Research Article

Intestinal Amoebiasis among Resistant Ulcerative Colitis

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Abstract

Background and aim of the work: Ulcerative colitis **(**UC**)** is a chronic gastrointestinal health problem that can be mistaken for many other infective and non-infective chronic diarrheal diseases. Its relation to chronic intestinal amoebiasis needs to be investigated especially if the cases are out of control. This work aims at evaluating the relation between intestinal amebiasis and UC. **Patient and methods:** A group of twenty- five patients diagnosed as uncontrolled ulcerative colitis and another group of twenty- five patients with controlled ulcerative colitis were included in this study. Both groups were subjected to detailed history taking, clinical examination, routine laboratory investigations, abdominal ultrasound and colonoscopy with biopsy taking and histopathological examination. Fresh stool samples were collected for measurement of calprotectin by enzyme linked immunosorbent assay (ELISA) and detection of Entamoeba histolytica (E. histolytica) by microscopy and antigen detection. Data were collected, tabulated and analyzed using Chi- square test for comparison between groups. $P < 0.05$ was considered statistically significant. **Results:** Most of UC signs and symptoms were more presented among the uncontrolled than the controlled group patients. E. histolytica was found to be more prevalent among the uncontrolled (52%) than the controlled (8%) UC patients. **Conclusion:** E. histolytica is more prevalent among uncontrolled cases of UC than the controlled cases and could play an important role in failure of treatment.

Keywords: Ulcerative colitis, chronic diarrheal diseases, amoebiasis

Introduction

Ulcerative colitis (UC) is a chronic idiopathic autoimmune inflammatory disease involving the mucosa and submucosa of the colon. It usually manifests by recurrent attacks with exacerbations and remissions usually induced by treatment. The rectum is the predilected site of pathology that may spread to affect the whole colon. UC patients present mainly with bloody diarrhea, urgency, fecal incontinence, tenesmus, and abdominal pain but gross bleeding is not so frequent[1,2]. The exact pathogenesis is still not clearly identified. Multiple factors were suspected to play an important role in deciding susceptibility to the pathogenic process of IBD such as impaired mucosal barrier, bacterial infection, defective immune system and genetic factors[3].

Amebiasis is an infectious disease caused by Entamoeba histolytica (E histolytica).[4,5]. Amebiasis ranges from asymptomatic infection - with subclinical colonization - to severe or even fulminant amoebic colitis with severe - sometimes bloody- diarrhea and high mortality[6,7]. Amebiasis is mainly a food- water born infection but sometimes sexually transmitted[8, 9]. It is one of the main causes of infectious diarrhea worldwide especially among military recruits and those coming back from endemic areas in addition to the general population[10- 12].

E histolytica has a simple life cycle with only two forms, the infectious stage (cyst) and the invasive stage (trophozoite).[12,13]. The patho-genesis of amoebic colitis is triggered by adherence to the host cells (intestinal epithelium) inducing the release of different immune modulators causing cell death, inflammation and trophozoite invasion[14-18].

Symptomatic amebic colitis usually presents with watery or bloody diarrhea, abdominal

cramps with tenderness and weight loss. Amebic colitis may involve ascending colon or the caecum but it can be fulminant extensive colitis with friable ulcerated mucosa, bowel necrosis, perforation, peritonitis and toxic megacolon. Clinically, manifestations of amoebic colitis are closely similar and easily mistaken with those of IBD including UC. Interestingly different investigations including inflammatory markers, imaging and even colonoscopy – in many instances- cannot differentiate between the two diseases. Misdiagnosis of amebic colitis with UC carries multiple grave outcomes -especially with the use of steroids- including flaring up of amebiasis, colectomy or even death[7,19].

Disease activity, treatment outcome and Mucosal healing of UC are usually assessed by clinical, serological and endoscopical methods. However, the apparent endoscopic mucosal healing is sometimes not consistent with the remaining histological activities[20-22]. There are many reports about diagnosed cases of UC who underwent repeated courses of medical treatment yet stayed out of proper control. Those patients were found to be positive for amebiasis and responded dramatically to anti-amebic medications. These accumulating data suggest that intestinal amoebiasis could be the underlying cause of failed UC treatment and/ or that amebic colitis can be masked by the presence of UC[23- 25].

This work aims at investigating the relationship between uncontrolled cases of UC and the presence of intestinal amebiasis.

Patients and Methods

Inclusion criteria: fifty patients- already diagnosed as UC- attending to Tropical Medicine and Internal Medicine Departments, Zagazig University Hospitals- from January 2014 to December 2015- were enrolled in this study. The fifty UC patients were twenty-five uncontrolled cases - after failure of successive courses of treatment for more than six months- plus twenty-five duly controlled cases under treatment. They must be controlled for at least six months.

Exclusion criteria: included the presence of other chronic illness or the recent control of cases (less than 6 months). Both groups were subjected to detailed history taking, clinical examination and the related investigations. Clinical activity of UC was assessed by Mayo scoring system (range 0- 9)[26].

- Routine laboratory investigations included stool analysis, complete blood count (CBC), erythrocyte sedimentation rate (ESR), serum albumin and C- reactive protein (CRP). Samples were collected within three days before or after collection of stool samples for measurement of calprotectin.
- Stool analysis for measurement of calprotectin was done by quantitative enzyme-linked immunosorbent assay (ELISA) using immune-chromatographic point of care test (OOCT) technology (POCT; Quantum Blue® Calprotectin; Bühlmann Laboratories AG)[27]. Fecal calprotectin level more than 500- 600 Ug/ mg is a strong indication of active UC[28].
- Stool Microscopy with fresh stool samples was also used for detection of E. histolytica trophozoites (single nucleus) or cysts (four nuclei) by an experienced eye. Wet mounts or stained preparations were used.
- Stool Antigen Detection or the Techlab E. histolytica II test (Blacksburg, VA), which detects E. histolytica–derived Gal/GalNAc-specific lectin, can exclude nonpathogenic E. dispar, as can the Cellabs CELISA Path (Brookvale, Australia). E. Histolytica Quik Chek immunochromatographic (IC) assay was also used to confirm the results.
- Abdominal ultrasound was done to exclude extra- intestinal amoebic involvement.
- Colonoscopy after bowel preparation using polyethylene glycol- based electrolyte solution with biopsy taking was done (PENTAX EC3890). Activity of UC and grading of mucosal inflammation was done according to The Mayo endoscopic subscore which is calculated as inactive disease and normal mucosa (0), mild disease (1), moderate disease (2), or severe disease (3).[29]. Colonoscopy was also used for detection of characteristic flask-shaped ulcer of E. histolytica and the tropho-zoites can be visualized at the margin of the ulcer or within the tissue. Periodic acid-Schiff staining or immunoperoxidase staining with specific anti-E. histolytica antibodies was used.
- Immunohistochemical xamination for UC was performed using a primary monoclonal antibody targeting calprotectin (clone MAC387; cat. no. M0747; Dako, Glostrup, Denmark) of specimens of healed or inflamed colonic tissue

of UC patients to detect increased numbers of calprotectin-positive cells, including inflammatory leukocytes - mainly monocytes and macrophages - in the colonic mucosa to confirm the diagnosis and grading of UC and amebic colitis and to assess for what extent UC is controlled. The biopsy specimens were also graded using the Geboes grading system which is graded from 0- 5.4[30]. The higher the scores the more severe the inflammation, and UC is considered to have an active histological inflammation with a Geboes score of ≥3.1 (uncontrolled). Those with a score < 3.1 are considered inactive (controlled) cases. The patients were classified according to Geboes score into controlled and uncontrolled groups.

Ethical approval.
Informed consent was taken from each patient. The research protocol was duly approved by the ethical committee of Zagazig University Hospitals.

Statistical analysis.
Data were collected, tabulated and analyzed using SPSS (Statistical package for social science) version 15. Qualitative data were presented as numbers and percent. Chi- square test was used for comparison between groups. Normally presented data were presented as mean +/- SD. Student - t test was used to compare the two groups. No parametric data was presented such as min – max and median. Mann - Whitney test was used for comparing between groups. $P < 0.05$ was considered statistically significant.

Results
This study was conducted for two years to assess the prevalence of E. histolytica infection and uncontrolled or resistant cases of UC. This was a collaborate work between Tropical Medicine Department, Clinical Pathology Department and Pathology Department, Faculty of Medicine, Zagazig University.

Table (1): Comparison between studied groups as regards patients' characteristics.

Study groups / Clinical findings	Controlled UC N=25	uncontrolled UC N=25	T	P
Age: mean (SD, IQR)	39 (4.7)	37.5 (3.19)	0.19	0.84
Gender (male)	10 (40%)	9 (36%)	0.7	0.79
Duration of treatment (years)	3.8 ± 0.4	3.9 ± 0.7	0.75	0.51
Duration of UC (years)	3.6 ± 0.36	3.7 ± 0.68	0.73	0.47
BMI (SD)	25.2(5.2)	25.4 (5.4)	0.18	0.8

Age range= 20- 65 UC= ulcerative colitis SD= standard deviation. IQR= inter quartile ratio.
Comparison of patients' characteristics between both study groups showed no significant difference between them (table- 1).

Table (2): Comparison between both study groups regarding common symptoms and signs of UC patients.

	Controlled UC (N=25)		Uncontrolled UC (N=25)		X_2	P
	N	%	No	%		
Abdominal pain	13	52 %	23	92%	9.9	0.002*
Bloody diarrhea	0	0 %	21	84 %	18.1	<0.001*
Bleeding/ rectum	0	0 %	1	4 %	17.3	<0.001*
Tenesmus	4	16 %	19	76 %	11.6	0.003*
Dysentery	2	8 %	11	44 %	10.009	0.007*
Urgency	2	8 %	12	48%	9.92	0.002*
Fecal incontinence	2	8 %	16	64 %	17.34	<0.001*

* highly significant. UC= ulcerative colitis
The main signs and symptoms of UC were more prevalent among the uncontrolled UC group than the controlled one and the differences were highly significant (table- 2).

Table (3): Comparison between study groups regarding their investigations.

	Controlled UC N= 25	Uncontrolled UC N= 25	T	P
Hemoglobin (mg/dl)	14.7± 1.4	12.1± 1.3	1.2	0.2
Platelets (x1000/mm)	117± 23	230.6± 68	5.6	<0.001*
WBCs(x1000MM)	7.8 ± 4.3	7.5± 3.1	0.2	0.85
Albumin(gm/dl)	3.9± 0.68	3.52± 0.3	0.75	0.48
Patients with High ESR	2 (8%)	13 (52%)	12.1	0.002*
Patients with Fecal calprotectin > 500 Ug/mg	4 (16%)	19 (76%)	11.6	0.003*
Patients with high CRP >200 mg/l	2 (8%)	12 (48%)	9.92	0.002*
Extent of UC.				
-Proctitis	9 (36 %)	22 (80 %)	17.3	0.001*
-Left sided colitis	16 (64 %)	2 (8 %)		
-Extensive colitis	0 (0%)	1 (4%)		

* highly significant. UC= ulcerative colitis WBCs= white blood cells. CRP= C- reactive protein. ESR= erythrocytes sedimentation rate.

Most of investigations conducted on both study groups showed higher levels of acute phase reactants among the uncontrolled group than the controlled one. The differences were highly significant regarding platelets count, ESR, CRP and fecal calprotectin. Colonoscopic examination showed left sided UC involvement was more common among patients of the controlled UC group, while proctitis and extensive colitis are more common among the patients of uncontrolled UC group. The differences were highly significant (table- 3).

Table (4): Prevalence of E. histolytica among study group.

Study groups / E. histolytica	Controlled UC N= 25	Uncontrolled UC N= 25	X_2	P
Positive	2 (8%)	13 (52%)	12.05	0.002*
Negative	23 (92%)	12 (48%)		

* highly significant. UC= ulcerative colitis.

According to Geboes score, the prevalence of E. histolytica was more common among patients of uncontrolled UC group than the controlled one and the difference was highly significant. E. histolytica was diagnosed by stool tests and confirmed by endoscopy and histopathological examination. (table- 4).

Table (5): Prevalence of E. histolytica according to the extension of UC.

Study groups / E.histolytica	Controlled UC N=25			Uncontrolled UC N= 25			P
	Proctitis N= 9	Left sided colitis N= 16	Extensive colitis N= 0	Proctitis N= 22	Left sided colitis N= 2	extensive colitis N= 1	
Positive	1 11.1%	1 6.2%	0 0 %	11 50 %	1 50 %	1 100 %	<0.05*
Negative	8 88.8 %	15 93.7%	0 0 %	11 50 %	1 50 %	0 0 %	

* significant. UC= ulcerative colitis.

E. histolytica was found to be more prevalent among patients of uncontrolled UC group than the controlled UC group and the differences were significant regarding the subgroups of colonic UC extension where it is more prevalent among the more extensive UC (table- 5).

Table (6): Prevalence of E. histolytica among UC patients according to Geboes histopathoogical classification and Mayo endoscopic subscore.

Study groups / E. histolytica		Mayo endoscopic subscore				P
		Normal (0)	Mild (1)	Moderate (2)	Severe (3)	
Positive	Controlled UC (N= 2)	1 50%	1 50 %	0 0 %	0 0 %	*< 0.05
	Uncontrolled UC (N= 13)	0 0 %	1 7.6%	1 7.6 %	11 84.6 %	
Negative	Controlled UC (N= 23)	15 65.2 %	8 34.7 %	0 0 %	0 0 %	
	Uncontrolled UC (N= 12)	0 0 %	0 0 %	1 8.3 %	11 91.6 %	

* significant. UC= ulcerative colitis.

The severe forms of UC were more common among the uncontrolled UC patients' group than those of the controlled group and the differences were significant (according to Geboes score). Moreover, E. histolytica was more prevalent among the more severe forms than the milder ones especially among patients of the uncontrolled UC group. The differences were also significant (table- 6).

Discussion

Ulcerative colitis is a chronic inflammatory disease of the gastrointestinal tract[31]. Intestinal amebiasis is a diarrheal disease that has a very close manifestations to UC[32]. It is important to exclude intestinal amebiasis especially in those resistant UC patients staying out of control despite the successive therapeutic courses.

This study showed that the prevalence of E. histolytica infection of the colon was more common among patients of uncontrolled UC group than the controlled one (Geboes score) and the difference was highly significant. This result agrees with that of Sebnem Ustun and Chan et al., who found that IBD patients have more prevalent E. histolytica infection than normal populations[33,24]. From their studies, if E. histolytica is more prevalent among patients with UC so we can predict more prevalence among the uncontrolled cases who keep resistant to successive therapeutic courses than the controlled cases. Chan et al., described three patterns of relationships between UC and E. histolytica infection of the colon. These patterns are coexisting infection, asymptomatic carrier and superinfection that causes exacerbation of colitis[24]. However, this result does not agree with that of Vukobrat-Bijedic et al., who denied any relation between colonic E. histolytica infection and the grade of activity of UC [34]. This can be explained by the different environmental factors of the studies that can affect the host immunity and/ or response to amoebic infection.

This study showed that E. histolytica infection of the colon was more common among patients with extensive UC of the colon. This result agrees with that of Vukobrat-Bijedic et al., and canan et al., who found that amoebic infection can be a co- factor in extending the localization of UC[34,35]. This can be explained by the substantial and/or the activating effect of E. histolytica infection on the colonic mucosa.

This study showed that the more severe endoscopical forms of UC were more common among the more severe histopathological forms. This result agrees with that of Dae Bum Kim et al., who found strong correlation between endoscopic activity and histological activity[36].

On the other hand, E. histolytica was more prevalent among the endoscopical more severe forms than the milder ones (Mayo endoscopical subscore) especially among patients of the uncontrolled UC group. The differences were also significant. This result can also be explained by the combined effect of UC and E. histolytica infection on colonic mucosa. Such effect can be a major cause in failure of treatment of UC, a condition that was encountered in many studies and case reports in which we could easily expect a dramatic response after treatment with anti- amebic medications[19, 23, 37].

Conclusion

E. histolytica is more prevalent among resistant or uncontrolled cases of UC than the controlled cases and could play an important role in failure of treatment. Moreover, E. histolytica should be excluded in all resistant cases of UC especially before corticosteroids administration.

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