0.004***	10 (100)	**0.000***	11 (73.34)	0.751
Female	11 (55)		00 (0)		4 (26.66)	
Education level						
literate	13 (65)	**0.011***	1 (10)	**0.029***	8 (54)	0.291
Illiterate	7 (35)		90 (90)		7 (46)	
Animal contact#						
Yes	20 (100)	**0.000***	1 (10)	**0.005***	9 (60)	0.454
No	0 (0)		90 (90)		6 (40)	
Abdominal pain						
Yes	20 (100)	**0.000***	4 (40)	0.198	11 (73.34)	0.190
No	0 (0)		60 (60)		4 (26.66)	
Diarrhea						
Yes	20 (100)	**0.000***	1 (10)	**0.006***	9 (60)	0.382
No	0 (0)		90 (90)		6 (40)	

Data presented as No. and percentage. **#:** Farm animals, *: Significant (P<0.05).

Associated risk factors for *H. pylori* co-infection with IPs among HIV-positive patients: To reveal potential contributing risk factors that could explain the link between *H. pylori* and certain IPs, several environmental markers that indicate fecal-oral transmission pathway exposure were studied. Univariate analysis showed statistically significant association between farm animal contact and concomitant *H. pylori* and *Cryptosporidium* spp. (0.007 for both pathogen). Diarrhea and abdominal pain were two of the primary symptoms of co-infection. Statistically, there was a strong association between coinfection of *Cryptosporidium* spp. with *H. pylori* and abdominal pain (P=0.019) and diarrhea (P=0.005) (Table 3).

Table 3. Socio-demographic data, and symptoms potentially recorded in *H. pylori* co-infection with *Cryptosporidium* spp.

	Co-infection of *H. pylori* with *Cryptosporidium* spp.	
	No. (%)	**Statistical analysis**
Total number	7	
Age		
16-30	2 (28.55)	**0.054**
31-45	0 (0)	
46-60	5 (71.45)	
Sex		
Male	3 (42.85)	**0.099**
Female	4 (57.15)	
Education level		
literate	5 (71.45)	**0.089**
Illiterate	2 (28.55)	
Animal contact#		
Yes	7 (100)	**0.007***
No	0 (0)	
Abdominal pain		
Yes	7 (100)	P =0.019, OR=4.11, 95% CI=1.81-8.35
No	0 (0)	
Diarrhea		
Yes	7 (100)	P =0.005, OR=3.70, 95% CI=1.69-8.41
No	0 (0)	

Data presented as No. and percentage. **#:** Farm animals, *: Significant (P<0.05).

DISCUSSION

According to our up-to-date review of the literature, our study is the first to outline the detection rates and contributing factors of opportunistic intestinal parasites, and *H. pylori* coinfection among HIV/AIDS patients in Egypt. In our investigation, intestinal parasites were recorded in 74.3% of HIV participants, which exceeds rates reported from Nigeria (22.7% and 5.3%)[17,18], Ghana (35%)[19], Kenya (50.9%)[20], and from Ethiopia 30.6%[21], but is lower than that recorded in Cameroon (82.6%)[22], and in 9 out of 10 patients attending Assiut University Hospitals in Egypt[23]. Registered variations from all over the world may be attributed to difference in geographical regions, socioeconomic conditions, sample sizes, and cultural practices. In our study, *I. belli* was found in 14.3% of the HIV patients complaining of diarrhea, which is consistent with the report of Certad *et al*.[24], but is higher than other reports[25-27].

Molecularly, the detected rate of *H. pylori* in 21.4% of HIV patients was apparently lower than that in prior studies from India[10], Iran[28], Ghana[29], Nigeria[30,31],

and Ethiopia[32]; but it is higher than the 8.3% rate recorded by Perry *et al*.[33] from Romania. Due to the associated immunological deficiencies in HIV patients, this coexistence may be mechanically or pathologically related. Our recording of statistically significant correlation between *H. pylori* infection and intestinal and opportunistic polyparasitism in HIV patients may support this hypothesis.

Even in the absence of HIV, gastrointestinal parasites and *H. pylori* are believed to share the same risk factors[34]. In Sudan, *H. pylori* was found to frequently coexist with intestinal and opportunistic parasites[35]. Furthermore, with statistical significance, it was discovered that the percentage of cryptosporidiosis co-infection with *H. pylori* was (53.85%), followed by *E. histolytica/E. dispar* (23.07%), *G. lamblia* (15.38%), and *I. belli* (7.7%)[36]. Ibrahim *et al*.[37] observed a prevalence of cryptosporidiosis co-infection with *H. pylori* (60%) and giardiasis (58%) in children.

Such variable detection rates are governed by differences in study populations, with conflicting socio-behavioral and demographic variables linked to *H. pylori* and intestinal parasites. In other reported studies, age, gender, education level, and animal contact showed a significant association with *I. belli* and *Cryptosporidium* spp., however, there was no significant association with *H. pylori*[38-40].

In conclusion, although the sample size is small because of the availability of HIV patients, our study observed that co-infection of IPs with *H. pylori* may synergistically compromise the HIV patient's health. Our study highlighted that C. *parvum* was the most prevalent OIP encountered in HIV patients. Gender, diarrhea, abdominal pain, and polyparasitism have all been identified as risk factors for co-infection with *H. pylori*. The efficient therapy protocol for HIV patients should include testing and treatment of co-existing gastrointestinal infectious agents in addition to *H. pylori*. Further studies using other more specific staining techniques (e.g., trichrome stain for detection of opportunistic *Microsporidium* spp.) as well as molecular screening of concomitant IPs infections and *H. pylori* are recommended.

Author contribution: Ibrahim A and Ramadan ME suggested the topic. All authors contributed to the practical work, analysis of data, and writing of the manuscript. Ramadan ME collected the sociodemographic (age, gender, animal contact) and clinical data including symptoms and treatment. All authors revised the manuscript and approved the final version of the manuscript before publication.

Conflict of interest: The authors declare that there is no competing interest.

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