Intestinal Amebiasis in a Group of Patients with Ulcerative Colitis: Influence on Clinical Course of the Disease

Zora Vukobrat-Bijedic¹, Azra Husic-Selimovic¹, Nina Bijedic², Ivana Bjelogrlic³, Aleksandra Djuran⁴
Gastroenterohepatology Department, University Hospital Sarajevo, Sarajevo, Bosnia and Herzegovina¹
University „Dzemal Bijedic“, Mostar, Mostar, Bosnia and Herzegovina²
CHC Lukavica Istocno Sarajevo, Bosnia and Herzegovina³
Pathology Department University Hospital Sarajevo, Sarajevo, Bosnia and Herzegovina⁴

Introduction: Ulcerative colitis (UC) is a common disease with a chronic and relapsing presentation requiring regular clinical follow up. Epidemiological and microbiological studies suggest that enteropathogenic microorganisms play a substantial role in the clinical presentation and extent of inflammatory bowel disease. Goal: To evaluate the presence of intestinal infections by Entamoeba hystolitica in patients with ulcerative colitis, their impact on clinical outcome, and to identify associated risk factors.

Material and methods: A total of 31 patients hospitalized on Gastroenterohepatology Department with pathohistologically proved ulcerative colitis were studied. Fresh feces samples taken from 20 patients were examined immediately using Eosin and Lugol-staining methods and analyzing the presence of vegetative and MIFC (Meriolat and Iod staining).

Results: A total of 16 female and 15 male hospitalized UC patients were analysed in a period of two years (2010-2011). The mean age at diagnosis was 43 years. We analyzed relation of amoeba infection with localization of ulcerative colitis. Our results indicate that amoeba infection is related to extent of disease (they were mostly present in pancolitis). Presence of amoeba is not related to age nor gender. Furthermore, presence of amoeba was not associated with more severe clinical course of disease.

Discussion and conclusion: Amoeba infections in UC patients treated at Gastroenterohepatology Department was not related to the grade of disease activity, and other clinical variables such as gender, age and parameters of inflammation. These microorganisms could be a contributing cause of extended localization of disease.

Key words: ulcerative colitis, entamoeba hystolitica, inflammation.

Corresponding author: Zora Vukobrat-Bijedic, MD, PhD, Professor of Internal medicine, Head of Gastroenterohepatology clinic, Clinical center of University of Sarajevo; e-mail: zoravukobratbijdic@yahoo.com

1. INTRODUCTION

Ulcerative colitis is autoimmune disease the cause of which is yet undiscovered. It has a long and clinically unpredictable course, and is accompanied by remissions of different duration and unexpected relapses.

The onset is usually in the rectum to extent proximally, occupying all parts of the colon (pancolitis), with the reaction in the terminal part of the ileum. The basic symptoms are diarrhea with blood and mucus, abdominal pain, tenesmus and weight loss. Diarrhea as a symptom does not accompany ulcerative proctitis, but extending proximally, the number of stools increases. Diarrhea is anyway accompanied by loss of liquid, minerals, Fe, blood and proteins. Diarrhea is attributed to the disorder in the absorption and electrolytes, reduced activity of Na+, K+ ATP of the pump with increased permeability of the mucous membrane and with changes of mucous phospholipids (1).

Colon motility is changed due to the inflamed mucous membrane and characterized by rapid passage of the intestinal content through the inflamed mucous membrane. Muscular contractions in the colon wall cause pain.

Diagnostic procedures include, beside endoscopy, targeted colon mucous membrane biopsy, an obligatory parasitological and microbiology analysis of the stool as well as stool tests against the presence of amoebas and fungi.

1.1. Pathogenesis of intestinal amoebiasis

Contemporary approach to diseases leads us via molecular-biological status and biochemical and genetic one. However, it must be admitted that the position on amoeba and its influence on ulcerative colitis, the activities thereof in particular, has not been clearly defined. Whether it is just an incidental inhabitant of intestines or a disease trigger is still discussed and has not been defined yet. Pathohistological developments in acute amebic colitis are related to macroscopic findings on the
mucous membrane of the colon. The acute Entamoeba hystolitica (E. hystolitica) infection is accompanied by fecal ulcerations of intestinal mucosa resulting in dysentery (diarrhea with blood and mucus). The mechanisms involved in the production of focal lytic lesions include multi factor processes in which lectins facilitate adhesion of proteins enzymes, degrading extra cellular matrix components. E. hystolitica develops mechanism of immune response modulations in the course of acute infection. It is protozoa, a parasite capable of invading intestinal mucosa and can expand to other organs, most frequently the liver. Entamoeba dispar, which is morphologically similar to E. Hystolitica, also colonizes human intestine and since recently has become recognized as a special species with non invasive capacity. A large number of asymptomatic infections are cause by this amoeba (2). Invasive amoebiasis with E. hystolitica is more frequent in developing countries due to poor health care awareness, over crowdedness and poor hygiene, low standard of living, poor sanitary conditions and water contaminated with amoebas. Transmission is fecal-oral. Vegetative form of E. Hystolitica lives in the lumen of the colon, where it is multiplied into a cyst responsible for the transmission of the infection. The cysts are secreted from a stool and may be ingested by contaminated food or water, finding a new host. In terminal ileum, the parasite is in vegetative form. There are 4 clinical forms of intestinal amoebiasis, most of which are acute: a) Dysentery or hemorrhagic diarrhea; b) Colitis with fulminant course; c) Amebic appendicitis; d) Ameboma of the colon. The incidence of dysentery and diarrhea syndrome is in 90% of patients with invasive intestinal amebiasis. Patients have up to 5 stools with mucous stools with blood a day, accompanied by pain and tenesmi. Fever and systemic manifestations of the disease are absent. Invasive amebiasis is frequently accompanied by amebic abscess of the liver. Ameba migrates into the liver from the colon by portal circulation and is 10 times more frequent in adult patients, and 3 times more frequent in case of male patients. The diagnosis of invasive amoebiasis is based on microscopic identification of E. Hystolitica in a stool. Active intestinal amoebiasis may be related to ulcerative colitis, toxic mega colon, amebas and appendix abscesses.

1.2. Macroscopic lesions
Typical malignant ulcers were found in colon, primarily in cecum, rectum and sigma. There are two types of ulcers: nodular and irregular. Nodular lesions are small, from 0.1 to 0.5 cm in size. They are round, insignificantly elevated from the mucous membrane, with irregular necrotic centers and surrounded by hard and edematous tissue. The necrotic centre may be hemorrhagic as well, but it is more frequently filled with yellowish mucous material. The lesions may cover a larger part of the mucous membrane of the colon. Irregular and serpiginous ulcers may be from 1 to 5 cm long and are more frequent in the cecum and ascending colon. They are characterized by wide elevated edges and are filled with fibrin.

1.3. Microscopic results
In initial stadium, amebas create non specific lesions characterized by thinned mucous membrane, glandular hyperplasia and stromal edema. There is also presence of medium infiltration of exudates placed around and between capillaries, as well as minor infiltration of exudates with neutrophils (3). Lymphoid aggregation indicates reactive hyperplasia with numerous histiocytes. Amebas are present in smaller number in surface exudate. In case of a lesion progression, it will be accompanied by mucopenic depression caused by the loss of mucin on glandular epithelia cells. Mucin also declines in column and cuboid cells. A larger neutrophilic infiltrate is also evident, with numerous plasma cells, eosinophils, macrophages with lots of lymphocytes in surface layer of colon mucous membrane. There is also quite a presence of amebas, along with epithelia lesions, on the wall.

The invasion of the colon mucous membrane and cecum by E. Hystolitica starts in the inter glandular epithelia forming early lesions with surface ulceration. Depending on the extent of the damage, those could be small interglandular focuses of the micro invasion with low-tissue necrosis and inflammatory cell infiltration. Neutrophil infiltration of the lamina propria occurs in early invasive lesions, like superficial ulcerations. In 90% of cases those are circulating granulocytes, epithelia cells also produce active cytokines, soluble factors, including IL-8, monocyte hemotact protein 1, granulocyte macrophage stimulating colon factor and alpha factor necrosis factor (TNF-Alpha).

1.4. The role of neutrophils
Cell infiltration around amoebas leads to fast lysis of inflammatory cells, accompanied by tissue necrosis. The reasons of neutrophils’ incapacity to destroy the ameba are still unknown. In case of other parasite infections, neutrophils kill microorganisms with both 02-dependent and independent mechanisms. They produce strong oxidizing destruction and their secreting granules contain strong cytotoxic molecules.

1.5. Late invasive lesions
Late invasive lesions are accompanied by deep ulcerations. Mucous ulcer, while progressing, penetrates deeply into the sub mucosa and the inflamed infiltration invades the sub mucous part of the colon wall.

1.6. The role of macrophage
The role of macrophage in acute intestinal amoebiasis remains unexplained as well. In vitro experiments with human polymorphonuclear leucocytes, peripheral monoclonal cells, monocytes rapidly destroy E. hystolitica. Prostaglandin E2 (PGE2) is a modulator of immune response, with pro inflammatory effects increasing the edema and infiltration of leucocytes. It inhibits some of macrophage functions, like IL-1 and TNF-alpha production and Class II MHC gen complex expression.

2. MATERIAL AND METHOD
Our research involved 31 patient hospitalized at the Clinic for gastroenterohepatology in the past two years (period 2009-2011). The results obtained in 16 male patients and 15 female were analyzed. The mean age was 43 years. All the patients underwent colonoscopy, targeted biopsy and they all had histologically confirmed clinical
diagnosis of ulcerative colitis. The stool was tested before the commencement of therapeutic treatment. Microbiological tests were undertaken and tests for parasite and amoeba presence. The stool for analysis was taken by provocation and the intestinal aspirate during the colonoscopy, when macroscopic results indicated intestinal amoebiasis.

At the Institute for Microbiology, the presence of amoeba was confirmed by applying the methods of Eosin and Lugol dyeing and MIFC method (meti- lat and iodine dyeing for the concentra tions of cystitic forms of E. hystolitica).

We analyzed Powell Tuck index of activities, serum inflammation parameters, presence of anemia and extent of the infection of the intestinal wall by pathological process and Association of intestinal amoebiasis with examined parameters, and its impact on the course of the disease.

3. RESULTS

Powell-Tuck was ranked from 1-3; CRP from 0 (referent) to 3 (severe inflammation). Leukocytes were either 0 (referent) or 1 (mildly elevated), Anemia was ranked as 0 (no anemia) to 2 (medium). We grouped age in four categories: younger than 30 (1), 31-40 (2), 41-50 (3) and older than 50 (4).

We can see almost statistically significant correlation between Powell-Tuck and CRP. The only other noticeable correlation is weak negative correlation between Age group and CRP, indicating that younger people encounter more severe inflammation.

From the regression equation it is obvious that the largest marginal contribution to the presence of amoeba is from the presence of anemia, in the sense that absence of anemia can be insignificantly related to the presence of amoeba. Furthermore, it can be deduced that absence of inflammation favors presence of amoeba, even though this interpretation is also insignificant. The only positive marginal contribution to presence of amoeba is from elevated number of leukocytes. But this is not statistically relevant either. Since the obtained regression F statistics (0.7906) is greater than the critical F value (0.2157), the regression is relevant.

Nevertheless, the regression R square is very low (0.2202), so the results have to be retested in future research, preferably with a more complete data set. We also calculated the risk ratio (RR) for the most influential regression parameter, that is, anemia. The interpretation of the obtained RR is that the presence of amoeba can be related to absence of anemia, as it can be concluded from the regression as well.

This result is not statistically significant, mostly due to the small number of data, and we hope to confirm the result in the next research.

4. DISCUSSION

Ulcerative colitis is a form of chronic-non specific inflammatory bowel disease. Along with other numerous risk factors, the ethiopathogenesis of ulcerative colitis is also contributed by: genetic predisposition, food, allergy, bacterial or virus infection and environmental factors. The above factors have an impact to the mucous membrane of the colon and lead to disarrangement in the intestinal immunological system (3).

In our research, we analyzed the impact of the infection with E. hystolitica on a clinical course of the disease, and the manifested extent of the disease activity. Powell Tuck index of activity was calculated and the correlation between this score and the activities of infection serum parameters, where statistically significant correlation between CRP and Powell-Tuck was found. We analyzed the presence of anemia in our sample of patients. There was no significant difference in the detection of anemia in patients with amoeba infection. At the same time, the minimal impact of the amoeba to the elevated values of leukocytes was found, but the determined parameter is not statistically relevant. Unlike the results in the study by Polinsky et al. (4), where the reduced values of the thrombocytes were determined, we did not find significant deviations in thrombocyte values in our sample.

Analyzing the extent of the inflammatory process in the colon wall in relation to the amoeba infection, we, unlike the study by C. Alkim et al. (5), found no statistically significant difference. Based on the results of our study, the presence of amoebas may contribute to a larger scale inflammatory process, but a more significant impact was not proved.

5. CONCLUSION

Intestinal infection by E. hystolitica in a group of patients with ulcerative colitis treated at the Gastroenterohepatology Clinic was not in correlation with either the degree of the disease activity, or other clinical parameters (sex, age, inflammatory parameters, except CRP). The presence of amoeba may contribute to a larger degree of infection of the colon wall.

REFERENCES: