

A Parasite That Should not be Neglected in Patients with Ulcerative Colitis: *Entamoeba histolytica*

Ülseratif Kolit Hastalarında İhmal Edilmemesi Gereken Bir Parazit: *Entamoeba histolytica*

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ABSTRACT

Objective: To determine the prevalence of amoebiasis, which has been neglected in recent years according to the World Health Organization, in ulcerative colitis patients and investigate the relationship between amoebiasis and ulcerative colitis.

Methods: The study included 150 individuals, including 100 ulcerative colitis patients and 50 healthy individuals without gastrointestinal complaints. The samples collected were first analyzed macroscopically and then using native-Lugol, trichrome staining, and enzyme-linked immunosorbent assay (ELISA).

Results: In the microscopic examination, *Entamoeba* spp. cysts were found in 22% of the ulcerative colitis patients and 2% of those in the control group. *Entamoeba histolytica* (*E. histolytica*) adhesin antigen was detected by ELISA in 31% of the ulcerative colitis patients and 4% of those in the control group. A significant correlation was found between the incidence of *E. histolytica* and ulcerative colitis in the statistical evaluation.

Conclusion: *E. histolytica* should not be neglected in ulcerative colitis patients and should be investigated in the presence of diarrhea, bloody diarrhea, and abdominal pain.

Keywords: Amoeba, dysentery, *E. histolytica*, ulcerative colitis

ÖZ

Amaç: Bu çalışma, Dünya Sağlık Örgütü'ne göre son yıllarda ihmal edilen amoebiasisin, ülseratif kolit hastalarındaki yaygınlığını belirlemek ve amoebiasis ile ülseratif kolit arasındaki ilişkiyi araştırmak amacıyla yapıldı.

Yöntemler: Çalışmaya ülseratif kolit tanısı konmuş 100 hasta ve gastrointestinal şikayeti olmayan 50 sağlıklı birey olmak üzere toplam 150 kişi dahil edildi. Çalışmaya dahil edilen örnekler önce makroskopik olarak, sonrasında nativ-Lugol, trikrom boyama ve enzimle bağlı immünosorbent deneyi (ELISA) yöntemleri kullanılarak incelendi.

Bulgular: Mikroskopik bakı ile ülseratif kolitli hastaların %22'sinde, kontrol grubunun %2'sinde *Entamoeba* spp. kisti saptandı. ELISA yöntemi ile ülseratif kolitli hastaların %31'inde, kontrol grubunun ise %4'ünde *Entamoeba histolytica* (*E. histolytica*) adezin antijeni saptandı. *E. histolytica* görülme sıklığı ile ülseratif kolit arasında yapılan istatistiksel değerlendirmede anlamlı bir ilişki saptandı.

Sonuç: Ülseratif kolit hastalarında *E. histolytica*'nın ihmal edilmemesi ve hastalarda ishal, kanlı ishal ve karın ağrısı varlığında mutlaka *E. histolytica*'nın ayırıcı tanı ile araştırılması gerektiği kanaatine varıldı.

Anahtar Kelimeler: Amip, dizanteri, *E. histolytica*, ülseratif kolit

INTRODUCTION

Inflammatory bowel disease (IBD) is a general term used for chronic inflammatory diseases involving the gastrointestinal system caused by immunological,

genetic, and environmental factors, the etiology of which is not yet well understood. Ulcerative colitis, categorized in IBD, is a chronic disease characterized by diffuse inflammation of the mucosa of the colon and rectum. The exact cause of ulcerative colitis is



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unknown. However, it is thought that the risk of developing the disease may increase in the presence of bacterial, viral, or parasitic infections (1,2).

Clinical symptoms of ulcerative colitis include abdominal pain and diarrhea with or without blood. There are no pathognomonic symptoms, signs, or tests for its diagnosis. Diagnosis can be made by evaluating the clinical symptoms in detail and excluding other diseases that may simulate the disease. *Entamoeba histolytica*, *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, *Mycobacterium tuberculosis*, *Clostridium difficile*, Norovirus, Adenovirus, Rotavirus, and Cytomegalovirus should be excluded, especially in differential diagnosis (3-5).

Amoebiasis is a common parasitic disease caused by *E. histolytica*, affecting approximately 10% of the world's population. Transmission of the disease occurs through food and drinks contaminated with the cyst form of the parasite. The disease is usually asymptomatic but can cause clinical symptoms ranging from cramping abdominal pain, watery or bloody diarrhea, weight loss, and amebic colitis. In addition, *E. histolytica* can colonize in the large intestine, cross the mucosal epithelial barrier, spread to extra-intestinal organs, and cause abscesses. In particular, abscesses may occur in the liver, lungs, brain, skin, or perianal region. Acute amoebic colitis is clinically similar to IBD. Amoebiasis can exacerbate IBD symptoms or have a negative impact on the course and treatment of the disease (6-9).

The aim of the current study was to determine the prevalence of amoebiasis, which has been neglected in recent years according to the World Health Organization (10), in ulcerative colitis patients and investigate the relationship between amoebiasis and ulcerative colitis.

METHODS

Study Design

This cross-sectional study was conducted between July 2020 and October 2021 at the Van Yüzüncü Yıl University Faculty of Medicine Parasitology Laboratory. The study was initiated after patient consent was obtained. Prior to the research, permission was obtained with the decision of Van Yüzüncü Yıl University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (2021/06-11). The study was designed with a patient group and a control group. Patients who applied to the Gastroenterology Outpatient Clinic of Van Yüzüncü Yıl University Dursun Odabaş Medical Centre and were diagnosed with ulcerative colitis based on endoscopic, radiological, histopathological, and clinical findings were included in the patient group, and individuals without gastrointestinal complaints were included in the control group.

Sample Size

The sample size of this prospective study was calculated using G*Power 3.1.9.7 statistical software within the scope of chi-squared (χ^2) goodness-of-fit tests (9). In the calculations, a minimum of 50 samples in each group was determined when the power was 0.80, the effect size was 0.4 (χ^2 test effect size interval value) and the type 1 error (α) was 0.05.

Study Population and Sample Collection

In the study, patients diagnosed with ulcerative colitis were included in the patient group, and healthy individuals without

any chronic disease were included in the control group. The age and clinical findings such as diarrhea and abdominal pain of each study participant were recorded. Stool samples were collected and brought to Van Yüzüncü Yıl University Faculty of Medicine, Department of Parasitology Research Laboratory, and stored in the refrigerator at +4 °C.

Examining Stool Samples

Stool samples were first examined macroscopically (shape, consistency and color of the stool) and then microscopically. The stool samples were analyzed using both native-Lugol and trichrome staining to evaluate cystic and trophozoitic forms of *Entamoeba* species. Trichrome staining was performed using Wheatley's trichrome staining kit (Gul Biology Laboratory, İstanbul, Türkiye), following the manufacturer's instructions.

Adhesin antigen was detected for *E. histolytica* seropositivity in the stool samples using an enzyme-linked immunosorbent assay (ELISA) kit (TechLab Systems Inc., Blacksburg, VA, USA), following the manufacturer's instructions.

Statistical Analysis

The χ^2 test, 2-ratio Z test of the ratios, and Fisher's Exact test were used in the statistical analyses. In the calculations, statistical significance was accepted as $p < 0.05$. IBM SPSS Statistics for Windows 26.0 (IBM Corp., Armonk, NY, USA) and MINITAB 14.0 were used for the calculations.

RESULTS

The study included 100 ulcerative colitis patients. Since it was difficult to reach healthy volunteers, only 50 individuals were included in the control group. The mean age of the 100 ulcerative colitis patients was 41.5 ± 14.7 (range: 18-79) years and that of the 50 individuals in the control group was 38.6 ± 17.8 (range: 18-81) years. There was no statistical difference between the age distributions in the groups ($p = 0.548$).

Macroscopic examination revealed that 71 (47.33%) stool samples were positive for diarrhea (type 7 according to Bristol stool scale) and 38 (25.33%) were positive for bloody diarrhea. *Entamoeba* spp. cysts were detected in 15.3% of the 150 stool samples using native-Lugol (Figure 1) and trichrome (Figure 2) staining. No trophozoite forms were observed in the microscopic examination of the stool samples. With trichrome staining revealed *Entamoeba* spp. cysts in 22 (22%) stool samples of the ulcerative colitis patients and one (2%) of those in the control group. There was a significant difference between the frequency of *Entamoeba* spp. in the ulcerative colitis patients and the control group ($p = 0.001$).

E. histolytica adhesin antigen was detected in 33 (22%) of the 150 stool samples via ELISA. *E. histolytica* was detected in 31 (31%) of the ulcerative colitis patients and two (4%) of those in the control group (Table 1). A statistically significant difference was found between the ulcerative colitis patients and control group in terms of *E. histolytica* positivity ($p = 0.001$).

E. histolytica was detected in 23 (32.39%) of 71 patients with diarrhea and eight (27.58%) of 29 patients without diarrhea (Table 1). No statistically significant difference was found between *E. histolytica* positivity and diarrhea ($p = 0.63$). In addition, *E. histolytica* was detected in 11 (28.95%) of 38 patients with bloody diarrhea and 20 (32.25%) of 62 patients without bloody diarrhea (Table 1). No significant difference was found between in terms of

E. histolytica positivity between patients having bloody diarrhea and not ($p=0.726$).

Abdominal pain was present in 45 (54%) patients with ulcerative colitis. *E. histolytica* was detected in 18 (40%) of 45 patients with abdominal pain and 13 (14.29%) of 55 patients without abdominal pain (Table 1) ($p=0.072$).

DISCUSSION

It was reported that the distribution of *E. histolytica* worldwide varies between 1% and 10% and in some regions, this rate is as high as 50%. In Türkiye, the prevalence of amoebiasis is between 0.4% and 18.4% and it is endemic in the southeastern region (11). The incidence of ulcerative colitis is quite high in European countries, but recent studies have shown that the incidence has plateaued or even decreased in these countries. However, a significant increase in the incidence of the disease was reported in South America, Asia, Middle East, and Africa, especially in developing countries (1,12).

In studies investigating the prevalence of amoebiasis in ulcerative colitis patients in different countries, *E. histolytica* positivity was stated as 1.4-14.3% (2,9,13,14). In Türkiye, *E. histolytica*/*E. dispar*

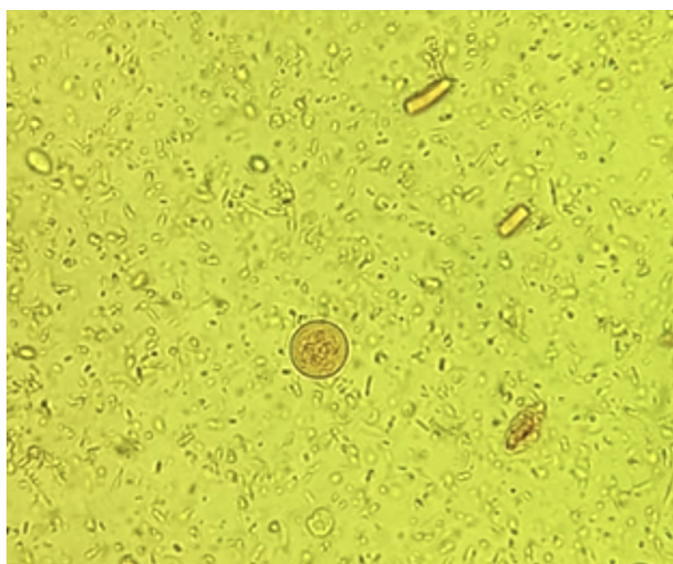


Figure 1. *Entamoeba* spp. cyst detected on a Lugol-stained preparation in a patient

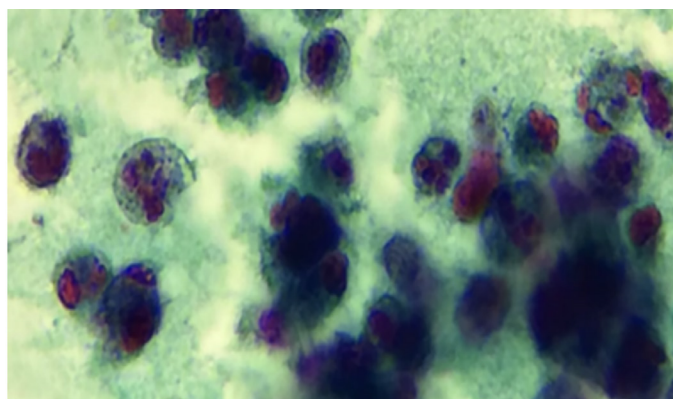


Figure 2. *Entamoeba* spp. cysts detected in a trichrome-stained preparation in a patient

Table 1. Comparison of the *E. histolytica* positivity rates

Variable		<i>E. histolytica</i> adhesin antigen		p-value
		Positive n (%)	Negative n (%)	
Working group	Ulcerative colitis patients (n=100)	31 (31.00)	69 (69.00)	0.001
	Control group (n=50)	2 (4.00)	48 (96.00)	
Diarrhea	Yes (n=71)	23 (32.39)	48 (67.61)	0.63
	No (n=29)	8 (27.58)	21 (72.42)	
Bloody diarrhea	Yes (n=38)	11 (28.95)	27 (71.05)	0.726
	No (n=62)	20 (32.25)	42 (67.75)	
Abdominal pain	Yes (n=45)	18 (40.00)	27 (60.00)	0.072
	No (n=55)	13 (23.63)	42 (76.36)	

was detected at rates as high as 10-31.5% in studies conducted on ulcerative colitis patients (4,6,15,16). In the current study, *E. histolytica* positivity was found in 31% of the ulcerative colitis patients. While the western part of Türkiye is similar to the socio-economic structure of developed countries, the eastern and southeastern regions have a lower socio-economic structure. We believe that the high amoebiasis rate found in the ulcerative colitis patients in the current study may be due to the socio-economic or hygiene habits of the region. However, since the main aim of the study was to investigate whether there is a relationship between ulcerative colitis and amoebiasis, the *E. histolytica* positivity rate in ulcerative colitis patients on its own is insufficient. Therefore, positivity rates in control groups are important. In the current study, *E. histolytica* positivity rate in the control group was 4%. When this positivity rate was compared with that in the ulcerative colitis patients, the difference was statistically significant ($p=0.001$). This indicates that there may be a relationship between *E. histolytica* and ulcerative colitis. In previous studies, the *E. histolytica* positivity rate was higher in the ulcerative colitis patients compared to the control groups (9,15,16). However, the relationship between ulcerative colitis and amoebiasis is not clearly known because the endoscopic appearance of amoebic colitis may be confused with that of ulcerative colitis. The diagnosis of ulcerative colitis is primarily based on endoscopic findings showing inflammation from the rectum to the colon and is confirmed by biopsy specimens showing chronic colitis. It is usually diagnosed by sigmoidoscopy without parasitological examination, and therefore, amoebic colitis is often overlooked. Histopathological findings of amoebic colitis and ulcerative colitis may also resemble crypt abscess (17). Therefore, we believe that one of the reasons for the high rate of *E. histolytica* in ulcerative colitis patients is the lack of a clear distinction between amoebic colitis and ulcerative colitis. Hence, since misdiagnosis of amoebic colitis as ulcerative colitis and subsequent treatment with corticosteroids can be fatal, a differential diagnosis for *E. histolytica* should definitely be made in patients diagnosed with ulcerative colitis.

In the present study, no statistically significant relationship was found between abdominal pain ($p=0.072$), diarrhea with blood ($p=0.726$), and diarrhea ($p=0.63$) and *E. histolytica* positivity. The clinical symptoms of ulcerative colitis include abdominal pain and diarrhea with or without blood (2). The same symptoms

are observed in symptomatic intestinal amoebiasis (18). For this reason, ulcerative colitis and amoebiasis may coexist and be misdiagnosed due to the similar clinical course of both diseases. In addition, it should be kept in mind that the steroids used in the treatment of amoebiasis misdiagnosed as ulcerative colitis may cause exacerbation of the disease and the formation of multiple liver amoebic abscesses (19).

In developing countries, the microscopic method is more frequently used in the diagnosis of the disease. However, the reliability of this method is debated. In the microscopic method, the presence of food residues and/or leucocytes in the feces, deformation of the parasite in stored feces, insufficient sample quantity, and inadequate experience of the person performing the microscopic examination result in misdiagnosis of *E. histolytica* (4,18). Furthermore, microscopic methods cannot distinguish between *E. histolytica* and *E. dispar* and *E. moshkovskii* and *E. bangladeshi*, which are morphologically similar to *E. histolytica*. In particular, the native Lugol method is the most commonly used method in microscopic examination. However, this method alone is insufficient to diagnose the parasite and must be supported by other methods. In the differential diagnosis of *E. histolytica*, serological methods or molecular methods based on the principle of detecting *E. histolytica*-specific antigens in the feces or parasite-specific antibodies in the serum are used. ELISA is the most preferred serological method in routine diagnosis due to its easy applicability, and high diagnostic sensitivity and specificity (20-22). In studies comparing the methods used in the diagnosis of the parasite (23-25), different results were obtained. In the current study, *Entamoeba* spp. cysts were detected in 15.3% of the stool samples under microscopic examination, while *E. histolytica* was detected in 22% of the samples via ELISA. Therefore, since microscopic methods may give misleading results in the differential diagnosis of amoebiasis in ulcerative colitis patients, we believe that the results should be supported by serological methods. Ayrıca Because of the similarity of clinical symptoms between ulcerative colitis and amoebiasis, amoebiasis should be distinguished from ulcerative colitis. Microscopic methods should not be evaluated alone because they are both misleading and inadequate when used alone. Although it is stated that the ELISA method and the adhesin antigen examination provide a definitive diagnosis for *E. histolytica*, it should not be forgotten that *E. histolytica* will not cause infection while continuing to live in the intestinal cavity. It was concluded that the most reliable evaluation should be the use of advanced methods together with the clinical picture of the disease and that a single method should not be used in diagnosis.

CONCLUSION

In conclusion, *E. histolytica* was detected in 31% of the ulcerative colitis patients. It was concluded that *E. histolytica* should not be neglected in ulcerative colitis patients. It should be excluded by differential diagnosis in the presence of diarrhea, bloody diarrhea, and abdominal pain in this patient group, and the result should be supported by serological methods since microscopic methods can give misleading results in diagnosis.

*Ethics

Ethics Committee Approval: Prior to the research, permission was obtained with the decision of Van Yüzüncü Yıl University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee (2021/06-11).

Informed Consent: The participants were informed about the research and their consent was obtained.

Footnotes

This article is an abridged version of Murat Soylu's Master Thesis titled "Investigation of *Entamoeba histolytica* Prevalence in Ulcerative Colitis Patients".

*Authorship Contributions

Concept: M.S., A.E., Ö.A., Ş.Y., Design: A.E., S.A., Ö.A., Data Collection or Processing: M.S., A.E., Analysis or Interpretation: M.S., A.E., S.A., Ş.Y., Literature Search: M.S., A.E., Ş.A., Writing: M.S., A.E., S.A., Ş.Y., Ö.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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