Tropical Biomedicine 40(2): 199-207 (2023)
https://doi.org/10.47665/tb.40.2.012

RESEARCH ARTICLE

Post-COVID-19 cryptosporidiosis: A serious risk or mere association?

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ARTICLE HISTORY

Received: 9 November 2022
Revised: 8 March 2023
Accepted: 9 March 2023
Published: 30 June 2023

ABSTRACT

Post-COVID-19 conditions encompass a wide range of health problems, including enteritis, but their association with parasitic infections has not yet been investigated. This study analyzed gastrointestinal symptoms, medical histories, fecal *Cryptosporidium* oocysts, and the history of COVID-19 infection in patients who attended the Faculty of Medicine, Cairo University, from January to July 2021. Fecal biomarkers, including *H. pylori*, occult blood, fecal calprotectin (FCAL), and TNF-α, were measured, and *Cryptosporidium* spp. genotypes were molecularly characterized among post-COVID-19 patients using RFLP. Preliminary results from 210 post-COVID-19 patients revealed that group 1 (*Cryptosporidium*-positive) (n = 49) and group 2 (*Cryptosporidium*-negative) (n = 161) showed no significant difference in the prevalence rate of diabetes mellitus (DM). While group 2 was linked to diarrhea, only infections with *Cryptosporidium* post-COVID-19 were related to chronic diarrhea, vomiting, and weight loss. A total of 220 healthy subjects served as negative controls. Administering azithromycin, hydroxychloroquine, and ivermectin was significantly related to an increased risk of *Cryptosporidium* infection in group 1, whereas only azithromycin was more frequently recorded in group 2. Antioxidant supplementation insignificantly affected the incidence of cryptosporidiosis. Cryptosporidiosis with a history of COVID-19 was linked to *H. pylori* infections, increased inflammatory biomarkers (FCAL and TNF-α), and occult blood when compared with group 2. *Cryptosporidium* genotype 1 was the most commonly occurring subset in individuals with post-COVID-19. The findings demonstrated that aggravating gastrointestinal manifestations, increased fecal biomarkers and anti-COVID-19 therapeutic interventions are significantly related to the existence of *Cryptosporidium* oocysts in patients with post-COVID-19, indicating the predominance of.

Keywords: COVID-19; *Cryptosporidium* genotype 1; inflammatory biomarkers; *H. pylori* infections; occult blood.

INTRODUCTION

Cryptosporidium spp. are a group of apicomplexan protozoa that infect the intestinal epithelial cells, causing villous atrophy, crypt hyperplasia, and increased fluid, electrolyte, and mineral excretion (Pinto & Vinayak, 2021). The disease occurs following the ingestion of *Cryptosporidium* oocysts, sporozoite excystation, adhesion, and parasite internalization inside the apical cell membrane of the intestinal epithelium. The COVID-19 novel coronavirus, which causes the severe acute respiratory syndrome, first appeared in China and rapidly outbreak worldwide in 2019 (Song *et al.*, 2020). The primary method of transmission of COVID-19 is through the respiratory droplets of infected individuals. The World Health Organization (WHO) classified it as a severe infectious virus due to its high infectivity and rapid spread, resulting in a pandemic. As a result, several pulmonary histological abnormalities also involve a wide range of effects, including inflammation, vasoconstriction, and hypercoagulability (Jain, 2020). According to the WHO, 756,291,327 verified COVID-19 cases had been reported worldwide as of February 2023, with 6,841,640 fatalities (WHO).

In 2020, meta-analysis research provided evidence of digestive symptoms and mortality in individuals suffering from COVID-19 (Zhang *et al.*, 2020). Remarkably, some cases experience vomiting or nausea as the primary clinical presentation of COVID-19, which is frequently disregarded. In addition, significant weight loss and malnutrition were reported in seriously diseased individuals (McMorrow *et al.*, 2020; Anker *et al.*, 2021). Increased recognition of these warning signs and well-timed intervention would aid in combating the pandemic (Zhang *et al.*, 2021). Recently, several reports have been conducted to demonstrate the adverse post-COVID health consequences involving gastroenteritis (CDC, 2021; Silva Andrade *et al.*, 2021).

Recent research has focused on the shared symptoms between COVID-19 and intestinal parasitic infections (Rajan *et al.*, 2020). Since early studies, cryptosporidiosis has been defined as the acute onset of watery diarrhea (Nime *et al.*, 1976; Colebunders, 2009), weight loss, and malnutrition in developing nations (Mølbak *et al.*, 1997; Shrivastava *et al.*, 2017). However, in 2021, O'Leary et al. suggested that the true prevalence and impact of cryptosporidiosis were often underreported, raising the possibility of the parasite's prevalence with COVID-19 infections (O'Leary *et al.*, 2021).

The prevalence of diabetes mellitus (DM) has been a risk factor linked to high morbidity (Zoppini *et al.*, 2018) and hospitalization (Shah & Hux, 2003) due to infection. In 2007, Benfield et al. reported the relationship between hyperglycemia and infectious diseases related to the respiratory system, skin, and urinary tract (Benfield *et al.*, 2007). However, few epidemiological studies have investigated the relationship between DM and intestinal parasitic infections (Mohtashamipour *et al.*, 2015). The improper functioning of adaptive and innate immune responses hinders the control and death of invading organisms, resulting in a continuous state of mechanical and physiological dysfunction and low-grade inflammation. Consequently, DM appears to be significantly impactful in controlling some diseases and medication resistance (Fisher-Hoch *et al.*, 2013). In tropical countries, research on the interactions between DM and the burden of accompanying infectious diseases is essential to boost health efforts (van Crevel *et al.*, 2017), particularly during COVID-19 (Suwanwongse & Shabarek, 2021).

Evaluation of the prevalence of different infectious agents among various populations of drug users has been the focus of recent research. A single drug may cause multiple adverse effects that collectively impact gastrointestinal and immunological functions and the course of infections (Jones *et al.*, 2017; Schrezenmeier & Dörner, 2020); for instance, the effect of immunomodulatory drugs on individuals suffering from rheumatic diseases (Monti & Montecucco, 2020) or their use as prophylaxis against COVID-19 (Sarzi-Puttini *et al.*, 2021). Furthermore, zinc and vitamin C supplements have been shown to reduce oxidative stress (Barbosa *et al.*, 2009).

Considering the pathogenicity of *Cryptosporidium* parasites and COVID-19, it is crucial to investigate the comorbidity caused by cosmopolitan *H. pylori* infection. A study mentioned that around 4.4 billion people, or 60.3% of the global population, have *H. pylori* (Hooi *et al.*, 2017). According to another research, *H. pylori* infection can develop at any age, starting in childhood and continuing throughout adulthood (Zamani *et al.*, 2018). Furthermore, screening for fecal occult blood to assess the possibility of enteropathogens causing intestinal bleeding has been previously demonstrated (Okamoto *et al.*, 2005; Eligail *et al.*, 2010; Bustinduy *et al.*, 2013).

Previous research on the host defense mechanisms for cryptosporidiosis suggested that neutrophils, lymphocytes, and macrophages could infiltrate the lamina propria (Di Genova & Tonelli, 2016; Shrivastava *et al.*, 2017). Additionally, bloody diarrhea and hemorrhagic colitis have been determined as initial COVID-19 symptoms (Carvalho *et al.*, 2020). In this regard, assessing inflammatory biomarkers such as calprotectin and TNF-α in cryptosporidiosis and COVID-19 may provide additional value for their diagnostic profiles.

Cryptosporidium spp. may infect a wide range of vertebrate hosts, with over 37 species and 70 genotypes (Ryan *et al.*, 2021). Evaluating the genotype and species diversity of *Cryptosporidium* spp. and its prevalence in COVID-19 infections may help clarify the potential association between *Cryptosporidium* and COVID-19.

This study aimed to evaluate the prevalence of (1) cryptosporidiosis in individuals having a COVID-19 history, (2) gastrointestinal manifestations, DM, and various therapeutic medications, (3) *H. pylori* antigen, fecal occult blood (FOB), and the inflammatory biomarkers (fecal calprotectin (FCAL) and TNF-α), and (4) the distribution of *Cryptosporidium* genotypes with COVID-19 infection among Egyptian patients.

MATERIAL AND METHODS

Ethics approval

This research was carried out on the 1964 Helsinki Declaration and the ethical standards of the National Research Committee. Ethical approval was granted by the Faculty of Medicine at Cairo University and the Institutional Review Board at the Armed Forces College of Medicine, Ministry of Defense, Cairo, Egypt (protocol number 178). Only stool samples were used in this study.

Study settings

This research was carried out at the Medical Parasitology Department and the Department of Clinical and Chemical Pathology at the Faculty of Medicine, Cairo University, from January to July 2021.

Study population and sample collection

The research conducted in this study involved designing a questionnaire to gather data on various factors related to COVID-19 infection and gastrointestinal manifestations. The first section of the questionnaire included information about the participants' gender, age, and history of COVID-19 infection. The second section examined any history of diabetes and drug treatments among the participants, and the prevalence of gastrointestinal symptoms, including weight loss, diarrhea, and vomiting ($\geq$ 3 loose stools per 24 hours). The study included adult participants older than 18 having a COVID-19 history of positivity. Children, pregnant women, cancer patients, and individuals with previously diagnosed inflammatory bowel disease were excluded. In total, 210 adult patients with a positive COVID-19 history participated in the study, and 220 healthy adults were negative controls. The research team considered the Ethical Standards of the Faculty of Medicine at Cairo University, and the ethical guidelines of the 1964 Helsinki Declaration. All study participants provided their informed consent.

Laboratory procedures

The specimens were split into two groups for analysis. The first group was utilized for stool analysis and permanent acid-fast staining. At the same time, the other was frozen for eventual evaluation of fecal biomarkers and genotyping of *Cryptosporidium* parasites in microscopically positive cases. To ensure sample quality, exclusion criteria were established to exclude unfit, contaminated, and mislabeled specimens.

Assessment of fecal biomarkers

Each participant received instructions on properly using the test kits according to the manufacturer's guidelines. Before the stool collection, dietary restrictions were implemented, including discontinuing vitamin C, aspirin, and nonsteroidal anti-inflammatory medications three days before the test. Fecal specimens were not collected three days before, during, or after a menstrual cycle if the participant had bleeding hemorrhoids or hematuria. Samples were collected using the provided gathering probes from multiple regions of the same bowel movement. Within four days of collection, the specimens were kept at 4°C in storage.

Detection of occult blood in stool

The Immunochromatography (ICT) test was performed by the commercial test kit (Bowel Colon Cancer Test Kit Faecal Occult Blood (FOB) Home Tests - One Step, China) according to the manufacturer's instructions. The ICT test was considered positive when the control (A) and test (C) lines were apparent.

The test was considered negative when just the control line (A) was present. The test was deemed invalid when the control line (A) was missing.

a- Detection of H. pylori antigen

The ICT test was implemented using the commercial test kit OnSite *H. pylori* Ag Rapid Test, San Diego County, California, following the manufacturer's guidelines. Readings were performed in a way similar to those within the kits of the FOB.

b- Measurement of TNF-α in fecal samples

This was determined utilizing fecal samples and an ELISA test (Eagle Biosciences, KR9610, USA) per the manufacturer's guidelines (Nicholls *et al.*, 1993). According to Kapel *et al*., undetectable fecal TNF-α is normal (Kapel *et al.*, 2005).

c- Measurement of fecal calprotectin (FCAL)

The FCAL level was assessed quantitatively using frozen stool samples. The manufacturer's instructions were followed to perform a solid-phase sandwich ELISA using the DRG: HYBRiDXL Calprotectin Kit, German, Cat. No. HYE-5767). FCAL readings higher than 50 µg/g were positive (Kennedy *et al.*, 2015).

Genotyping of *Cryptosporidium* oocysts

Genomic DNA was obtained from fecal specimens by the FavorPrepTM stool DNA isolation Mini Kit (Favorgen Biotech Corporation, Ping-Tung, Taiwan, Cat. No. FASTI 001,). The extracted copro-DNA was amplified by nested PCR (nPCR) targeting the *Cryptosporidium* oocysts wall protein (COWP) gene. BCOWPF (5'-ACC GCT TCT CAA CAA CCA TCT TGT CCT C-3') and BCOWPR (5'-CGC ACC TGT TCC CAC TCA ATG TAA ACC C-3') were the primers used to generate the 796-bp fragment (Pedraza-Díaz *et al*., 2001). Cry-15 (5'-GTA GAT AAT GGA AGA GAT TGT G-3') and Cry-9 (5'-GGA CTG AAA TAC AGG CAT TAT CTT G-3') were employed as nested primers to amplify the 553-bp fragment (Spano *et al.*, 1997). The total reaction volume in the primary and secondary reactions was 25 µL. Except for the template DNA, the reagents employed in the primary and secondary reactions were identical. The amplified amplicons of 553 bp generated by the secondary reaction were electrophoresed on a 1.5 percent agarose gel and examined under a UV transilluminator after being stained with ethidium bromide.

To identify the *Cryptosporidium* genotypes, positive nPCR samples were submitted to RFLP with restriction enzyme cleavage (Rsa I). The reaction was carried out in 30 µL. Among the utilized ingredients were 17 µL nuclease-free water, 1 µL Rsa I enzyme, 2 µL green buffer, and 10 µL PCR products (target DNA). If Rsa I digestion produced four bands: 34 bp, 106 bp, 125 bp, and 285 bp, genotype 1 was considered, whereas genotype 2 was recognized if Rsa I digestion produced three bands: 34 bp, 106 bp, and 410 bp (Fathy *et al.*, 2014; Abdelrazek *et al.*, 2016; Amin *et al.*, 2021).

Statistical analysis

The data were coded and entered using SPSS v28 (IBM Corp., Armonk, NY, USA). Quantitative data were summarised using median, standard deviation, mean, minimum, and maximum, whereas categorical data were summarized using frequency (count) and relative frequency (%). The non-parametric Mann-Whitney test was utilized to compare the quantitative variables (Chan, 2003a). To compare categorical data, a chi-squared ($\chi 2$) test analysis was used. If the expected frequency was < 5, the exact test was performed (Chan, 2003b). Statistical significance was defined as p-values < 0.05.

RESULTS

Characterization of the study population

Of the 210 post-COVID-19 patients, 49 (23.3%) were positive for *Cryptosporidium* and belonged to group 1 (*Cryptosporidium* positive), while 161 (76.6%) patients tested negative for *Cryptosporidium* and belonged to group 2 (*Cryptosporidium* negative). When comparing both sex and age, insignificant differences were identified between the groups.

Gastrointestinal manifestations

Insignificant difference was found in the duration of diarrhea between individuals with and without a *Cryptosporidium* infection. Nevertheless, vomiting, weight loss, and mean duration of diarrhea (6.9 ± 5.2 days, ranging from 2 to 20 days) were significantly more frequent in patients with cryptosporidiosis than in the control group ($p < 0.05$).

Incidence of preexisting DM

An investigation into the frequency of comorbidity with DM showed a notable similarity in the significant variation between both group 1 and group 2 (20.5% and 18.3%, respectively). This was confirmed by the non-parametric Mann–Whitney test, which demonstrated a statistically non-significant p-value ($p > 0.05$).

Previous medical therapies

Individuals who were administered ivermectin, hydroxychloroquine, or azithromycin were more likely to develop a *Cryptosporidium* spp. infection ($p \leq 0.05$) in group 1. Nevertheless, an insignificant difference was identified in the history of antioxidant intake (Zinc + vitamin C) between the groups, as shown in Table 1.

Table 1. Distribution of group 1 and group 2 regarding the past anti-COVID-19 therapeutic history

		Group 1		Group 2		
		Count	%	Count	%	P-value
Zithromycin	P[1]	14	28.6%	13	8.1%	< 0.001
	N[2]	35	71.4%	148	91.9%	
Ivermectin	P	19	38.8%	20	12.4%	< 0.001
	N	30	61.2%	141	87.6%	
Hydroquinne	P	20	40.8%	29	18.0%	0.001
	N	29	59.2%	132	82.0%	
vit c+Zinc	P	9	18.4%	35	21.7%	0.612
	N	40	81.6%	126	78.3%	

[1]P: positive; [2]N: negative.

Table 2. Fecal tests between group 1 and group 2

		Group 1 (49 cases)		Group 2 (161 cases)		
		Count	%	Count	%	P-value
H. pylori	P	23	46.9%	27	16.8%	< 0.001
	N	26	53.1%	134	83.2%	
FCAL	P	32	65.3%	4	2.5%	< 0.001
	N	17	34.7%	157	97.5%	
Occult blood	P	21	42.9%	40	24.8%	0.015
	N	28	57.1%	121	75.2%	

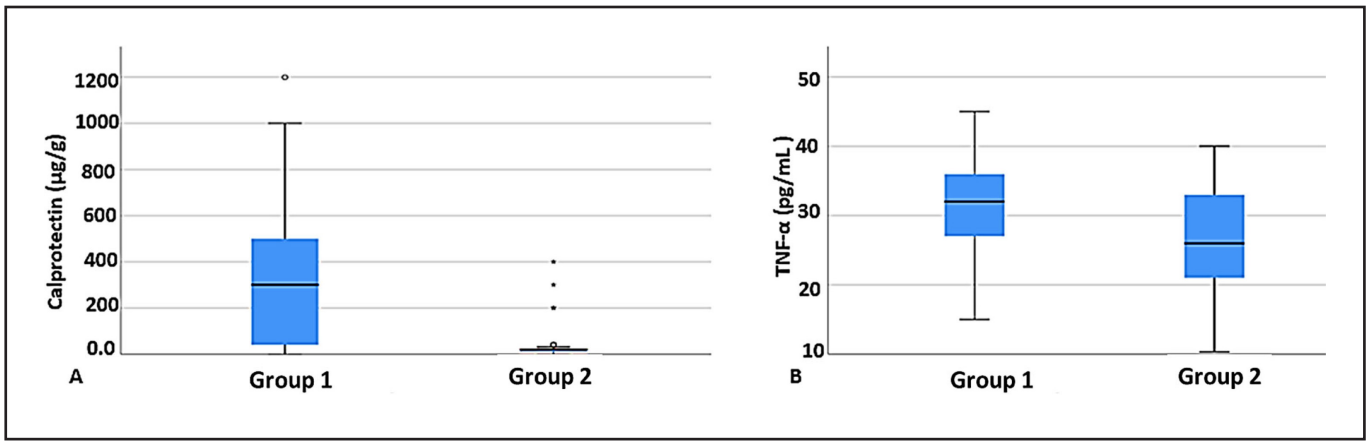

Figure 1. The distribution of fecal inflammatory biomarkers in groups 1 and 2. (A) FCAL and (B) TNF-α.

Table 3. The distribution of *Cryptosporidium* genotypes in post-COVID-19 patients

Genotype	(n = 49)	%	P-value
1	43	87.7%	< 0.05
2	6	12.2%	

Assessment of fecal biomarkers

Regarding group 1, a significant overall increase in the proportions of infection with *H. pylori*, positive occult blood, and FCAL was observed compared to group 2 (Table 2). However, both groups had significantly higher proportions than the controls.

In parallel, levels of FCAL and TNF-α were significantly lower in group 2 than in group 1 (Table 3, Figure 1).

Molecular characterization of *Cryptosporidium* spp.

Of the 49 positive specimens, 42 (85.7%) generated visible bands indicating genotype 1, while only seven samples (14.3%) generated genotype 2 ($p \leq 0.05$) (Table 3).

DISCUSSION

Knox et al. mentioned that the COVID-19 pandemic lockdown was responsible for preventing the spread of *Cryptosporidium* from person to person (Knox *et al.*, 2021). However, zoonotic transmission remained predominant, and subsequently, the secondary anthroponotic transmission of the parasite occurred among contacts of zoonotic cases. Intriguingly, Elsaftawy *et al*. proposed an inquiry into the capability of parasites to be infected with COVID-19 through endosymbiosis (Elsaftawy *et al.*, 2021). In 1997, Khramtsov *et al*. showed the existence of double-stranded RNAs in *C. parvum* (Khramtsov *et al.*, 1997). Similarly, protozoans such as *Naegleria gruberi* (Schuster, 1969), *Entamoeba histolytica* (Mattern *et al.*, 1972), *Trichomonas vaginalis* (Parent *et al.*, 2013), *Giardia lamblia* (Janssen *et al.*, 2015), *Eimeria stiedai* (Xin *et al.*, 2016), *Leishmania guyanensis* (Bourreau *et al.*, 2015), and *Plasmodium* Rosenke spp. (Rosenke *et al.*, 2016) were infected with double-stranded RNA virus-like particles. Moreover, a recent study found that DNA from large DNA viruses has been incorporated into the genome of *Acanthamoeba* species (Aherfi *et al.*, 2016). Griffiths *et al*. determined that the host's innate immunity can detect many viruses through several inflammatory sequences (Griffiths *et al.*, 2011). In addition, alterations in gut microbiota seemed to be induced by both intestinal parasites (Elsaftawy & Wassef, 2021) and COVID-19 (Dhar & Mohanty, 2020), which can exacerbate the condition.

Overall, *Cryptosporidium* infections in post-COVID-19 patients was correlated with severe gastrointestinal involvement. Cama *et al*. documented that vomiting and chronic diarrhea coincided with *C. parvum* (Cama *et al.*, 2007). In contrast, diarrhea was the predominant manifestation associated with *C. felis*, *C. canis*, and the subtype (Id) of *C. hominis*. Unlike our findings, prior research revealed that cryptosporidiosis in infancy was associated with weight loss in both males and females, and no significant catch-up growth was provided to compensate for this weight loss (Mølbak *et al.*, 1997). The inhibition of TLR2 and TLR4, and Th1 cytokine responses are associated with parasitic enteric infections and the vicious cycle of malnutrition (Costa *et al.*, 2011) and the induced expression of Fas/Fas ligand on infected and uninfected cells (Motta *et al.*, 2002; Costa *et al.*, 2011).

An earlier meta-analysis demonstrated the connection between various gastrointestinal symptoms and the mortality and morbidity of COVID-19 patients (Zhang *et al.*, 2020). During the COVID-19 infection, it was discovered that the gastrointestinal epithelium had a high expression of the angiotensin-converting enzyme-2 (ACE2) receptor, which is thought to be the gateway to the virus and may cause the onset of nausea and vomiting (Zhang *et al.*, 2021). In this regard, Anker *et al*. focused on the necessity of nutritional support whenever possible. Although there are no specific anti-cachectic treatments for COVID-19, they constitute a high medical priority to avoid long-term disability (Anker *et al.*, 2021). However, Eskandarian et al. found the absence of a significant relationship between gastrointestinal manifestations and COVID-19 infections (Eskandarian *et al.*, 2021).

The present study demonstrates a relative consensus regarding the incidence of cryptosporidiosis and COVID-19 infections in diabetic and nondiabetic individuals. The current outcomes agree with prior research that reported *Cryptosporidium* spp. in less than 5% of diabetic patients (Mohtashamipour *et al.*, 2015; Fadl *et al.*, 2021). *Blastocystis hominis* (Mohtashamipour *et al.*, 2015; Tangi *et al.*, 2016; Fadl *et al.*, 2021), *G. lamblia* (Elnadi *et al.*, 2015; Mohtashamipour *et al.*, 2015), and *E. histolytica* (Mohtashamipour *et al.*, 2015; Tangi *et al.*, 2016; Fadl *et al.*, 2021) were found to be the most frequent intestinal parasites in prior studies, with a significantly increased prevalence in diabetic individuals than in nondiabetic people. However, the incidence of *Cryptosporidium* infections in diabetic patients appeared to be higher in childhood (Rady *et al.*, 2019) and in uncontrolled diabetes status (Fadl *et al.*, 2021) due to immune dysregulation (Htun *et al.*, 2018).

Concerning COVID-19, Chen *et al*. reported a relationship between SARS-CoV-2 infections and uncontrolled diabetes, and the comorbidity of COVID-19 with diabetes was linked to an increased risk of morbidity (Htun *et al.*, 2018; Chen *et al.*, 2020). This is likely due to insulin resistance caused by an over-activated inflammatory response, as confirmed by increased levels of CRP, IL6, pro-calcitonin, and serum ferritin (Li *et al.*, 2021).

In the current research, no significant differences were identified in the incidences of cryptosporidiosis and COVID-19 infections among patients who received corticosteroids. In a previous study, corticosteroid administration was linked to a decreased mortality rate compared to usual care (Arabi *et al.*, 2020). Corticosteroids are immunosuppressive medications that regulate immune responses and reduce inflammation in viral diseases, such as severe MERS and SARS infections (Angus *et al.*, 2020). Previous studies have reported increased incidences of *Cryptosporidium* infections in immunocompromised individuals, including those having HIV (Shrikhande *et al.*, 2009) or who have undergone organ transplantation (Rodr□guez Ferrero *et al.*, 2010). A subset of COVID-19 patients who experience a higher death rate may have cytokine storm syndrome (Mehta *et al.*, 2020). Therefore, corticosteroids have been suggested for treating severe COVID-19 individuals in China (Zhao *et al.*, 2020). However, the use of corticosteroids in COVID-19 has been widely debated (Johnson & Vinetz, 2020).

The present research indicated a significant increase in *Cryptosporidium* infections among patients who received azithromycin after being infected with COVID-19. Azithromycin is a weak base that remains in acidic cell organelles, including lysosomes, and may prevent the virus from replicating by interfering with pH-dependent processes (Molina *et al.*, 2020). In addition, certain substances can induce macrophages to adopt an anti-inflammatory M2 phenotype (Derwand & Scholz, 2020).

Our findings revealed widespread *Cryptosporidium* infections in patients receiving hydroxychloroquine. Hydroxychloroquine is an antimalarial drug with activity against rheumatoid arthritis, systemic lupus erythematosus (SLE), and other inflammatory rheumatic diseases (Schrezenmeier & Dörner, 2020), as well as bacterial (Brorson & Brorson, 2002), fungal (Wang *et al.*, 2023), and viral infections (Gao *et al.*, 2020). Nevertheless, it is known that hydroxychloroquine interferes with lysosomal function and interacts with membrane stability, inhibiting the generation of cytokines and immunomodulation (Schrezenmeier & Dörner, 2020). Concerning COVID-19 infections, hydroxychloroquine inhibited SARS-CoV replication and spread in an *in vitro* study (Keyaerts *et al.*, 2004) by impairing the activation of angiotensin-converting enzyme-2 and hindering virus binding to cells (Vincent *et al.*, 2005). Nevertheless, using immunosuppressants as prophylaxis for COVID-19 is still inconsistent (Sarzi-Puttini *et al.*, 2021).

Similar to azithromycin and hydroxychloroquine, ivermectin appears to be related to a significant increase in the prevalence of parasitic infection. Ivermectin is a macrocyclic lactone derivative with broad antiparasitic (Chhaiya *et al.*, 2012), antiviral (Momekov & Momekova, 2020), and anti-cancer therapeutic bioactivities (Juarez *et al.*, 2020). Ivermectin was determined to be an effective option for managing individuals with mild-to-moderate COVID-19 infection since it is effective in in vitro studies against several RNA viruses (Mohiuddin Chowdhury *et al.*, 2020). Caly *et al*. approved ivermectin to inhibit the replication of SARS-CoV-2 *in vitro* by around 5000-fold at 48 h (Caly *et al.*, 2020). Additionally, it is active against *Cryptosporidium* and *Giardia* infections in animal models. Moreover, for mass treatment of malaria, it was found to kill mosquitoes sucking blood from recently treated patients (Smit *et al.*, 2018). However, in the present research, ivermectin was linked to various incidences of parasites.

This study showed insignificant differences in the proportion of individuals having or do not have a parasitic infection in the post-COVID-19 group when antioxidant supplements of zinc and vitamin C were administered. Few studies have considered the effect of minerals and vitamins on cryptosporidiosis infection. In a recent study, Zn showed an impressive reduction rate of *Cryptosporidium* oocysts (Gaber *et al.*, 2022). However, the modulation of inflammation and production of cytokines through Zn as immune nutrition is an innovative concept of treatment for COVID-19 (Pal *et al.*, 2021). Additionally, Kim and Yeom proposed vitamin C as a possible therapy for COVID-19 (Kim & Yeom, 2020). Moreover, the protective effect of vitamin C in treating COVID-19 was emphasized to lower the risk of mortality (Fowler *et al.*, 2019) with multiple supplements involving zinc, ascorbic acid, N-acetyl-cysteine, and vitamin D (Bauer *et al.*, 2020).

The current research demonstrated a significantly increased proportion of *Cryptosporidium* and *H. pylori* co-infections in post-COVID-19 patients. Gastric juice, under normal circumstances, forms an inhospitable environment for pathogenic germs. *H. pylori* infection alters gastric acid output. Depending on the position and duration of infection, the bacteria can undermine the gastric mucosal barrier, providing a suitable environment for intestinal pathogens (Wroblewski *et al.*, 2010; Waldum *et al.*, 2016). Co-infections of the parasite with *H. pylori* were highly prevalent in adults (Ghallab & Morsy, 2020). In contrast, in childhood, concomitant cryptosporidiosis was non-significant (Rady *et al.*, 2019). Jones *et al*. mentioned that the clinical manifestations of *H. pylori* are associated with its virulence and the host's immune system (Jones *et al.*, 2017). Furthermore, *H. pylori* increased ACE-2 receptors' expression in the gastrointestinal tract, which is directly related to the extent and infection severity. Furthermore, immune factors, such as IL-10, IL-8, IL-6, and TNF-α may increase via virulent factors (Sugimoto *et al.*, 2012; Muhammad, 2015) that might impact the disease's clinical course (Balamtekin *et al.*, 2021).

Concerning our research, we found that *Cryptosporidium* infection in people who recovered from COVID-19 was associated with significantly higher fecal occult blood (FOB) results. Previous studies have reported that 18% of *Cryptosporidium* parasite-infected

cases had occult blood (Newman *et al.*, 1999). Additionally, similar findings were observed in cases of amebic colitis (Okamoto *et al.*, 2005) and active *S. mansoni* infection (Bustinduy *et al.*, 2013).

FOB is an easily accessible screening biomarker for gastrointestinal COVID-19 infection, even though the exact cause of diarrhea in COVID-19 infection is unclear. Higher positivity for FOB has been observed (Du *et al.*, 2021). Reports suggested that anticoagulants may be a contributing factor (Cavaliere *et al.*, 2020; Melazzini *et al.*, 2020; Mauro *et al.*, 2021), particularly in the upper gastrointestinal tract (Mauro *et al.*, 2021).

In cases of non-variceal upper gastrointestinal hemorrhage and COVID-19, only medical therapy can be utilized for treating them (Sethi *et al.*, 2020; Mauro *et al.*, 2021) through intravenous proton pump inhibitors (Cavaliere *et al.*, 2020).

The current research found that *Cryptosporidium* infections in people who recovered from COVID-19 are associated with increased inflammatory biomarkers, specifically fecal calprotectin (FCAL) and tumor necrosis factor-alpha (TNF-α). Moreover, Effenberger *et al*. reported a relationship between increased production of fecal FCAL and fecal SARS-CoV-2 RNA (Effenberger *et al.*, 2020). *Cryptosporidium* infection is linked to increased levels of proinflammatory cytokines, such as interleukin IL-1β and TNF-α, which enhance prostaglandin synthesis (Kandil *et al.*, 1994) and influence diarrhea by altering chloride secretion (Robinson *et al.*, 2001). Silvin *et al*. mentioned a positive correlation between FCAL, neutrophil counts, and disease severity (Silvin *et al.*, 2020). Enteric infections alter gut microbiomes that inhibit the action of proinflammatory cytokines, including TNF-α, IL-6, and IL-1β, and enhance the anti-inflammatory activity of IL-10 (Song *et al.*, 2020; Elsaftawy & Wassef, 2021).

Furthermore, the invasion of SARS CoV 2 is linked to a decline in mucosal ACE2, leading to an increase in angiotensin II, low angiotensin (1–7) levels, and elevated inflammatory cytokines (Okoloko *et al.*, 2021). Song et al. hypothesized that mucosal inflammation primarily occurs due to high expression of TNF-α, IL-1β, and IL-6 (Song *et al.*, 2020). In COVID-19, FCAL data should be investigated carefully as dysentery, and hemorrhagic colitis has been identified as initial symptoms in several cases, leading to poor outcomes (Carvalho *et al.*, 2020; Mahler *et al.*, 2021).

Consistent with our findings, Amin et al. mentioned a higher prevalence of genotype 1 compared to genotype 2 (Amin *et al.*, 2021). The results demonstrated that cryptosporidiosis genotype 1 infections were significantly higher in cases of simultaneous co-infection with COVID-19. Interestingly, in a previous study, bovine coronavirus (BCoV) entry appeared to be higher when *Cryptosporidium* sporozoites were present, as the specific binding between BCoV and the sporozoites occurred (Shakya *et al.*, 2022).

CONCLUSIONS

In the present research, post-COVID-19 patients experienced exacerbated gastrointestinal symptoms in the presence of *Cryptosporidium* infections, despite diarrhea being the predominant symptom in most cases. Furthermore, we found that fecal biomarkers, including FCAL, TNF-α, *H. pylori* Ag, FOB, and medications related to COVID-19 treatment, were significant factors in this population. Notably, *Cryptosporidium* genotype 1 was the most prevalent. We speculate whether these findings could be explained by the preceding pathology of the COVID-19 virus in the enteric mucosa, a subject that warrants further molecular investigations to comprehend the perplexing paradox of COVID-19. Depending on the research findings, the direct evaluation of *Cryptosporidium* infection in the follow-up profile of post-COVID-19 individuals suffering from severe gastrointestinal symptoms is recommended.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding declaration: There is no fund or grant obtained from funding agencies.

Consent to publish and consent to participate

The patients were informed of their participation and provided verbal consent to have their data published.

Data availability

The corresponding author can provide the datasets created or analyzed during the current study upon reasonable request.

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