Journal of the Egyptian Society of Parasitology, Vol.49, No. 1, April 2019
J. Egypt. Soc. Parasitol. (JESP), 49(1), 2019: 153 – 162

PREVALENCE OF INTESTINAL PROTOZOA AMONG PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND IRRITABLE BOWEL SYNDROME

By
SAMIRA METWALLY MOHAMMAD[1], HOWAYDA S. F. MOAWAD[1], And MAGDA M. SHERIF[2]
Departments of Medical Parasitology[1] and Internal Medicine[2], Faculty of Medicine, Zagazig University, Egypt (*Correspondence: samirametwally1971@gmail.com)

Abstract

Intestinal parasites have a great influence on patients with gastro-intestinal disorders especially those with inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS). This study detected the difference in prevalence of intestinal protozoa among these two groups of patients and evaluated the injury degrees caused by protozoa in gastrointestinal tract (GIT) by measuring the fecal calprotectin (FC) level. This study was done on 219 patients; 101 were IBD patients and 118 were IBS cases attended to the gastroenterology clinics, Zagazig University Hospitals in the period from May 2017 till May 2018 with gastrointestinal symptoms. Stool samples were taken. Microscopic examination for direct wet preparation using 0.9 % of NaCl and 1% of Lugols iodine for each stool specimen was done then confirmed by formol ether sedimentation technique. Fecal smears from sediments of the concentrated samples were stained with modified Ziehl-Neelsen and Trichrome stains. Special ELISA kit was used for calprotectin (FC) fecal detection. Blood sample was taken from each case in EDTA tube for detection of total white blood count, neutrophil percentage, and the hemoglobin content.

Out of 219 stool samples, 84 were positive for intestinal protozoa (38.4%); 58(57.4%) of them were patients with IBD & 26 (22%) were patients with IBS. *Entamoeba histolytica/dispar, Giardia lamblia, Blastocystis hominis*, *Cryptosporidium parvum* and *Cyclospora cayetanensis* are the protozoa which were found among the positive cases. Prevalence among IBD was 27(27%), 14(14%), 10(10%), 6(6%) and 1(1%) respectively while among IBS were 10 (8%), 6(5%), 7(6%), 2(2%) and 1(1%) respectively. High rates of *E. histolytica*/*dispar* (27%), *G. lamblia* (14%), and *B. hominis* (10%) were among IBD patients as compared with IBS patients (8%, 5%, 6%), $P<0.05$. Mean fecal calprotectin levels ≥50ng / ml was considered positive and detected in 15 cases (40.5 %) for *E. histolytica*, followed by 4(20 %) for *G. lamblia*, 3(17.6 %) for *B. hominis* and 1(14%) for *C. parvum;* meanwhile FC level was negative for *C. cayetanensis*. There was significant correlation $P<0.05$ among FC, protozoan infections, total WBCs count and neutrophil%.

Keywords: Patients, IBD, IBS, prevalence, Intestinal protozoa, ELISA, Faecal calprotectin.

Introduction

Gastrointestinal tract (GIT) is an important system in human body affected by many diseases as inflammatory bowel disease (Kaser *et al*, 2010), and irritable bowel syndrome with symptomatic confusion (Fengming and Jianbing, 2014). IBD is a chronic autoimmune inflammatory disease characterized by attacks of relapse and remission, with two subtypes ulcerative colitis (UC) and Crohn's disease (CD), and different signs and symptoms (Ponder and Long, 2013). Crohn's disease can affect any part from mouth to anus (Lennard-Jones, 1989), but the ulcerative colitis affects the rectum and colon mucosa causing typical ulcers (Parray *et al*, 2012).

At turn of the 21st century, inflammatory bowel disease has become a global disease with accelerating incidence in newly industrialized countries whose societies have become more westernized. Although incidence is stabilizing in western countries, burden remains high as prevalence surpasses 0·3% (Ng *et al*, 2018). Helminthes high rate were in early childhood in developing countries might protect them against IBD by stimulation and modification of the immune responses (Adisakwattana *et al*, 2013).

The abnormal immunological response is due to the interaction between environmental and genetic agents causing in intestinal inflammation (Baumgart and Carding, 2007). A significant environmental factor in occurrence of IBD is the compositional changes in the intestinal microbiota. If these changes

are harmful, they can produce an improper immune response which leads to intestinal epithelial damage and loss of its integrity (Maloy and, Powrie, 2011). Also, animal proteins may correlate with increased incidence of IBD and relapses (Andersen *et al,* 2012). The characteristic symptoms of IBD are abdominal pain, diarrhea, rectal bleeding, and weight loss, always with anemia (Wang *et al,* 2012).

Irritable bowel syndrome (IBS) is a GIT functional disorder without underlying lesion evidence, manifested by abdominal discomfort, bloating and changed bowel habits. It is highly prevalent and with unknown exact causes (Wilson *et al,* 2004). It's been linked to oversensitive gut nerves, stress, and a family history of IBS (Borgaonkar *et al,* 2006). There are variable triggers for it, which may include certain hormones, medications, foods or an acute gastroenteritis episode (Longstreth *et al,* 2006).

The global prevalence of IBS is nearly 12% (Mertz, 2003). It is higher in industrialized countries and lower in developing ones (Gwee, 2005). Abdominal pain and diarrhea are common symptoms between patients with IBD and IBS. Colonoscopy is needed to rule out IBD, as negative endoscopy finding diagnose IBS (Hammood *et al,* 2016).

Amoebiasis is a protozoan infection caused by *E. histolytica* and affects nearly 10% of world's population (Lehmann *et al,* 2015). It is more prevalent in developing countries (Lau *et al,* 2013). Its symptoms can overlap with those of the inflammatory bowel disease causing difficulty in diagnosis and treatment of IBD (Hansen and Lund 1998). The risk of opportunistic infection with amebias is was higher in IBD patients due to the wide use of immunosuppressive drugs and corticosteroids treatment (Babić *et al,* 2016).

The occurrence of post-infectious IBS (PI-IBS) subsequent to infectious gastroenteritis ranges from 4% to 31%, with an average incidence of 10% (Thabane *et al,* 2007). Different parasites as *E. histolytica, B. hominis, Giardia* spp., and *Trichinella* spp. are implicated agents in the development of PI-IBS. In a historical cohort study of *G. lamblia* infected patients, prevalence of IBS was 46.1% as long as 3 years after exposure, compared with the controls 14% (Wensaas *et al,* 2012). The PI-IBS following *G. lamblia* infection is also linked with an elevated duodenal mucosal cholecystokinin (CCK) (Dizdar *et al,* 2010).

Blastocystis hominis is one of the most widespread intestinal protozoa in developing and developed nations (Fletcher *et al,* 2012), usually in patients with diarrhea predominant IBS (Cekin *et al,* 2012). Its potential role in the development of IBS was suggested due to its penetration of mucosal layer (Stark *et al,* 2007), present in 67% of the IBS patients and this could be a serious diagnostic problem for IBS (Dogruman *et al,* 2010).

Cryptosporidium is transmitted via oocysts ingestion with contaminated food and/or water but, usually a self-limiting disease in immunocompetent patients (Griffiths, 1998). In patients with acquired immunodeficiency syndrome and transplant recipients, it involves as one of the most important opportunistic infections (Wolska-Kusnierz *et al,* 2007). Due to the increasing use of immunosuppressive drugs, IBD patients are more exposed to opportunistic infections. In the absence of adequate stool studies, these patients could be misdiagnosed and treated as a relapse. *Cryptosporidium* was associated with relapses in adult IBD patients (Vadlamudi *et al,* 2013), with the beginning of gastrointestinal symptoms after an acute cryptosporidiosis in spite of recovery and parasite disposal (Engsbro *et al,* 2014). Symptoms following *C. parvum* infection are the same as those of IBS patients indicating that possibility parasite causing PI-IBS (Jadallah *et al,* 2017).

Cyclospora cayetanensis is one of protozoa parasites causing diarrhea in developing countries (Eberhard *et al,* 1999), and foodborne disease in industrial countries (Mansfield and Gajadha, 2004) and travelers' diarrhea back from developing nations (Dre-

naggi *et al,* 1998). In contrast to *Cryptosporidium*,which are infectious after excretion, *Cyclospora cayetanensis* speculate in soil before becoming infectious, by consumption of contaminated food and/or water. In immunocompetent hosts, self-limiting diarrhea is common with asymptomatic infections but may be fatal in children and AIDS patients (Shields and Olson, 2003). Microscopically, *Cyclospora* and *Cryptosporidium* are similar but *Cyclospora* oocysts are twice larger (Varea *et al,* 1998).

Fecal calprotectin (FC) is an antibody produced by neutrophil white blood cell during its challenge with the pathogens which cause IBD. It is a neutrophil cytoplasmic calcium-binding protein (Quail *et al,* 2009). It is also present in monocytes and early macrophage stage, response to local inflammation, degranulation when occurs in the intestinal lumen (Foell *et al,* 2009). FC is diagnosed serologic by ELISA, a level >50ng/ml in severe GIT damage indicating endoscopic intervention (Kopylov *et al,* 2016). Also, it is detected in stool to differentiate between IBD and other non-inflammatory illness, as well as an accurate indicator for disease activity and follow-up (Sherwood, 2012). A negative result of this non-invasive diagnostic test is probably a true negative safely exclude IBD in children with chronic GIT symptoms (Holtman *et al,* 2016). Delay in diagnosing IBD leads to subsequent delay in proper medication causing many complications as irreversible growth failure, anemia and late sexual maturity (Kim and Ferry, 2004).

This study aimed to determine the prevalence of intestinal protozoa in IBD patients and IBS ones and to evaluate the injury degrees caused by these protozoa on the gastrointestinal tract by measuring fecal calprotectin (FC) level.

Material and Methods

Study design: The study was done from May 2017 to May 2018 on 219 patients (116 males & 103 females), 101 IBD patients and 118 IBS ones, attended Gastroenterology Clinics, Zagazig University Hospitals with GIT suggestive complications.

Ethical aspects: The Ethics Committee of Faculty of Medicine, Zagazig University was considered and, written consents were taken from patients whom were informed by the purpose and procedures of this study.

Stool samples were collected from patients and subjected to: a- Macroscopic examination for stool consistency, odor, color, and blood or mucous, and b- Microscopic examination of fecal specimens by direct smear and Lugol's iodine followed by formol-ether sedimentation (Cheesbrough, 2009). Also, modified Ziehl-Neelsen (Adegbola *et al,* 1994) and trichrome stains (Cheesbrough, 1987) were used.

Quantitative detection of fecal calprotectin by ELISA (Epitope Diagnostic Inc. Co, USA): The sandwich ELISA was used with two particular antibodies fixed to various human calprotectin epitopes.According to the manufacturer instructions,the standards, controls and specimens of patients were added to the microtiter plate wells which were coated with calprotectin antibodies, incubation of the plat for a short period followed by washing. Human calprotectin specific monoclonal antibody was conjugated to the horseradish peroxidase (HRP) enzyme which was added to all wells. A sandwich was made following a second incubation period and it consisted of a solid phase antibody, human calprotectin and a horseradish peroxidase conjugated monoclonal antibody. After that, washing was done to eliminate the buffer matrix and the unbound monoclonal antibodies. Subsequently, the substrate solution was added and incubated in a timed reaction. The immune-complex was measured by the spectrophotometric microplate reader. The immune complex enzymatic activity was directly proportional to the quantity of human calprotectin in the examined sample.

A standard curve was performed with the assessment of the FC concentration in the samples from this curve. FC below 50ng/ml

was considered a negative result.

Venous blood from every patient was taken into an EDTA tube. Samples were used to evaluate hemoglobin content (gm/dl), total leucocytic count and neutrophils%.

Statistical analysis: Data were analyzed by SPSS Version 17. Values were expressed as percentages. Chi-square test was used to study the variances between the studied parameters. Hemoglobin content and total leucocytic count were given as mean ±standard deviation (SD) and analyzed using student T-test. Significance was at P- value <0.05.

Results

The results are given in tables (1, 2, & 3) and figures (1 & 2)

Table 1: Demographic data and parasitosis in IBD and IBS patients

Variables	IBD	IBS	Total	X^2 test	P-value
Sex: Male	61 (60%)	55 (46.6%)	116 (53%)	4.152	0.042*
Female	40 (40%)	63 (53.4%)	103 (47%)		
Ages: 15-35y	48 (47.5%)	39 (33%)	87 (40%)		
36- 50y	31 (30.7%)	52 (44%)	83 (38%)	5.468	0.065
> 50 y	22 (21.8%)	27 (23%)	49 (22%)		
Residence: Rural	59	67	126		
Urban	42	51	93	0.059	0.807
Parasitiosis: +ve	58 (57.4%)	26 (22%)	84 (38.4%)	28.83	0.00001*
-ve	43 (42.6%)	92 (78%)	135 (61.6%)		
Total	101	118	219		

*Significant differences at P-value < 0.05

A total of 116 (53%) were males and 103 (47%) females. Males showed highest IBD (60%), females showed highest IBS (53.4%) with a significant difference. As to ages, IBD affected age group was from 15 to 35 years (47.5%), while the IBS affected age group was from 36 to 50 years (44%) without significant difference. Most of IBD and IBS cases were from rural areas (126) cases without significant difference.

Table 2: Fecal calprotectin antibody levels among parasitic infected patients:

Parasite	Fecal calprotectin antibody		Total	X^2 - test	P- value
	+ve	-ve			
Entamoeba histolytica/dispar	15 (40.5%)	22(59.5%)	37(40%)		
Blastocystis hominis	3 (17.6%)	14 (82.4%)	17(20.2%)		
Giardia lamblia	4 (20%)	16 (80%)	20(23.8%)	5.77	0.217
Cryptosporidium parvum	1(14%)	7 (86%)	8 (9.5%)		
Cyclospora caytanensis	0	2 (100%)	2 (2.4%)		
Total number	23 (27.4%)	61 (72.6%)	84 (100%)		

Parasitic infections: 58(57.4%) in IBD patients & 26(22%) in IBS, with a significant difference. Parasites were *E. histolytica/dispar, G. lamblia, B. hominis, C. parvum* and *C. caytanensis* as (27)27%, (14)14%, (10) 10%, (6) 6% and (1)1% in IBD patients, (10)8%, (6) 5%, (7)6%, (2) 2% and (1)1% in IBS ones respectively.

Table 3: Hematological parameters in relation to fecal calprotectin antibody levels in IBD and IBS cases:

Variables	Fecal calprotectin antibody		Statistical analysis	P-value
	+ve	-ve		
IBD	57 (83%)	44 (29%)	X^2 53.98	0.000*
IBS	12 (17%)	106 (71%)		
Anemia +ve	43 (62%)	33 (22%)	X^2 33.9	0.000*
-ve	26 (38%)	117 (78%)		
Hb (gm/dl) M±SD	10.63±0.73	11.42±1.8	t-test 1.202	0.128
Mean total leucocytes	11463	8270	t-test 3.82	0.002*
Neutrophil (%)	72.9 %	61.3 %		

*Significant differences at P-value < 0.05

Calprotectin antibody levels were positive (≥ 50ng/ml) in 69 cases. Highest proportion of positive was in IBD patients 57(83%) and 12(17%) in IBS ones with a significant dif-

ference. Among calprotectin positive cases, 62% were anemic with Hb (10.63±0.73) and mean total leucocytes and neutrophil percentage 11463, & 72.9% respectively with a significant difference.

Among 84 parasitic infected cases, calprotectin levels were positive in 23(27.4%): *E histolytica/dispar* 15(40.5%), *G. lamblia* 4 (20%), *B. hominis* 3(17.6%), and *C. parvum* 1(14%).

Discussion

Intestinal parasitosis has a strong effect on gastrointestinal tract especially by an invasive parasitic agent (Ali *et al,* 2018). Lower intestinal symptoms, as chronic abdominal pain or discomfort with diarrhea or constipation, are common symptoms in different pathological and functional bowel disorders including inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) respectively (Walsham and Sherwood, 2016).

Calprotectin is a small calcium-binding protein among the S100 family of zinc-binding proteins, mainly released from degranulated neutrophils but to lesser extent from other phagocytic cells as monocytes and macrophages during inflammation (Hanauer, 2017). In active intestinal inflammation, leakage of neutrophils and calprotectin pass into the lumen and excrete in feces. So, the fecal calprotectin concentration reflects the number of participating neutrophils in this inflammation and depends on the severity of the intestinal inflammation (Walsham and Sherwood, 2016).

Fecal calprotectin is used as a good indicator of intestinal mucosal inflammation. It is easy, rapid, and non-invasive laboratory test along with simple microscopic examination of stool, can be used as an indicator of intestinal inflammation and to differentiate the IBD from the IBS (Duman *et al,* 2015).

The aim of this study is to determine the prevalence of intestinal protozoa among patients with IBD and IBS and to evaluate the degree of injuries caused by these protozoa on the gastrointestinal tract by measuring the fecal calprotectin (FC) level.

In this study, 219 cases were 101 IBD cases and 118 IBS cases, 116 (53%) males and 103 (47%) females. Male patients represented the highest proportion of IBD patients (60%), while females represented the highest proportion of IBS (53.4%) with a significant difference between. The results agreed with Ali *et al.* (2018) and Morgan *et al.* (2012) who reported that males were more affected by IBD but females were more affected by IBS. In the present study, the most affected age group with IBD was from 15 to 35 years (47.5%), while the most affected IBS age group was from 36 to 50 (44%) without significant difference. These results more or less agreed with Walsham and Sherwood (2016) who reported that the typical age of IBD was between 15 years and 30 years, but up to 20% during childhood, and the most common age of IBS was between 20 years and 30 years. In the present study, IBD & IBS (126) cases were from rural areas without significant difference.

In the present study, 84 (38.4%) cases were infected by different parasites. Parasites were detected in 58(57.4%) IBD patients and in 26(22%) IBS patients with a significant difference. The protozoan parasites detected were *Entamoeba histolytica/dispar, Giardia lamblia, Blastocystis hominis, Cryptosporidium parvum* and *Cyclospora cayatanensis.* Prevalence among IBD were (27) 27%, (14)14%, (10)10%, (6)6%, and (1)1% while among IBS were (10)8%, (6)5%, (7) 6%, (2) 2% and (1)1% respectively.

In the present study, *E. histolytica/dispar* was the most prevalent protozoa, 37 (44%), more prevalent in IBD (27%) than IBS (8%) cases. No doubt, the invasive parasite could penetrate intestinal mucosa causing marked inflammation and flask shaped ulcers. This agreed with Salman *et al,* (2017) who found amoebiasis represented 43.21%. Nevertheless, the present data were higher than those recorded as 5.11%, (Morsy *et al,* 1991), 4.9% (Hamzy *et al,* 2003), 21.2% (Al-Nak kas *et al,* 2004), 4.57% (Ismaiel, 2011) and 16% (Babic *et al,* 2016).

Salman *et al.* (2016) reported that the liquid diarrhea picture in acute cases mimic that of IBS, also, the color and even the odor of the positive *Giardia* stool samples were the same. While in chronic giardiasis, clinical picture resemble IBD mainly epigastric pain and the other sequels. In this study, *G. lamblia* infection was in 20 cases (23.8%). It was more prevalent in IBD (14%) than IBS (5%). Salman *et al.* (2017) found *G. lamblia* (17.66%) cases more among IBD patients than IBS ones. Also, the present results agreed with Grazioli *et al.* (2006), who found that *G. lamblia* infections occurred in 6.5% of people with IBS symptoms. Also, Dizdar *et al.* (2010) found increased post-infectious bowel dysfunction, IBS patients after *Giardia* infection. But, the present results were higher than that reported by Morgan *et al.* (2012) and Bujanda *et al.* (2002) 3.0% and 2.7% respectively among IBS cases.

In the present study, *B. hominis* infection was in 20.2%, 10% in IBD patients and 6% in IBS ones. This result agreed with Cekin *et al,* (2012) who reported higher *Blastocystis* infection among IBD patients (8.35%) compared to those with IBS (5.82%). These results were higher than that of Salman *et al.* (2017) who detected (11.65%) of cases more in IBD patients than IBS ones. But, Hammood *et al.* (2016) found higher rate of *B. hominis* (37.75%) infection. The difference might be due to higher number of patients (608) examined that was higher among IBD patients than IBS ones. Others reported a moderately strong association between *B. hominis* infection and symptomatic IBS that varied among the geographic areas (Grazioli *et al,* 2006; Yakoob *et al,* 2010; Coyle *et al,* 2012).

In the present study, *C. parvum* infection rate was 9.5%, affected 6% of IBD patientss and 2% of IBS ones. These results disagreed with Jadallah *et al.* (2017) who detect *C. parvum* in 12.8% of IBS patients. Also, Vadlamudi *et al.* (2013) detected *C. parvum* infection in 18% of the IBD patients and accounted 4.6% of all IBD relapses.

Khaldi *et al*. (2009) reported that cryptosporidiosis in suckling rats caused jejunal hypersensitivity to distension associated with accumulation of activated mast cells at 50 d post-infection. This showed that cryptosporidiosis is diarrhea-predominant. IBS cases have a marked increase in mast cells number and elevated tryptase concentrations in jejunal mucosa (Guilarte *et al,* 2007).

In the present study, *C. caytanensis* was 2.4% of infected cases. Only 1% of IBD and IBS cases were infected. Cyclosporiasis gave symptoms resembling celiac disease and irritable bowel syndrome (Karanja *et al,* 2007)

Kopylov *et al.* (2016) reported that FC antibody levels normal (10-50ng/ ml) and levels above 50ng/ml denoted damage in GIT indicating endoscopy intervention.

In the present study, fecal calprotectin antibody levels were positive in 69 cases. The highest proportion was among IBD patients 57(83%) while only 12(17%) among IBS ones with a significant difference. These results agreed with Hanauer (2017) who reported that 99% of patients with active IBD gave elevated fecal calprotectin levels while, 15% to 20% of IBS patients gave mild levels. Also, Tursi *et al.* (2011) and Sydora *et al.* (2012) reported that fecal calprotectin levels among IBS patients were much lower than in patients with IBD, without significant different from healthy individuals.

In the present study, calprotectin positive cases, 62% were anemic, Hb (10.63±0.73) with mean total leucocytes and neutrophil% were elevated 11463, 72.9 % respectively with a significant difference between calprotectin positive and negative cases. This data agreed with Salman *et al* (2017) who re- prted high total WBC count and neutrophilia among patients with elevated fecal calprotectin due to acute *E. histolytica* infection. Also, Ali *et al*. (2018) reported decreased mean Hb level and neutrophilia among patients with elevated fecal calprotectin level but decreased mean total white blood cells due to parasitic infection.

In the present study, among 84 parasitic infected cases, calprotectin levels were positive in 23(27.4%) patients stool samples with *E. histolytica/dispar* 15(40.5%), *G. lamblia* 4(20%), *B. hominis* 3(17.6%), *C. parvum* 1(14%). These results agreed with Salman *et al.*(2017) who reported elevated fecal calprotectin in (41.75%) of *E. histolytica* infected cases followed by (21.27%) of *G. lamblia* and (3.22%) of *B. hominis* infected cases.. Also, the results agreed withGol *et al.* (2018) and Hanevik *et al.* (2007) who noted that some parasitic diseases as *G. duodenalis* increased the level of fecal calprotectin among patients with IBD. Besides, increased in fecal calprotectin was due to *Schistosoma mansoni* (Bustinduy *et al,* 2013) and *Dientamoeba fragilis* (Munasinghe *et al,* 2013).

Conclusion

Undoubtedly, intestinal protozoa infections are more prevalent among patients with IBD when compared to those with IBS. The degree of damages in GIT due to protozoa infections was clarified efficiently by measuring FC as a dependent test.

References

Adegbola, RA, Demba, E, Deveer, G, Todd, F, 1994: *Cryptosporidium* infection in Gambian children less than 5 years of age. Am. J. Trop. Med. Hyg. 97:103-7.

Adisakwattana, P, Nuamtanong, S, Kusolsuk ,T, Chairoj ,M, Yenchitsomanas, PT, *et al*, 2013: Non-encapsulated *Trichinella spp*., *T. papuae*, diminishes severity of DSS-induced colitis in mice. Asian Pac. J Aller. Immunol. 31, 2:106-14.

Ali, OS, Mohammad, SA, Salman, YJ, 2018: Relationship between *Entamoeba histolytica* and Fecal Calprotectin. Egypt. J. Med.Microbiol. 27, 2:49-56.

Al-Nakkas, E, Al-Mutar, EM, Shweiki, HM, Sharm, PN, Rihan, S, 2004: Parasitic infection in Kuwait. A study based on primary care center. Med. East. J. Fam. Med. 3:7-12.

Andersen, V, Olsen, A, Carbonnel, F, Tjønneland, A, Vogel, U, 2012: Diet and risk of inflammatory bowel disease. Digest. Liver Dis. 44, 3:185-94.

Babić, E, Bevanda, M, Mimica, M, Karin, M, Volarić, M, *et al,* 2016: Prevalence of amebiasis in inflammatory bowel disease in University Clinical Hospital Mostar. Springer-Plus. 5, 1: 1586 doi: 10.1186/s40064-016-3261-7

Baumgart, DC, Carding, SR, 2007: Inflammatory bowel disease: cause and immunobiology. Lancet 369, 9573:1627-40**.**

Borgaonkar, MR, Ford, DC, Marshall, JK, Churchill, E, Collins, SM, 2006: The incidence of irritable bowel syndrome among community subjects with previous acute enteric infection. Dig. Dis. Sci. 51:1026-32.

Bujanda, L, Guti´errez-Stampa, MA, Caballeros, C H, Alkiza, ME, 2002: Gastrointestinal disorders in Guatemala and their relation with parasitic infections. Ana. de Med. Int..19, 4:179-82.

Bustinduy, AL, Sousa-Figueiredo, JC, Adriko, M, *et al*, 2013: Fecal occult blood and fecal calprotectin as point-of care markers of intestinal morbidity in Ugandan children with *Schistosoma mansoni* infection. PLoS Negl Trop Dis. 7, 11:25-42.

Cekin, AH, Cekin, Y, Adakan, Y, Tasdemir, E, Koclar, FG, *et al*, 2012: Blastocystosis in patients with gastrointestinal symptoms: a case-control study. BMC Gastroenterol.12:122.

Cheesbrough, M, 1987: Medical Laboratory Manual for Tropical Countries: ELBS Tropical Health Technology Butterworth.

Cheesbrough, M, 2009: District Laboratory Practice in Tropical Countries: vol.1. New York, Cambridge University Press.

Coyle, CM, Varughese, J, Weiss, LM, Tanowitz, HB, 2012: *Blastocystis*: to treat or not to treat. Clin. Infect. Dis. 54, 1:105-10.

Dizdar, V, Spiller, R, Singh, G, Hanevik, K, Gilja, O, *et al*, 2010: Relative importance of abnormalities of CCK and 5-HT (serotonin) in *Giardia*-induced post-infectious irritable bowel syndrome and functional dyspepsia. Aliment. Pharmacol. Ther. 31:883-91.

Dogruman, AF, Simsek, Z, Boorom, K, Ekici, E, Sahin, M, *et al,* 2010: Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD Patients in Ankara, Turkey. PLoS One; 5:e15484.

Drenaggi, D, Cirioni, O, Giacometti, A, Fiorentini, A, Scalise, G, 1998: Cyclosporiasis in a traveler returning from South America. J Travel Med. 5:153-5.

Duman, M, Gencpinar, P, Biçmen, M, Arslan, N, Özden, Ö, *et al*, 2015: Fecal calprotectin: can be used to distinguish between bacterial and viral gastroenteritis in children? Am. J. Emerg. Med. 33, 10:1436-9.

Eberhard, ML, Nace, EK, Freeman, AR, Streit, TG, da Silva, AJ, *et al*, 1999: *Cyclospora cayetanensis* infections in Haiti: a common occurrence in the absence of watery diarrhea. Am J Trop Med Hyg. 60:584-6.

Engsbro, AL, Stensvold, CR, Vedel Nielsen, H, Bytzer, P, 2014: Prevalence, incidence, and risk factors of intestinal parasites in Danish primary care patients with irritable bowel syndrome. Scand. J. Infect. Dis. 46: 204-9.

Fengming, Y, Jianbing, W, 2014: Biomarkers of inflammatory bowel disease. Dis. Markers 710915.

Fletcher, SM, Stark, D, Harkness, J, Ellis, J, 2012: Enteric protozoa in the developed world: a public health perspective. Clin. Microbiol. Rev**.** 25:420-49**.**

Foell, D, Wittkowski, H, Roth, J, 2009: Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut 58:859-68.

Gol, SM, Mirjalali, H, Aghdaei, HA, Zali, MR, 2018: Can *Giardia* infection impair the diagnostic level of fecal calprotectin in patients with inflammatory bowel disease? A case report. Iranian J, Parasitol. 13,3 :505-509.

Hanevik, K, Hausken, T, Morken, MH, *et al*, 2007: Persisting symptoms and duodenal inflammation related to *Giardia duodenalis* infection. J. Infect.55, 6:524-30.

Grazioli, B, Matera, G, Laratta, C, 2006: *Giardia lamblia* infection in patients with irritable bowel syndrome and dyspepsia: a prospective study. Wld. J. Gastroenterol. 12, 12:1941-4.

Griffiths, JK, 1998: Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment, and diagnosis. Adv. Parasitol. 40, 37:85-9.

Gwee, KA, 2005: Irritable bowel syndrome in developing countries a disorder of civilization or colonization? Neurogastroenterol. Motil.17, 3: 317-24.

Hammood, AM, Salman, YJ, Ahmed, BA, 2016: Possible association between *Blastocystis hominis* and bowel disorders among Iraqi Patients in Kirkuk Province. Int. J. Curr. Res. Acad. Rev. 4, 7:50-6.

Hamzy, M, Dabboussi,F, Al-Ali, K, Ourabi, L, 2003: Prevalence of intestinal parasites in Northern Lebanon. East. Medite. Hlth. J. 10, 3:343-6.

Hanauer, SB, 2017: The use of fecal calprotectin in inflammatory bowel disease. Gastroenterol. Hepatol. 13, 1:53-56.

Hansen, LH, Lund, C, 1998: Amebiasis-a differential diagnosis from inflammatory bowel disease. Ugeskr. Laeger. 160:5514-5.

Holtman, GA, Lisman-van Leeuwen, Y, Reitsma, JB, Berger, MY, 2016: Non-invasive tests for inflammatory bowel disease: a meta-analysis. Pediatrics137, 1:1-11.

Ismaiel, MT, 2011: Detection of intestinal parasites among primary shool children in Hama-Syria. J. Med. Lab. Diag. 10:1-9.

Karanja, RM, Gatei, W, Wamae, N, 2007: Cyclosporiasis: an emerging public health concern around the world and in Africa. Afr. Hlth. Sci. 7, 2:62-7.

Kaser, A, Zeissig, S, Blumberg, RS, 2010: Inflammatory bowel disease. Ann. Rev Immunol. 28:573-621.

Khaldi, S, Gargala, G, Le Goff, L, Parey, S, Francois, A, *et al*, 2009: *Cryptosporidium parvum* isolate-dependent postinfectious jejunal hypersensitivity and mast cell accumulation in an immunocompetent rat model. Infect Immun.77:5163-9.

Jadallah, KA, Nimri, LF, Ghanem, RA, 2017: Protozoan parasites in irritable bowel syndrome: A case control study. Wld. J. Gastrointest. Pharmacol.Ther. 8, 4:201-7.

Kim, SC, Ferry, GD, 2004: Inflammatory bowel diseases in pediatric and adolescent patients: clinical, therapeutic, and psychosocial considerations. Gastroenterology.126, 6:1550-60.

Kopylov, U, Yung, DE, Engel, T, Avni, T, Battat, R, *et al*, 2016: Fecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis Eur. J. Gastroenterol. Hepatol. 28, 10:1137-44

Lau, YL, Anthony, C, Fakhrurrazi, SA, Ibrahim, J, Ithoi, I, *et al*, 2013: Realtime PCR assay in differentiating *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* infections in Orang Asli settlements in Malaysia. Parasit. Vectors 6:250-2.

Lehmann, FS, Burri, E, Beglinger, C, 2015: The role and utility of faecal markers in inflammatory bowel disease. Thera. Adv. Gastroenterol. 8, 1:23-6.

Lennard-Jones, J, 1989: Classification of infl-

ammatory bowel disease. Scand. J. Gastroenterol. 24:2-6.

Longstreth, GF, Thompson, WG, Chey, WD, Houghton, LA, Mearin, F, *et al*, 2006: Functional bowel disorders. Gastroenterology 131, 2: 688-70.

Maloy, KJ, Powrie, F, 2011: Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature 474, 7351:298-306.

Mansfield, LS, Gajadhar, AA, 2004: Cyclospora cayetanensis, a food- and waterborne coccidian parasite. Vet. Parasitol. 126:73-90.

Mertz, HR, 2003: Irritable bowel syndrome. New Engl. J. Med. 349, 22:2136-46.

Morgan, DR, Benshoff, M, C´aceres, M, Becker-Dreps, S, Cortes, L, *et al*, 2012: Irritable bowel syndrome and gastrointestinal parasite infection in a developing nation environment. Gastroenterol. Res. Pract. 2012:343812

Morsy, TA, Farrag, AM, Sabry, A, Salama, MM, Arafa, MA, 1991: Ecto and endo-parasites in low primary schools in Qualyob City, Egypt. J. Egypt. Soc. Parasitol. 21, 2:391-401.

Munasinghe, VS, Vella, NG, Ellis, JT, Windsor, PA, Stark, D, 2013: Cyst formation and fecal-oral transmission of *Dientamoeba fragilis* the missing link in the life cycle of an emerging pathogen. Int. J. Parasitol. 43, 11:879-83.

Ng, SC, Shi, HY, Hamidi, N, Underwood, FE, Tang, W, 2018: Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet.390, 10114:2769-78.

Parray, FQ, Wani, ML, Malik, AA, Wani, S N, Bijli, AH, *et al*, 2012: Ulcerative colitis: A challenge to surgeons. Int. J. Preven. Med. 3:74-9.

Ponder, A, Long, MD, 2013: A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin. Epidemiol. 5: 237-47.

Quail, MA, Russell, RK, Van Limbergen, JE, *et al*, 2009: Fecal calprotectin complements routine laboratory investigations in diagnosing childhood inflammatory bowel disease. Inflamm. Bowel Dis. 15, 5:756 -9.

Salman, YJ, Al-Taee, AA, Mohammad, AA, 2016: Role the employee of some biological stains in detecting *Giardia lamblia* among Internal Iraqi Displaced Peoples in Kirkuk Province. Int. J. Curr. Microbiol. App. Sci. 5, 3:705-18.

Salman,YJ, Ali, CA, Abdul Razaq, AA, 2017: Fecal calprotectin among patients infected with some protozoan infections , Int. J. Curr. Microbiol. App. Sci. 6, 6:3258-74.

Sherwood, RA, 2012: Fecal markers of gastrointestinal inflammation. J. Clin. Pathol. 65:981-5.

Shields, JM, Olso, BH, 2003: *Cyclospora cayetanensis*: a review of an emerging parasitic coccidian. Int. J. Parasitol. 33**:**371-91.

Stark, D, van Hal, S, Marriott, D, Ellis, J, Harkness, J, 2007: Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. Int. J. Parasitol. 37, 1:11-20.

Thabane, M, Kottachchi, DT, Marshall, JK, 2007 : Systematic review and meta-analysis: the incidence and prognosis of postinfectious irritable bowel syndrome. Alim. Pharmacol.Therape. 26, 4: 535-44.

Sydora, MJ, Sydora, BC, Fedorak, RN, 2012: Validation of a point-of-care desk top device to quantitate fecal calprotectin and distinguish inflammatory bowel disease from irritable bowel syndrome. J. Crohn's Colitis 6, 2:207-14.

Tursi, A, Elisei, W, Giorgetti, G, Aiello, F, Brandimarte, G, 2011: Role of fecal calprotectin in the diagnosis and treatment of segmental colitis associated with diverticulosis. Minerva Gastroenterol. Dietol. 57, 3:247-55.

Vadlamudi, N, Maclin, J, Dimmitt, RA, Thame, KA, 2013: Cryptosporidial infection in children with inflammatory bowel disease. J. Crohn's Colitis 7, 9:337-43.

Varea, M, Clavel, A, Doiz, O, Castillo, FJ, Rubio, MC, *et al*, 1998: Fuchsin fluorescence and autofluorescence in *Cryptosporidium, Isospora* and *Cyclospora* oocysts. Int. J. Parasitol. 28:1881-3.

Walsham, NE, Sherwood, RA, 2016: Fecal calprotectin in inflammatory bowel disease. Clin. Exp. Gastroenterol. 9:21-9.

Wang, GF, Ren, JA, Liu, S, Chen, J, Gu, GS, *et al*, 2012: Clinical characteristics of non-perianal fistulating Crohn's disease in China: a single-center experience of 184 cases. China Med. J. 125,14: 2405-10.

Wensaas, KA, Langeland, N, Hanevik, K, Mørch, K, Eide, GE, *et al*,, 2012: Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. Gut 61, 2:214-9.

Wilson, S, Roberts, L, Roalfe, A, Bridge, P, Singh, S, 2004: Prevalence of irritable bowel syndrome: a community survey. Br. J. Gen. Pract.

54, 504:495-502.
Wolska-Kusnierz, B, Bajer, A, Caccio, S, Heropolitanska, Pliszka, E, *et al*, 2007: *Cryptosporidium* infection in patients with primary immunodeficiencies. J. Pediatr Gastroenterol. Nutr. 45:458-64.
Yakoob, J, Jafri, W, Beg, MA, *et al*, 2010: Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. Parasitol. Res. 106, 5:1033-8.

Explanation of figures

Fig. 1: Intestinal parasites among IBD patients.
Fig. 2: Intestinal parasites among IBS patients.