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Sonia R. Allam
Alexandria University
Salwa Talaat El-Mansoury
Alexandria University
Abeer Mahmoud Ali Ibrahim
Alexandria University
Maha M. Gomaa
dr.maha_kh@yahoo.com
Alexandria University
Samar A. Ibrahim
Alexandria University

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The Relationship Between Parasitic Infections and The Activity of Ulcerative Colitis

Sonia R. Allam [a], Salwa Ahmed Talaat El -Mansoury[a], Abeer Mahmoud Ali Ibrahim[b], Maha M. Gomaa [a*], Samar A. Ibrahim [a,]

[a] Department of Medical Parasitology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

[b] Department of Internal medicine, Gastroenterology unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt

[*] **Corresponding author**: Maha M. Gomaa, Department of Medical Parasitology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

E-mail: dr.maha_kh@yahoo.com

Abstract

Intestinal parasitic infections (IPIs) are a major global health issue, leading to substantial illness and death, particularly among the immunosuppressed individuals involving the ulcerative colitis (UC) cases. Some IPIs could alleviate UC, others might worsen the condition. This study investigated the prevalence of IPIs in UC patients and their relation to disease activity. 110 participants were recruited; 30 healthy controls and 80 UC cases (remission and relapse subgroups). All participants were subjected to health-history taking and laboratory investigations. Abdominal pain, rectal bleeding and diarrhea were significantly-dominant in relapse patients. Stool samples examination revealed a significantly-high prevalence of protozoal infections among UC cases. *Blastocystis hominis* and *Giardia lamblia* exhibited considerably-high odds ratios in relapse patients. These findings are supporting an association between *Giardia lamblia* and *Blastocystis hominis* and UC activity. Further researches are recommended to explore whether such protozoa are merely opportunistic, or their increased-prevalence is attributable to UC compromised gut-barrier.

Keywords:

Intestinal parasitic infections, ulcerative colitis, Relapse, Stool protozoa, Special stains, odds ratio.

Introduction

Intestinal parasitic infections (IPIs) are significant public health challenges impacting millions of individuals across the world. These infections are caused by various helminthic and protozoal parasites.(Saab et al., 2004) They can cause significant morbidity and mortality in endemic developing countries mainly in the young malnourished and immunocompromised individuals (Saab et al. 2004). The World Health Organization (WHO) estimates of global infections include 200 million cases of *Giardia lamblia*, 500 million of *Trichuris trichiura*, 500 million of *Entamoeba histolytica/ Entamoeba dispar,* 700 million of hookworm and 800 million of *Ascaris lumbricoides* (World Health Organization 2020). Intestinal parasitic infections impact over 3.5 billion people worldwide, with around 300 million experiencing severe health consequences, and around 4.5 billion people are estimated to be at risk (Tigabu et al. 2019). The prevalence of IPIs is high in the developing tropical and subtropical countries (Ahmed 2023).

Most of the intestinal parasites are transmitted by ingesting the infective stages through contaminated food, water or hands (Haque 2007). The pathogenesis of IPIs is complex. Parasites can cause the intestinal changes by mechanical disruption, toxin secretion, enzyme production and stimulation of the host immune response (Di Genova et al. 2016). Intestinal interactions between the microbiota and the parasite still remain poorly understood, yet this intestinal microbiota has a significant role in the pathogenesis of parasitic infections (Berrilli et al. 2012). These infections generally present with diarrhea, abdominal pain and bloating sensation. In chronic cases, diarrhea, steatorrhea and loss of weight are common (Ahmed 2023).

Ulcerative colitis (UC) is a chronic relapsing disease that involves the mucosa and submucosa of the distal colon and the rectum which could extend proximally in a continuous manner to involve the whole colon (Gisbert et al. 2019). The incidence of UC is bimodal showing an increasing trend globally. The main peak is between 15 and 30 years. A second, smaller peak occurs between 50 and 70 years with fewer hospitalization and milder course (Gajendran et al. 2019; Georgiou et al. 2021). The pathogenesis of UC is complicated, but the current studies favor a dysregulated immune response that is initiated by luminal antigens, involving intestinal parasites, bacteria and food antigens, in a genetically susceptible host (Mitsuyama et al. 2016). The inflammation due to abnormal immune response induces ulcers formation and disruption of the intestinal barrier that may lead to severe consequences (Hendrickson et al. 2002).

The “hygiene hypothesis” aimed to clarify the generational rise in immune dysregulation. The developed countries experienced a consistent increase in atopic diseases and immune disorders coinciding with a decline in infectious diseases. In contrast, developing countries exhibited the opposite trend, with lower immune dysregulation and a higher incidence of infectious diseases (Leong et al. 2016). Moreover, the correlation between

UC and intestinal parasites is still controversial. Some IPIs could alleviate UC; however, others might worsen the disease. The use of immune-modulators and biological agents for UC treatment has remarkably improved disease but unfortunately has increased the risk of opportunistic infections (Dave et al. 2014).

On a clinical basis, both IPIs and UC induce common manifestations such as: abdominal pain, diarrhea, bloody stool, malabsorption, anemia and weight loss. The diagnosis of IPIs relies on the clinical manifestations and definitely on the laboratory microscopic detection of different parasite forms in the fecal samples. In UC, medical history and clinical examination targeting the gastrointestinal, ocular and dermatologic systems are important. Laboratory investigations including CBC and inflammatory markers are also essential. However, ilio-colonoscopy and biopsy are mandatory for definite UC diagnosis and scoring. Characteristic features include mucosal friability, loss of vascular pattern, exudates, ulcerations and granularity in a continuous, circumferential pattern (Williams 1981).

The principal aim of the treatment in IPIs is to decrease morbidity and to improve the developmental and mental capacity in the pediatric age group. Anti-helminthic drugs include a wide range of medications such as piperazine, benzimidazole and ivermectin (Lacey 1988; Fennell et al. 2008; Adler et al. 2016). Among the antiprotozoal agents, nitroimidazole derivatives are widely used in addition to nitrofuran compounds (Gardner et al. 2001).

The chief goal of medical therapy of UC is the relief of clinical symptoms. This is through: attaining remission of active symptoms and maintaining this remission (Peyrin-Biroulet et al. 2015). The well-established conventional therapy involves 5-aminosalicylates (5-ASA), corticosteroids and thiopurine immune-modulators (Raine et al. 2022; Dubinsky 2024). The biologic therapies include several types of monoclonal antibodies such as anti-TNF and anti-interleukin biologics.

Probiotic therapy is recently applied in the treatment of UC and IPIs to modify the gut microbiota, maintain a balanced immune response and improve intestinal barrier functions (Wang et al. 2021). The most used micro-organisms are *lactobacilli*, bifid bacteria, *Streptococcus thermophiles*, *Escherichia coli* and certain yeasts as *Saccharomyces boulardii* (McSorley et al. 2012; Szkaradkiewicz et al. 2013).

The lack of helminthic exposure due to industrialization nowadays would have a profound effect on the gut immunity. While the areas where the helminths are endemic, there is much lower prevalence of immune mediated disease (McSorley and Maizels 2012). So, these helminths have a protective role. On the other hand, the increase in protozoal infections worldwide leads to an increase in immune dysregulation disease such as UC (Pestehchian et al. 2015). This contradiction elicited the need to investigate the IPIs in UC patients in the active and in the inactive phases to explore the possible relationship between such infections and the disease activity.

2. Material and Methods

2.1. Patients and diagnosis:

This study included 110 participants, who were referred to the Gastroenterology Department at Alexandria Main University Hospital. They were all subjected to a detailed history taking with emphasis on symptoms of gastrointestinal diseases such as abdominal pain, diarrhea, weight loss, tenesmus, urgency, stool incontinence, fever, rectal bleeding and perianal pain, extra-intestinal manifestations as joint swelling and eye affection. A structured questionnaire was used to collect personal data and treatment modalities.

They were categorized according to the disease activity that was assessed by the Simple Clinical Colitis Activity Index (SCCAI) and endoscopically by Ulcerative Colitis Endoscopic Index of Severity (UCEIS) (Ikeya et al. 2016; Marín-Jiménez et al. 2016). Thirty participants were reported to have normal endoscopic findings of the colon and were assigned as control group I. Eighty participants were diagnosed with UC, representing group II, that was divided into two subgroups: subgroup IIa includes 40 patients in inactive phase and subgroup IIb involves 40 patients in active phase. Laboratory investigations such as CBC, ESR, CRP, Serum albumin, liver enzymes and a quantitative assessment of fecal calprotectin by ELISA were performed for each participant.

2.2. Reagents for parasitological examination:

Schaudinn's solution was used for stool sample preservation; physiological saline 0.85% and lugol's iodine were beneficial in wet mount examination. Saline and diethyl ether were utilized for sample concentration. Modified trichrome stain (MTS), modified ziehl-neelsen stain (MZN), safranin stain and iron hematoxylin stain were applied in specimen permanent staining. All these reagents and stains were provided by the laboratory of Medical Parasitology Department, Faculty of Medicine, Alexandria University.

2.3. Fecal sample collection, preservation and examination

Three stool samples were collected separately from each participant on alternate days, in sterile screw capped, properly labelled plastic container. First, all samples were preserved in Schaudinn's fluid, then macroscopic observation of each sample regarding consistency, color, smell, presence of mucous, blood, worms, and segments was performed. The microscopic examination was performed as follows: direct wet mounting, saline ether concentration and special permanent staining. Finally, the prepared slides were examined under a light microscope using low, high powers and oil immersion lenses (X100, X400 & X1000, respectively) (Garcia et al. 2016).

2.4. Statistical analysis

Data was fed to the computer and analyzed using IBM SPSS software package version 23.0. Comparisons between groups for categorical variables were assessed using Chi-square test (Monte Carlo or Fisher Exact). One

Way ANOVA: The one-way analysis of variance (ANOVA) is used to compare between the means of three or more groups. OR to identify significant predictors of parasitic infection. The significance of the results obtained was judged at the 5% level.

2.5. Ethics statement

The consents were taken from all participants for completing the questionnaire and performing all laboratory investigation. This study was conducted following the guidelines of the ethics committee of Alexandria University, Faculty of Medicine with approval No 0107382.

3. Results

3.1. Demographic data (Age and gender)

Most of the participants' age ranged from 20 to 40 years old. In the control group GI, the percentage of male and female participants was equal. While in the inactive subgroup IIa, the percentage of the female participants was higher (57.5%) than the males (42.5%). In the active subgroup IIb, the percentage of male participants was slightly higher (55%) than the females (45%) **(Table 1).**

3.2. Clinical manifestations

Regarding the different clinical presentations of the disease among the studied groups, abdominal pain and diarrhea were significantly higher in subgroup IIb when compared to subgroup IIa ($p=0.001$). Rectal bleeding was significantly higher in subgroup IIb when compared to subgroup IIa ($p=0.037$) **(Fig. 1).**

3.3. Laboratory investigations

The mean Hb level was significantly decreased among the active subgroup IIb (9.8±1 g/dl) when compared to the inactive subgroup IIa (11.2±1mg/dl). On comparing IIa and IIb subgroups, the mean white blood cells (WBC) and platelet count was significantly higher among subgroup IIb in comparison to subgroup IIa **(Table 2).**

There was a statistically-significant elevation in the mean CRP level in UC cases in comparison to the controls. Furthermore, the mean CRP level in subgroup IIb was significantly higher than that in subgroup IIa **(Table 3).** Concerning the ESR, its mean showed no significant increase in both GI and GII with no statistically-significant difference between subgroups IIa and IIb **(Table 3, Fig. 2).** There was also a statistically-significant increase in the mean level of fecal calprotectin in UC patients when compared to the control group. There was a statistically-significant higher level of such calprotectin in GIIb than GIIa. **(Table 4).** Regarding liver function tests, the mean serum albumin level and the liver enzymes were normal among most of the studied participants with no statistically-significant difference between the studied groups. **(Table 5, Fig. 2)**

3.4. Treatment modalities

Among the 80 UC cases, 40 patients were on conventional therapy (5-Aminosalicylates, corticosteroids and thiopurines) while the other 40 patients were on biological therapy (anti-TNF such as infliximab, adalimumab or anti-integrin such as vedolizumab or jak inhibitors such as tofacitinib) with a statistically non-significant difference between GIIa and GIIb **(Fig. 3).**

3.5. Parasitological examination

Macroscopically, in the control group 67% of stool samples were well-formed and brown, with no mucus or blood detected; 83% had a normal odor. While in the inactive subgroup (GIIa), 50% of samples were semi-formed, 45% were reddish, and blood was present in 30% of samples, with 52.5% showing a normal odor. However, the active subgroup (GIIb) exhibited more evident characteristics: 55% of samples were loose, 47.5% reddish, blood was found in 37.5%, and 67.5% had an offensive odor. Statistically-significant differences were observed among the three groups regarding stool consistency, color, and smell **(Table 6).**

Through the microscopic examination, the collected samples revealed protozoal parasites only and no helminthic infections were detected. Direct saline smear (wet mount) revealed *Giardia lamblia* cyst, *Blastocyst*is *hominis* vacuolar form and *Cyclospora* oocyst. By special staining techniques, *Cyclospora* oocyst was identified using safranin stain. *Microsporidia* spores and *Blastocyst*is *hominis* vacuolar and granular forms were stained with modified trichrome. *Cryptosporidium* oocysts were evident with modified Zeihl-Nelseen, and *Giardia lamblia* both cyst and trophozoites appeared using the iron hematoxylin stain **(Fig. 4).** The prevalence of protozoal infections showed a statistically-significant increase in UC patients in comparison to the control group **(table 7).** The most significant protozoal infection in those patients is giardiasis followed by *Blastocystis hominis* infection **(Fig. 5)**.

In the active subgroup, *Giardia lamblia* was the most common parasite (55%), followed by *Blastocystis hominis*, *Cryptosporidium*, *Microsporidia*, and *Cyclospora*. While in the inactive subgroup, the predominant parasites were *Blastocystis hominis*, followed by *Microsporidia*, *Giardia lamblia*, and *Cyclospora* **(Table 8).**

Mixed infections were detected in the control group GI and the active GIIb subgroup, with a high statistically-significant prevalence in GIIb ($p<0.001$), particularly involving *Blastocystis hominis* with *Microsporidia* and *Giardia lamblia* **(Table 9).**

The odds ratios for *Giardia lamblia* and *Blastocystis hominis* infections were 4.667 and 3.0, respectively with a significant difference in UC patients when compared to the control ones **(Table 10).**

When comparing to the control group, the odds ratios in the active group were particularly high for giardiasis (11.455), *Blastocystis hominis* infection (6.111), and microsporidiasis (4.804) **(Table 11)**. Comparatively, the inactive subgroup showed obviously lower odds ratios for these infections when matched with the controls **(Table 12).**

4. Discussion

Enteric protozoa have emerged as fatal opportunistic parasites that can cause serious infections in the immunosuppressed individuals involving UC patients (Le Berre et al. 2023). Hence, in the current study the prevalence of IPIs in UC patients was investigated, to determine the possible relation between these infections and disease activity.

Among 110 patients enrolled in this study, the mean age was 38±11 years. This is supported by previous studies in Egypt reporting 30 ± 8.7 years as a mean age of the studied UC patients (Esmat et al., 2014; Moussa et al., 2021). Herein, the gender appears not to play a nuanced role in the prevalence and presentation of UC. This could be explained by the fact that genetic susceptibility and environmental triggers associated with UC are equally distributed across both genders. Studies documented minimal gender-specific differences in the incidence of UC. However, some differences in disease onset, presentation, and severity based on gender have been noted (Greuter et al. 2020). Smoking which is an established risk factor for the development and progression of UC, contributes to this disparity (Georgiou et al. 2021). Additionally, the potential impact of the menstrual cycle on the clinical course of UC has been recognized (Lungaro et al. 2023).

Abdominal pain and diarrhea showed a statistically-significant increase in GII, which was more noticeable in the active subgroup. This could be interpreted by the presence of mucosal ulcers due to chronic inflammatory cellular infiltration. Such inflammation affects the water reabsorption by the colon with electrolyte transport disruption leading to watery diarrhea. Moreover, the colonic muscle contractility becomes more frequently stronger enhancing watery diarrhea and inducing abdominal pain. These are comparable with previous results demonstrating higher incidence of abdominal pain in active UC subgroup than in the inactive subgroup (van Gils et al. 2025). Rectal bleeding exhibited a significant association in the active subgroup which could be explained by the fragility of the inflamed mucosal blood vessels. These clinical manifestations are supported by other workers reporting that UC involves excessive mucosal immune response with increased intestinal permeability and impaired barrier function (Kałużna et al. 2022).

The current laboratory results showed a statistically-significant decrease in the mean Hb level in the active subgroup when compared to the inactive subgroup. This is a unique example of the combination of chronic iron deficiency anemia and anemia of chronic disease. This is supported previously where the prevalence of anemia in the UC population was 40% (Uspenskiy et al. 2022). Regarding platelet's count, it was considerably higher in GII than GI with a marked elevation in subgroup GIIb compared to GIIa. These results coincide with the fact that the thrombocytosis was used as a predictor of the active in UC patients indicating active colonic inflammation. Herein, WBCs were significantly higher in GII when compared to control group. Moreover, WBCs were significantly higher

in GIIb patients than in GIIa coinciding with GüRler M *et* al. (Gürler et al. 2020). However, mild lymphopenia was reported in 43% of IBD patients receiving AZA or 6-mercaptopurin which could be an evident adverse effect of such immunosuppressive drugs (Abraham et al. 2015).

In the current study, the mean CRP and fecal calprotectin levels were significantly elevated in UC patients. Their levels were reported to have a significant relationship in the assessment of the morbidity in patients with colitis (Anindita et al. 2023).

Regarding liver functions, there was no statistically-significant difference between the studied groups. However, a significant affection of liver function with a decrease in serum albumin and increase in liver function tests were reported previously (Catanzaro et al. 2024). This contradiction could be a result of the use of the immunomodulatory drugs in the treatment causing hepatotoxic effect.

Herein, Schawdinn's solution was used for optimal immediate stool sample fixation after collection. It contains $HgCl_2$, ethyl alcohol, and glacial acetic acid; $HgCl_2$ stabilizes cellular structures, alcohol facilitates penetration, and acetic acid enhances nuclear clarity (Ash et al. 1987; Bhat et al. 2021). The saline-ether concentration technique enhances parasite recovery by eliminating fecal debris and dissolving fats, allowing sedimentation of parasitic elements. This can explain the high detection rate (81.25%) in the current study (Bora et al. 2016).

Light microscopy remains the diagnostic gold standard for IPIs due to 100% specificity and low cost. Direct saline smears detect motile trophozoites and helminth ova, though sensitivity is limited with single samples. To mitigate this, three samples were collected on alternate days, accounting for cyclical parasite shedding. Literature reports obviously improved detection rates for *E. histolytica* and *G. lamblia* with multiple specimens (Hiatt et al. 1995; Bayayibign et al. 2019). Final confirmation was conducted using permanent staining techniques for cytoplasmic and nuclear detail, exploiting differential affinities for dyes due to protein and nucleic acid content, and minimizing background interference via counterstaining, thus enhancing diagnostic sensitivity and specificity (Garedaghi 2020; Datta et al. 2024).

In this study, there is no helminthic infection detected in UC patients either in inactive or in active subgroups. This could be interpreted by the geographical factor, lifestyle changes with diet difference and diagnostic method used. The use of immunosuppressive drugs could reduce shedding of eggs or larvae leading to false negative results. The reduced exposure to parasites due to improved hygiene, modern sanitation and medical practices with recurrent vaccination leads to an increased risk of developing autoimmune diseases, could be another explanation for the absence of helminthic infection in our participants. The lack of exposure to those infections shifts the balance toward the Th2 response, increasing the likelihood of autoimmune diseases (Murdaca et al. 2021). The relationship of helminthic infections and UC has evoked much interest as a result of the recognition that certain helminths could be

used to treat patients with UC by different mechanisms (Chakraborty et al. 2024). *Schistosoma mansoni* reduces inflammation associated with UC by suppression of pro-inflammatory responses and the upregulation of IL-10 (Cortés et al. 2020; Smyth et al. 2023). *Trichuris trichiura* which can lower the intestinal lumen PH, promoting the growth of anaerobic bacteria, such as *Lactobacillus*. These microbial shifts reshape the gut microbiota and could maintain a more stable and less inflammatory environment during helminthic infection (Holm et al. 2015).

The present study revealed an obvious increase in the prevalence of protozoal infections in UC patients when compared to the control group. The most prevalent protozoal parasites were *Blastocystis hominis*, followed by *Microsporidia* and *Giardia lamblia*. This could be due to immune system dysregulation, genetic susceptibility or altered gut microbiota which are the main causative theories in UC. Similar findings were reported previously entailing the relatively-high prevalence of protozoal infections in UC patients (Pestechian et al. 2021; Abdel Hamed et al. 2023). Additionally, a significantly higher prevalence of *Blastocystis hominis* was observed in UC patients as compared to control individuals. On the contrary, Toychiev et al. (2021) showed that the prevalence of *Giardia lamblia* in UC patients didn't differ from the controls. Furthermore, various studies showed that *Blastocystis hominis* is usually associated with healthy gut microbiota, exhibiting low prevalence in UC patients when compared to control ones (Audebert et al. 2016; Coskun et al. 2016). That difference in parasitic prevalence among UC patients may be multifactorial. The geographical distribution, the pathogenicity of *Blastocystis hominis* subtypes and the diagnostic methods used may be attributing factors.

Herein, the active subgroup showed a significantly high prevalence of protozoal infections in comparison to the inactive subgroup with predominance of *Blastocystis hominis* followed by *Giardia lamblia*. This could be due to altered gut microbiota in UC active creating a favorable environment for opportunistic protozoa, impairing its immune defense against protozoal colonization. Another explanation could be the immunosuppressive environment during the active phase of UC. This is driven by activation of immune suppressor cells with presence of pro-inflammatory cytokines, growth factors and angiogenic factors (Rogovskii 2020). In addition, the therapeutic immunosuppression due to high intake of corticosteroids or biological therapy decreases the body's ability to fight off protozoal infections. Previous studies approved that the parasites had a role in the exacerbation of UC and the elevation of inflammatory biomarkers in such patients (Iyer et al. 2013; Abdel Hamed et al. 2023).

This means that the infection with *Giardia lamblia* increases the risk of UC activity by 11.455 times, while the *Blastocystis hominis* infection increases the risk of the disease activity by 6,111 times. It was reported that protozoal infections were significantly associated with moderate to severe UC with the relative risk of 1.686 having severe disease when compared to uninfected patients (Iyer et al. 2013).

Giardia lamblia infection compromises the intestinal mucosa through secreted cysteine proteases, impairing mucus integrity, and its protective function, contributing to gut dysbiosis and weakening mucosal defenses (Barash et

al. 2017). In spite of being primarily a small intestinal parasite, *G. lamblia* may influence colonic inflammation in UC by initiating upstream damage that cascades into colonic immune activation (Cotton et al. 2015). By disrupting villi and impairing fat and carbohydrate absorption, *G. lamblia* reduces barrier maintenance and promotes mucosal inflammation (Sosnowski 2024). Altered bile acid metabolism and shifts in pH and oxygen levels further disturb the colonic microbiota, aggravating UC symptoms (Fekete et al. 2020). Systemically, *G. lamblia* elicits macrophage and neutrophil recruitment and Th1 polarization, leading to increased IL-12/IL-23p40 expression, a cytokine axis critical in UC pathogenesis (Dann et al. 2018). The relationship between *Giardia lamblia* and the gut microbiota is bi-directional: *Giardia lamblia* alters the microbiota composition, virulence and balance leading to dysbiosis and the microbiota influences the outcomes and the severity of the infection (Torres et al. 2000; Buret et al. 2015).

Similarly, *Blastocystis hominis* infection promotes pro-inflammatory cytokine expression (IL-1β, IL-6, TNF-α) and elevates biomarkers like fecal calprotectin, serum IL-6, and CRP in UC patients (Ajjampur et al. 2016; Abdel Hamed et al. 2023). It also induces oxidative stress, activating the NLR inflammasome and triggering pyroptosis, contributing to UC onset and exacerbation (Tian et al. 2017; Chen et al. 2022).

It is not yet confirmed the direction of the immune reaction alteration by protozoa or helminths. Although, the prevalence of these protozoal infections in UC patients was the basis for evaluation of the efficiency of lines of UC management. Proposed that anti-parasitic medication could be a treatment option in UC management and documented a better mucosal healing with combined therapy than monotherapy (Toychiev et al. 2021).

In conclusion, the current study is reporting a link between IPIs especially *Giardia lamblia* and *Blastocystis hominis* infections with the development and activity of UC. Hence, clinicians should be informed about the role of protozoal infections in the course of such a disease. Consequently, the clinical prognosis of the UC patient could be improved through the development of preventive or early diagnostic strategies of the IPIs with periodic routine parasitological screening.

Further research is recommended to explore whether the observed protozoal infections in the current study are merely opportunistic, resulting from the immunosuppressive state induced in UC patients or if the increased prevalence is attributable to the compromised gut barrier integrity associated with UC itself. Additionally, further researches using larger sample size of UC patients are needed with genetic profiling use of parasitological molecular diagnostics to improve the diagnostic yield in such patients. That could help in better understand the potential role of intestinal parasites and pave the way for additive therapy options for UC patients.

Declarations of competing interests

The authors declare that they have no conflicts of interest of a financial or personal nature.

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Legend to figures:

Figure_1: Bar chart comparing the gastrointestinal complaints in the different studied groups.

Figure-2: Bar chart representing the laboratory investigations of the studied groups.

Figure_3: Bar chart representing the treatment modalities among the studied groups.

Figure_4: Light microscopic examination of stool samples: **Direct saline smears** (a, b): a) *G. lamblia* cyst (x400), b) *Blastocystis hominis* vacuolar form (x400). **Iodine-stained smears**: c) mixed infection with *G. lamblia* cyst and *Cyclospora* oocyst (arrow) (x400). **Special stained slides** (d-i): d) *Cyclospora* oocyst stained by safranin (x400), e) *Blastocystis hominis* vacuolar and granular forms stained by MTS (x1000), f) Microsporidia spores stained by MTS (x1000), g) Cryptosporedium oocyst stained by MZN (x1000), h) *G. lamblia* trophozoite stained by iron hematoxylin (x400), i) *G. lamblia* cyst stained by iron hematoxylin (x400).

Figure_5: Bar chart demonstrating the parasites detected in the control and ulcerative colitis groups.

Table 1: Distribution of the studied cases according to the demographic data

	G1 (n = 30)		GIIa (n = 40)		GIIb (n = 40)	
	n	**%**	**n**	**%**	**n**	**%**
Age						
20-40	20	67%	30	75%	26	65%
40-60	10	33%	10	25%	14	35%
Mean ±SD	38.07±13.55		35.10±13.27		38.55±11.32	
Gender						
Male	15	50.0%	17	42.5%	22	55.0%
Female	15	50.0%	23	57.5%	18	45.0%

G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number, SD: standard deviation.

Table 2: Comparison between the studied groups according to complete blood count

	G1 (n = 30)	GIIa (n = 40)	GIIb (n = 40)	Test of sig.	P
Hb					
Min. – Max.	9.0 – 14.0	8.0 – 12.5	7.5 – 11.0		
Mean ± SD	12.0±1.3	11.2±1.0	9.8±1.0	F = 38.218*	<0.001*
Median	12.0	11.5	10.0		
Sig. bet. groups	**P1=0.131, p2=0.029* p3= 0.764**				
Platelets					
Min. – Max.	177.0 – 420.0	240.0 – 558.0	390.0 – 570.0		
Mean ± SD.	279.9 ± 57.88	309.0 ± 89.54	506.33 ± 44.78	F= 7.634*	<0.001*
Median	272.5	265.0	510.0		
Sig. bet. groups	**P1=0.125, p2<0.001* p3<0.001***				
WBCs					
Min. – Max.	3.50 – 10.0	3.50 – 7.70	8.0 – 16.0		
Mean ± SD.	6.18 ± 1.80	5.69 ± 1.42	12.93 ± 2.22	F= 10.647*	<0.001*
Median	6.0	6.0	13.0		
Sig. bet. groups	**P1=0.837, p2<0.001* p3<0.001***				

F: F for one way ANOVA test for comparing the three groups,

P1: p value for Post Hoc Tukey for comparing between group I and IIa

P2: p value for Post Hoc Tukey for comparing between group I and IIb, P3: p value for Post Hoc Tukey for comparing between group IIa and IIb *: Statistically significant at $p \leq 0.05$, G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number, SD: standard deviation

Table 3: Comparison between the studied groups according to the inflammatory markers

	G1 (n = 30)		GIIa (n = 40)		GIIb (n = 40)		Test of sig.	P
CRP								
Min. – Max.	0.4 – 22.0		8.0 – 80.0		15.0 – 85.0			
Mean ± SD	6.2±6.1		12.3±11.6		26.3±13.2		H = 71.442*	<0.001*
Median	5.0		9.0		22.0			
Sig. bet. groups	**P1=0.011*, p2<0.001* p3<0.001***							
ESR								
Normal	25	83.3%	30	75%	26	65%	X_2 = 3.028	0.220
High	5	16.7%	10	25%	14	35%		

H=Kruskal-Wallis,

P1: p value for Post Hoc Test Dunn's test for comparing between group I and IIa

P2: p value for Post Hoc Test Dunn's test for comparing between group I and IIb

P3: p value for Post Hoc Test Dunn's test for comparing between group IIa and IIb

X^2 = chi square test, *: Statistically significant at $p \leq 0.05$, G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number, SD: standard deviation

Table 4: Comparison between the studied groups according to fecal calprotectin level

	G1 (n = 30)	GIIa (n = 40)	GIIb (n = 40)	H	P
Min. – Max.	56.0-115.0	158.0-280.0	361.0-810.0		
Mean ± SD	75.5±18.0	186.7±38.7	615.0±140.0	96.520*	<0.001*
Median	75.0	169.0	617.5		
Sig. bet. groups	**P1<0.001*, p2<0.001* p3<0.001***				

H: Kruskal Wallis test for comparing the three groups,

P1: p value for Post Hoc Test Dunn's test for comparing between group I and IIa

P2: p value for Post Hoc Test Dunn's test for comparing between group I and IIb

P3: p value for Post Hoc Test Dunn's test for comparing between group IIa and IIb

*: Statistically significant at $p \leq 0.05$, G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number, SD: standard deviation

Table 5: Comparison between the studied groups according to the liver function tests

	G1 (n = 30)		GIIa (n = 40)		GIIb (n = 40)		Test of sig.	P
Albumin								
Normal	27	90.0%	31	77.5%	34	85.0%	2.043	0.360
Low	3	10.0%	9	22.5%	6	15.0%		
Liver enzymes								
Normal	26	86.7%	30	75.0%	28	70.0%	2.703	0.259
High	4	13.3%	10	25.0%	12	30.0%		

X^2 = chi square test, *: Statistically significant at $p \leq 0.05$, G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number

Table 6: Macroscopic examination of all stool samples studied

		GI n=30		GIIa n=40		GIIb n=40		X^2	p
		n	%	n	%	n	%		
Consistency	Watery	1	3%	5	12.5%	12	30%	62.655*	<0.001*
	Loose	2	7%	7	17.5%	22	55%		
	Semi-formed	7	23%	20	50%	5	12.5%		
	Well-formed	20	67%	8	20%	1	2.5%		
	Sig. bet. groups	P1=0.001*, p2<0.001* p3<0.001*							
Color	Brownish	20	67%	12	30%	3	7.5%	43.013*	<0.001*
	Whitish	0	0%	0	0%	0	0%		
	Reddish	0	0%	18	45%	19	47.5%		
	Yellowish	8	26%	10	25%	16	40%		
	Greenish	2	7%	0	0%	2	5%		
	Sig. bet. groups	P1<0.001*, p2<0.001* p3= 0.026*							
Content	Mucus	0	0%	7	17.5%	10	25%	0.045	0.831
	Blood	0	0%	12	30%	15	37.5%		
	Worms and segments	0	0%	0	0%	0	0%		
Smell	Normal odor	25	83.3%	21	52.5%	13	32.5%	17.846*	<0.001*
	Offensive odor	5	16.7%	19	47.5%	27	67.5%		
	Sig. bet. groups	P1=0.007*, p2<0.001* p3= 0.070							

χ 2: Chi square test, P1: p value for Chi square test for comparing between group I and IIa
P2: p value for Chi square test for comparing between group I and IIb, P3: p value for Chi square test for comparing between group IIa and IIb, *: Statistically significant at $p \leq 0.05$, GI: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number.

Table 7: Comparison between control and ulcerative colitis groups according to different parasitic infections

	GI (n = 30)		GII (n = 80)		X^2	P
	n	%	n	%		
Giardia lamblia						
-ve	28	93.3%	60	75.0%	4.583*	0.032*
+ve	2	6.7%	20	25.0%		
Blastocystis hominis						
-ve	25	83.3%	50	62.5%	4.365*	0.037*
+ve	5	16.7%	30	37.5%		
Microsporidia						
-ve	26	86.7%	56	70.0%	3.194	0.074
+ve	4	13.3%	24	30.0%		
Cyclospora						
-ve	30	100.0%	72	90.0%	3.235	0.072
+ve	0	0.0%	8	10.0%		
E. histolytica						
-ve	30	100.0%	78	97.5%	0.764	1.000
+ve	0	0.0%	2	2.5%		
Cryptosporidium						
-ve	30	100.0%	70	87.5%	4.125	0.059*
+ve	0	0.0%	10	12.5%		

χ 2: Chi square test, *: Statistically significant at $p \leq 0.05$, GI: control group, GII: ulcerative colitis cases, n: number.

Table 8: Parasites detected in inactive and active subgroups as compared to control group

	GI (n = 30)		GIIa (n = 40)		GIIb (n = 40)		χ^2	^{MC}p
	n	%	n	%	n	%		
Giardia lamblia								
-ve	28	93.3%	38	95.0%	22	55.0%	24.583*	<0.001*
+ve	2	6.7%	2	5.0%	18	45.0%		
Sig. bet. groups	**P1=0.766, p2<0.001*p3<0.001***							
Blastocystis hominis								
-ve	25	83.3%	32	80.0%	18	45.0%	15.568*	<0.001*
+ve	5	16.7%	8	20.0%	22	55.0%		
Sig. bet. groups	**P1=0.723, p2=0.001* p3=0.001***							
Microsporidia								
-ve	26	86.7%	33	82.5%	23	57.5%	9.782*	0.008*
+ve	4	13.3%	7	17.5%	17	42.5%		
Sig. bet. groups	**P1=0.635, p2=0.008* p3= 0.015***							
Cyclospora								
-ve	30	100.0%	39	97.5%	33	82.5%	8.256*	0.009*
+ve	0	0.0%	1	2.5%	7	17.5%		
Sig. bet. groups	**P1=0.383, p2=0.017* p3=0.025***							
E. histolytica								
-ve	30	100.0%	40	100.0%	38	95.0%	2.376	0.337
+ve	0	0.0%	0	0.0%	2	5.0%		
Cryptosporidium								
-ve	30	100.0%	40	100.0%	30	75.0%	17.074*	<0.001*
+ve	0	0.0%	0	0.0%	10	25.0%		
Sig. bet. groups	**P1=-, p2=0.003* p3=0.001***							

P1: p value for Chi square test for comparing between group I and IIa
P2: p value for Chi square test for comparing between group I and IIb
P3: p value for Chi square test for comparing between group IIa and IIb
$\chi 2$: Chi square test, MC: Monte Carlo, *: Statistically significant at $p \leq 0.05$, GIIa: inactive subgroup, GIIb: active subgroup, n: number.

Table 9: Comparison between the studied groups according to the presence of mixed infection

Mixed infection	G1 (n = 30)		GIIa (n = 40)		GIIb (n = 40)		□□	MCp
	n	%	n	%	n	%		
No infection	20	66.7%	22	55.0%	3	7.5%		
Mono infection	9	30.0%	18	45.0%	10	25.0%	67.468*	<0.001*
Mixed infection	1	3.3%	0	0.0%	27	67.5%		
Sig. bet. groups	**P1=0.217, p2<0.001* p3<0.001***							
Type of infection								
One type								
Blastocystis hominis	4	13.3%	8	20.0%	5	12.5%		
Cryptosporidium	0	0.0%	0	0.0%	2	5.0%		
Cyclospora	0	0.0%	1	2.5%	0	0.0%		
E. histolytica	0	0.0%	0	0.0%	2	5.0%		
Giardia lamblia	1	3.3%	2	5.0%	0	0.0%		
Microsporidia	4	13.3%	7	17.5%	1	2.5%		
Mixed infection								
**Blastocystis +Cryptosporidium*	0	0.0%	0	0.0%	2	5.0%		
**Blastocystis +Microsporidia*	0	0.0%	0	0.0%	4	10.0%		
**Giardia lamblia +Blastocystis*	1	3.3%	0	0.0%	2	5.0%	94.824*	<0.001*
**Giardia lamblia +Blastocystis hominis+ cryptosporidium*	0	0.0%	0	0.0%	2	5.0%		
**Giardia lamblia +Blastocystis hominis +Cyclospora*	0	0.0%	0	0.0%	4	10.0%		
**Giardia lamblia +Blastocystis hominis +Microsporidia*	0	0.0%	0	0.0%	3	7.5%		
**Giardia lamblia +Cyclospora*	0	0.0%	0	0.0%	1	2.5%		
**Giardia lamblia +Microsporidia*	0	0.0%	0	0.0%	3	7.5%		
**Giardia lamblia +Microsporidia +Cryptosporidium*	0	0.0%	0	0.0%	1	2.5%		
**Giardia lamblia +Microsporidia +Cyclospora*	0	0.0%	0	0.0%	2	5.0%		
**Microsporidia +Cryptosporidium*	0	0.0%	0	0.0%	3	7.5%		
Sig. bet. groups	**P1=0.795, p2<0.001* p<0.001***							

χ2: Chi square test, MC: Monte Carlo, P1: p value for Chi square test for comparing between group I and IIa, P2: p value for Chi square test for comparing between group I and IIb
P3: p value for Chi square test for comparing between group IIa and IIb
*: Statistically significant at $p \leq 0.05$, G1: control group, GIIa: inactive subgroup, GIIb: active subgroup, n: number.

Table 10: Odds ratio for control group versus ulcerative colitis group regarding different detected parasites

	GI (n = 30)		GII (n = 80)		p value	OR	95% CI	
	N	%	n	%			LL	UL
Giardia lamblia								
-ve	28	93.3%	60	75.0%				
+ve	2	6.7%	20	25.0%	0.047*	4.667	1.019	21.362
Blastocystis hominis								
-ve	25	83.3%	50	62.5%				
+ve	5	16.7%	30	37.5%	0.043*	3.000	1.038	8.672
Microsporidia								
-ve	26	86.7%	56	70.0%				
+ve	4	13.3%	24	30.0%	0.082	2.786	0.877	8.852
Cyclospora								
-ve	30	100.0%	72	90.0%				
+ve	0	0.0%	8	10.0%	-	-	-	-
E. histolytica								
-ve	30	100.0%	78	97.5%				
+ve	0	0.0%	2	2.5%	-	-	-	-
Cryptosporidium								
-ve	30	100.0%	70	87.5%				
+ve	0	0.0%	10	12.5%	-	-	-	-

OR: Odds ratio quantifies the strength of the association between two events,
CI: Confidence interval, LL: Lower limit, UL: Upper limit, *: Statistically significant at $p \leq 0.05$, G1: control group, GII: ulcerative colitis group, n: number.

Table 11: Odds ratio for control group versus active subgroup regarding different detected parasites

	GI (n = 30)		GIIb (n = 40)		p value	OR	95% CI	
	N	%	n	%			LL	UL
Giardia lamblia								
-ve	28	93.3%	22	55.0%				
+ve	2	6.7%	18	45.0%	0.002*	11.455	2.398	54.726
Blastocystis hominis								
-ve	25	83.3%	18	45.0%				
+ve	5	16.7%	22	55.0%	0.002*	6.111	1.946	19.195
Microsporidia								
-ve	26	86.7%	23	57.5%				
+ve	4	13.3%	17	42.5%	0.012*	4.804	1.411	16.358
Cyclospora								
-ve	30	100.0%	33	82.5%				
+ve	0	0.0%	7	17.5%	-	-	-	-
E. histolytica								
-ve	30	100.0%	38	95.0%				
+ve	0	0.0%	2	5.0%	-	-	-	-
Cryptosporidium								
-ve	30	100.0%	30	75.0%				
+ve	0	0.0%	10	25.0%	-	-	-	-

OR: Odds ratio quantifies the strength of the association between two events,
CI: Confidence interval, LL: Lower limit, UL: Upper limit, *: Statistically significant at $p \leq 0.05$, G1: control subgroup, GIIb: active subgroup, n: number.

Table 12: Odds ratio for control group versus inactive ulcerative colitis subgroup regarding different detected parasites

	GI (n = 30)		GIIa (n = 40)		p value	OR	95% CI	
	n	%	n	%			LL	UL
Giardia lamblia								
-ve	28	93.3%	38	95.0%				
+ve	2	6.7%	2	5.0%	0.767	0.737	0.098	5.554
Blastocystis hominis								
-ve	25	83.3%	32	80.0%				
+ve	5	16.7%	8	20.0%	0.723	1.250	0.364	4.293
Microsporidia								
-ve	26	86.7%	33	82.5%				
+ve	4	13.3%	7	17.5%	0.636	1.379	0.364	5.222
Cyclospora								
-ve	30	100.0%	39	97.5%				
+ve	0	0.0%	1	2.5%	-	-	-	-
E. histolytica								
-ve	30	100.0%	40	100.0%				
+ve	0	0.0%	0	0.0%	-	-	-	-
Cryptosporidium								
-ve	30	100.0%	40	100.0%				
+ve	0	0.0%	0	0.0%	-	-	-	-

OR: Odds ratio quantifies the strength of the association between two events, CI: Confidence interval, LL: Lower limit, UL: Upper limit. *: Statistically significant at $p \leq 0.05$, G1: control subgroup, GIIa: inactive subgroup, n: number.

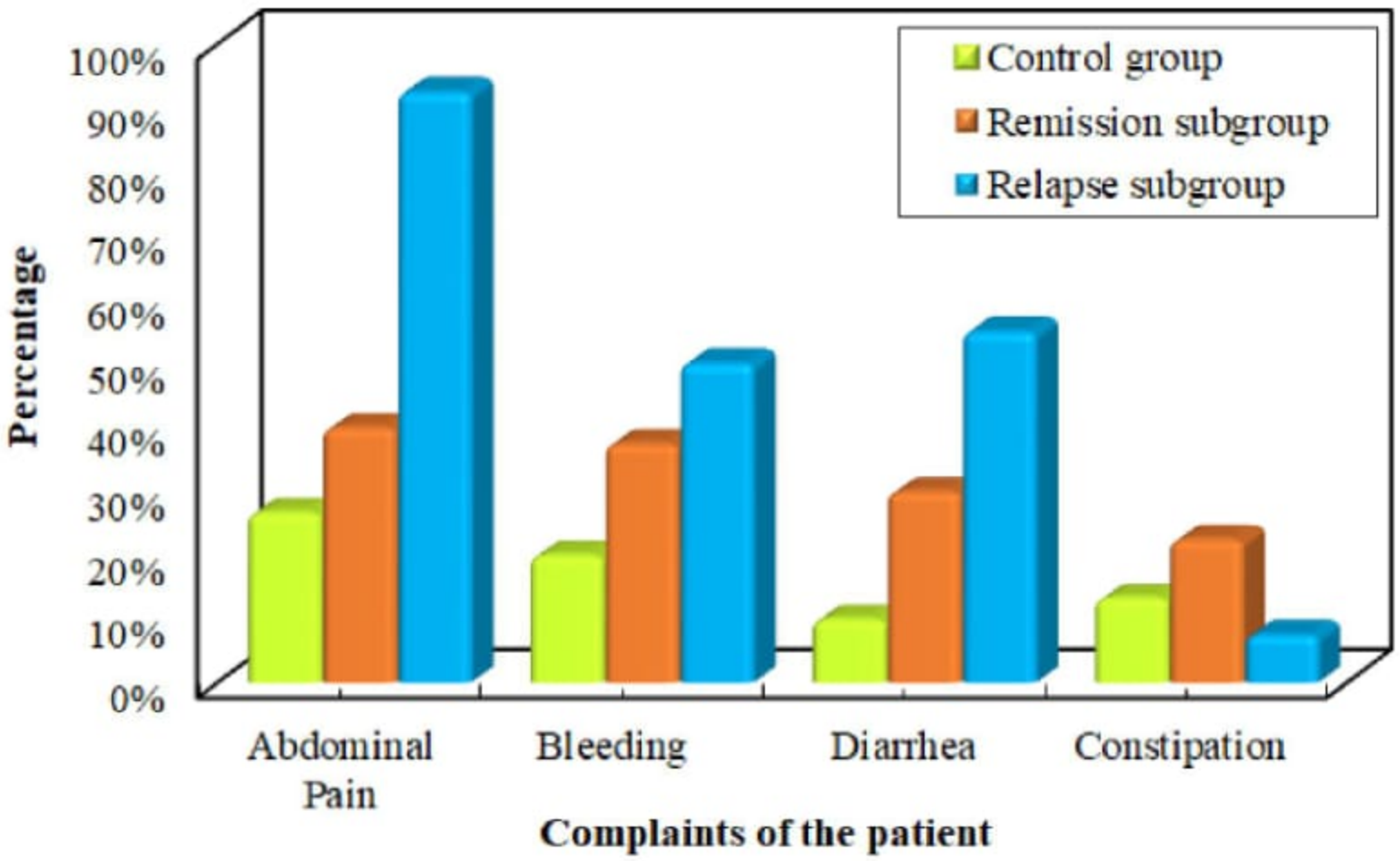


Fig. 1: Bar chart comparing the gastrointestinal complaints in the different studied groups.

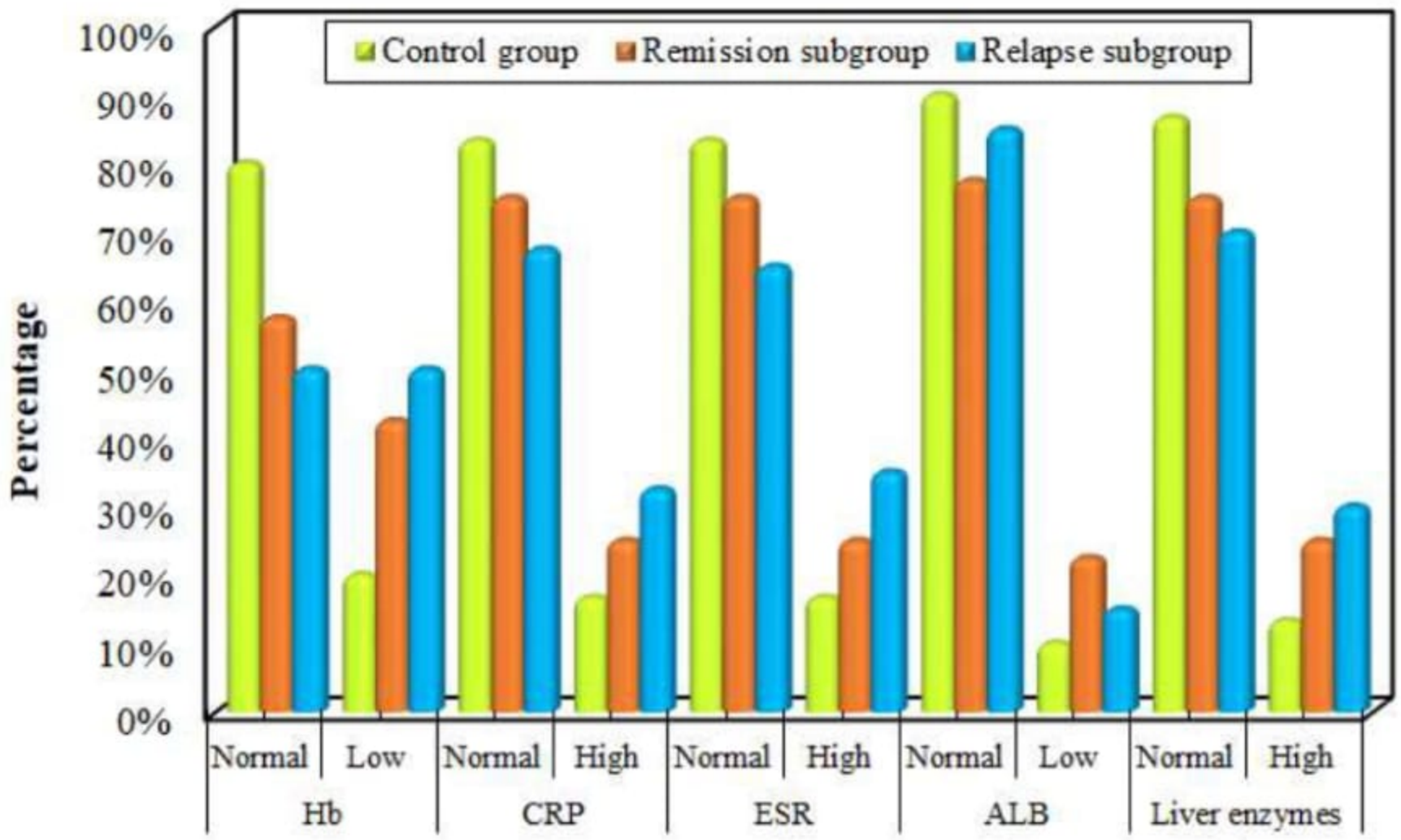


Fig. 2: Bar chart representing the laboratory investigations of the studied groups.

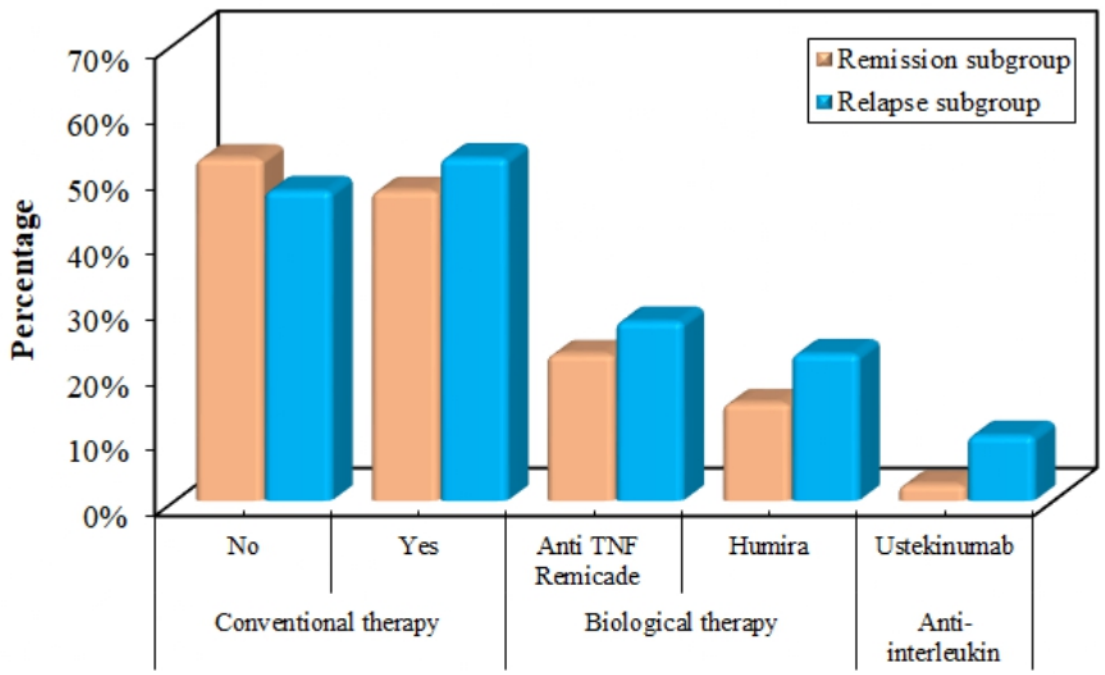


Fig. 3: Bar chart representing the treatment modalities among the different studied groups.

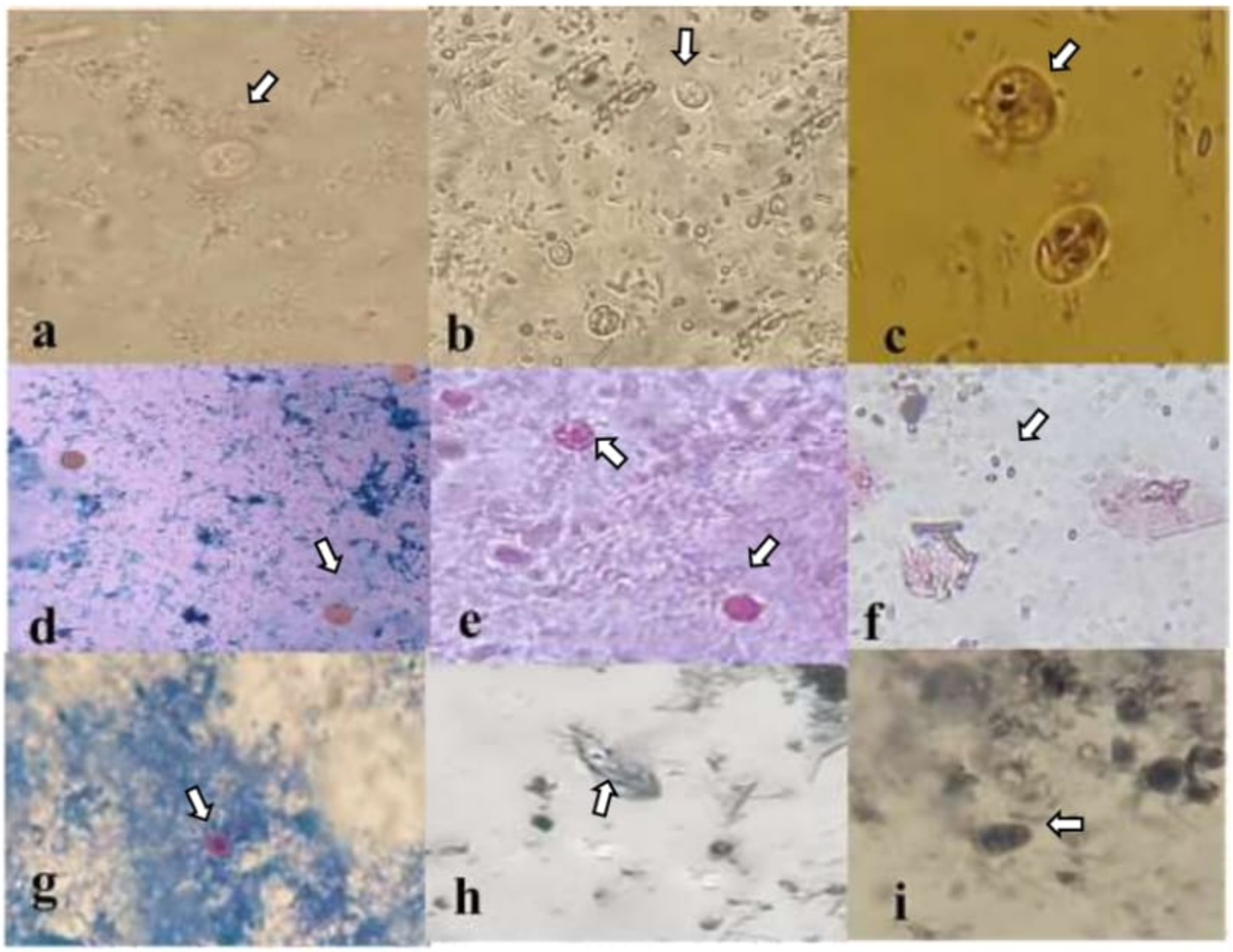


Fig. 4: **Microscopic examination of stool samples, direct saline smears (a&b): a) *Giardia lamblia* cyst (x400), *b) Blastocyst*is *hominis* vacuolar form (x400). Iodine-stained smears: c) mixed infection with *Giardia lamblia* cyst and *Cyclospora* oocyst (arrow) (x400). Special staining techniques (d-i): d) *Cyclospora* oocyst stained by safranin stain (x400), e) *Blastocystis hominis* vacuolar and granular forms stained by MTS(x1000), f) *Microsporidia* spores stained by MTS(x1000) , g) *Cryptosporidium* oocyst stained by MZN(x1000), h) *Giardia lamblia* trophozoites stained by iron hematoxylin stain (x400), I) *Giardia lamblia* cyst stained by iron hematoxylin (x400)**

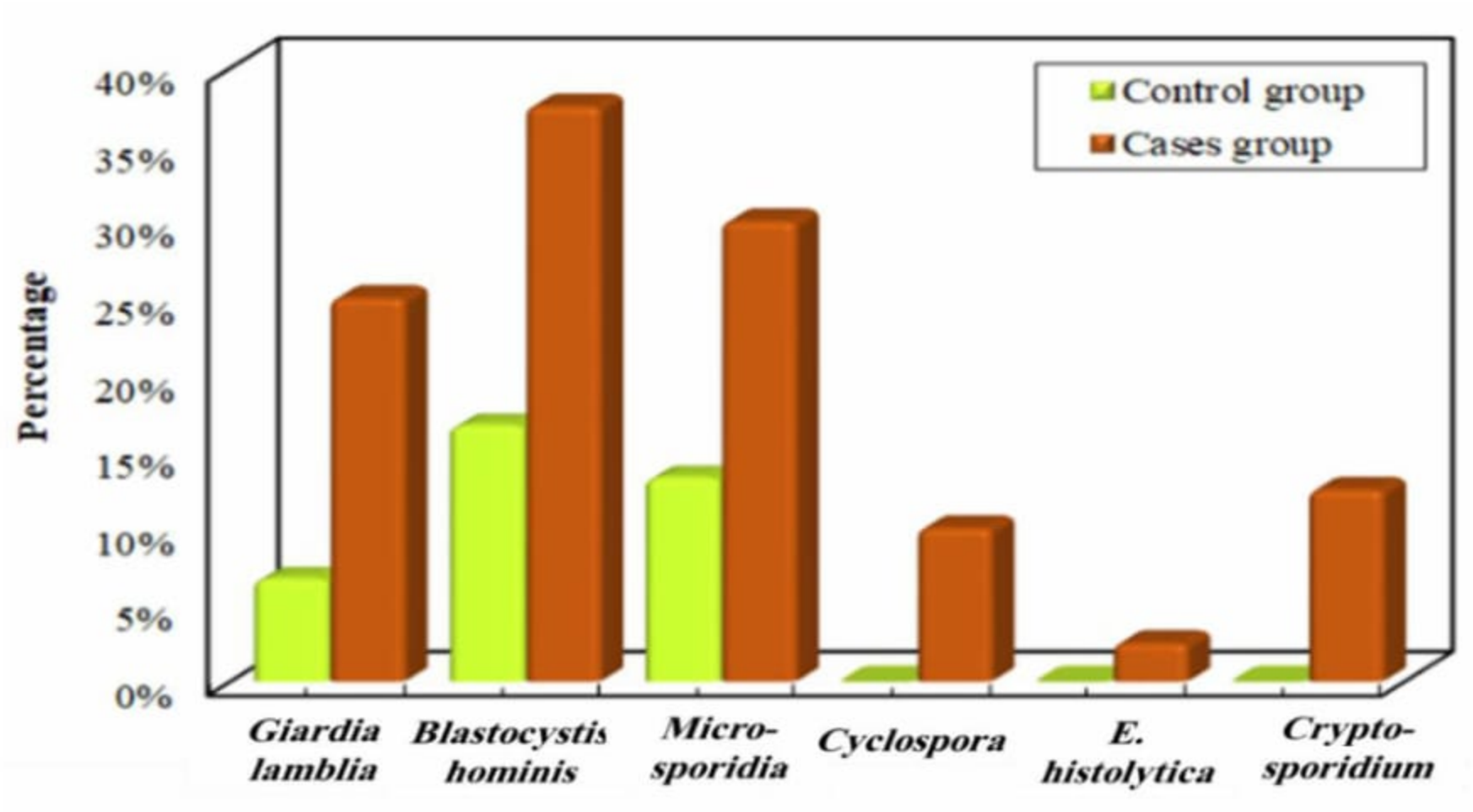


Fig.5: Bar chart representing the parasites detected in the control and ulcerative colitis groups.