Prevalence of cytomegalovirus in new and recurrent ulcerative colitis

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Abstract

Background: Identification of risk factors for exacerbation of ulcerative colitis (UC) can be helpful in the prevention and treatment of it. This study aimed to evaluate the epidemioclinical features of the patients with cytomegalovirus ulcerative colitis.

Methods: In this cross-sectional study, patients with UC referred to the gastrointestinal ward of Rasool-e Akram and Firoozgar hospitals were selected. After pharmaceutical treatment of ulcerative colitis, both newly diagnosed patients for colitis and patients who did not respond to treatment and returned to the hospital, Underwent colonoscopy, a colon biopsy was performed and a blood sample was taken for active cytomegalovirus (CMV) by ELISA. Tissue samples were examined by PCR.

Results: A total of 72 patients with ulcerative colitis (65 recurrent patients) were included in the study. In total patients with colitis, based on serological results, 11.1% of the patients had acute infection and 88.9% had CMV infection in the past, but based on the detection of CMV using PCR, the virus genome was revealed only in 2.8%. In general, the highest rate of CMV tracking was related to cases of pancolitis and the lowest rate was related to proctitis. The rate of CMV was not affected by other variables such as gender, age or Anti-colitis drug protocol.

Conclusion: The rate of CMV infection in patients with ulcerative colitis is directly related to the extent and severity of the disease but may not be affected by the treatment protocol.

Keywords: Cytomegalovirus, ulcerative colitis, inflammatory bowel disease

Introduction

Cytomegalovirus colitis is an uncommon finding in the patients with healthy immune systems, but its higher prevalence in those with immune-suppressed patients is routine. The first case of colitis caused by cytomegalovirus (CMV) was reported about 50 years ago [1]. In patients with ulcerative colitis, mucosal inflammation is often exacerbated and immunosuppressive steroids are prescribed to treat it. However, the role of CMV in exacerbating inflammatory bowel disease (IBD) remains unclear. In addition, treating patients with ulcerative colitis with cytomegalovirus infection is a challenging [2]. According to serological and pathological diagnosis, the prevalence of this infection in patients with moderate to severe ulcerative colitis is estimated to be 16% to 34% [3]. In cases of severe steroid-resistant ulcerative colitis, the prevalence is estimated to be 20 to 40% (4-7). Recent studies have shown that CMV infection increases the risk of exacerbating the course of ulcerative colitis, the risk of long-term hospitalization due to exacerbation of colitis and affects the therapeutic response of anti-colitis protocols [8, 9]. Patients with ulcerative colitis due to concomitant CMV infection have been shown to require more cumulative colectomy [10]. Some studies have shown that reactivation of cytomegalovirus in patients with moderate to severe colitis has no effect on the need for colectomy or delay the patient's recovery [11].

To the best of our knowledge, no study has been done on the relationship between CMV reactivation and recurrence of ulcerative colitis in Iran. Moreover, most of the studies on infection with CMV have been only serological-based in Iran. Therefore, the present study was performed to trace the CMV genome by PCR technique followed by its epidemioclinical features in a sample of Iranian patients' population.

Materials and methods

Study subjects

In this cross-sectional study, patients with ulcerative colitis referred to the gastrointestinal ward of Rasool-e Akram and Firoozgar hospitals were included into the study. After obtaining patients' satisfaction and filling out a demographic and clinical questionnaire, blood and biopsy samples were taken from colonic tissue. The initial diagnosis of ulcerative colitis was made by a gastroenterologist based on anal examination, complete abdominal examination, fecal examination, and colonoscopy. Since following the use of steroid and immunosuppressant drugs, CMV can be reactivated and causes recurrence of ulcerative colitis, after drug treatment of ulcerative colitis and follow up patients, a number of patients who did not respond to treatment and returned to the hospital were reassessed as recurrent ulcerative colitis by colonoscopy, biopsy of the colon and blood samples for detecting CMV reactivation through ELISA and PCR techniques. Inclusion criteria were ulcerative colitis (newly diagnosed and recurrent cases), age over 18 years, and signing the informed consent form by the patient. Exclusion criteria were cases of other colon diseases (such as the presence of cancer or polyps in the colon), the unsuitability of the sample for testing, or age less than 18 years.

Data collection

Demographic and clinical data used for collecting patients' information were age, gender, weight, marital status, cigarette smoking or hookah smoking (past or present) and its amount (pack-year), history of alcohol consumption, occupation, physical activity level, place of residence (urban or rural area), economic status, nutritional level, dietary regimens, history of ulcerative colitis, history of using steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs), history of gastrointestinal diseases, family history of ulcerative colitis, history of colon cancer in family, history of organ transplantation, history of receiving immunosuppressive drugs, having inherited immune deficiency, and having familiar history of cancer. Also, in patients with ulcerative colitis, severe diarrhea, fever, blood in the stool, rectal bleeding, ulcerative colitis (proctitis, left colitis or pancolitis), C-reactive protein level, albumin and hemoglobin levels, weight loss, constipation, abdominal pain, back pain, bloating, mouth ulcers, pruritus, headache, and insomnia were also determined.

Serological test

A total of 5 ml whole blood was obtained from each participant and serum was separated in vacuum tubes after clotting. Evaluation of IgM and IgG antibodies against CMV in serum samples of the patients with ulcerative colitis was performed using ELISA commercial kit (GENELABS DIAGNOSTICS CMV-IgG, CMV-IgM Equipar, Italy). After IgM-ELISA and IgG-ELISA tests, if the sample had anti-cytomegalovirus antibodies, color change was achieved by adding a substrate-chromogen solution. After stopping the reaction, optical density (OD) of all samples was read by ELISA reader. If the light absorption of the samples was higher than the cut-off value, it was considered positive and otherwise it was considered negative for IgM or IgG anti-cytomegalovirus antibodies.

CMV Polymerase Chain Reaction

A minimum of 25 mg of colonic tissue was collected in normal saline and frozen freshly at -70°c until further use. Extraction of genomic material was done by DNA extraction kit (QIAamp DNA FFPE Tissue Kit - QIAGEN) according to the kit instructions and then the extracted products were frozen at -20°C. PCR test was performed to determine the presence or absence of CMV genome in all samples. To perform this test, PCR reaction conditions were adjusted according to the protocol and primers released by Kishore *et al* [19]. The results were recorded by observing 406 bp bands on 2% Agarose gel against appropriate positive and negative controls.

Statistical analysis

For statistical analysis, results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using t-test or Mann-Whitney test whenever the data had not normal distribution. Categorical variables were compared using chi-square test. For the statistical analysis, SPSS version 23.0 for windows (IBM, Armonk, New York) was used. P value less than of 0.05 was considered significant.

Results

In this study, a total of 72 patients with ulcerative colitis (65 recurrent cases) were included in the study. Demographic characteristic, medical history and clinical manifestations of the population were summarized in Tables 1 and 2. Regarding gastrointestinal involvement, left colitis was found in 61.1%, pancolitis in 18.1%, proctitis in 1.4% and proctocolitis in 19.4% while these rates in the subgroup with recurrent colitis was 64.6%, 18.5%, 1.5% and 15.4%, respectively. Regarding anti-colitis medications, aspirin, NSAIDS, corticosteroids, and infliximab were prescribed in 93.1%, 40.3%, 31.9% and 5.6%, respectively in total population and 100%, 38.5%, 35.4% and 6.2%, respectively in 65 recurrent cases. The prevalence of anti-CMV IgM and Anti-CMV IgG based on the ELISA technique in patients with ulcerative colitis was 11.1% and 88.9%, respectively, and in patients with recurrent type was 12.3% and 89.2%, respectively. According to PCR technique, 2.8% and 3.1% of the cases with/without recurrence were positive for viral genome, respectively. According to the findings presented in Table 3, the highest and the lowest rates of CMV detection was revealed in the patients with pancolitis and proctitis indicating a close link between disease extension and rate of CMV positivity. However, CMV detection was independent to other baseline variables including gender, age, and medication protocols.

Table 1: Baseline characteristics of the patients with ulcerative colitis

Variable		Total patients with ulcerative colitis	Patients with recurrent ulcerative colitis
Sexual distribution	Male	43 (59.7)	39 (60.0)
	Female	29 (40.3)	26 (40.0)
Age		36.28±9.79	36.38±10.02
Marital status	Single	20 (27.8)	19 (29.2)
	Married	52 (72.2)	46 (70.8)
Number of children	0	22 (30.6)	20 (30.8)
	1	22 (30.6)	8 (27.7)
	2	25 (34.7)	24 (36.9)
	3 and higher	3 (4.2)	3 (4.6)
Level of Education	Illiterate	1 (1.4)	1 (1.6)
	Under diploma or diploma	36 (50.0)	32 (42.9)
	Collegiate	35 (48.6)	32 (49.2)
Occupational status	Employed	6 (8.3)	6 (9.2)
	Housewife	20 (27.8)	17 (26.2)
	Self-employed	34 (47.2)	30 (46.2)
	Student	6 (8.3)	6 (9.2)
	Unemployed	6 (8.3)	6 (9.2)
Income	< One million tomans	32 (44.4)	29 (44.6)
	One to three million tomans	12 (16.7)	11 (16.9)
	> Three million tomans	8 (38.9)	25 (38.6)
Regular physical activity		27 (37.5)	24 (36.9)
Weight		70.25±6.63	70.29±9.56
History of alcohol use		6 (8.3)	6 (9.2)

Table 2: Clinical symptoms of the patients with ulcerative colitis

Variable		Total patients with ulcerative colitis	Patients with recurrent ulcerative colitis
Clinical Manifestations	Diarrhea	34 (47.2)	35 (53.8)
	Fever	17 (23.6)	17 (26.2)
	Blood in the stool	35 (48.6)	33 (50.8)
	Anal bleeding	18 (25.0)	18 (27.7)
	Weight Loss	15 (28.0)	14 (21.5)
	Nervous stress	21 (29.2)	20 (30.8)
	Constipation	14 (19.4)	13 (20.0)
	Abdominal pain	41 (56.9)	37 (56.7)
	Back pain	12 (16.7)	12 (18.5)
	Flatulence	22 (30.6)	22 (33.8)
	Oral ulcers	2 (2.8)	2 (3.1)
	Pruritus	5 (6.9)	5 (7.7)
	Headache	15 (20.8)	14 (21.5)
	Insomnia	15 (20.8)	19 (29.2)
Medical History	History of gastrointestinal disease	67 (97.3)	63 (96.9)
	Previous history of ulcerative colitis	66 (91.7)	64 (98.5)
	Hospitalization history	17 (23.6)	16 (24.6)
	History of cancer in relatives	11 (15.3)	9 (13.8)
	History of ulcerative colitis in relatives	11 (15.3)	10 (15.4)
Involvement Site	Left colitis	44 (61.1)	42 (64.6)
	Pancolitis	13 (18.1)	12 (18.5)
	Proctitis	1 (1.4)	1 (1.5)
	Proctocolitis	14 (19.4)	10 (15.4)

Table 3: Frequency of anti-CMV IgM and IgG antibodies in patients with/without recurrent ulcerative colitis

Variable		Total patients with ulcerative colitis		Patients with recurrent ulcerative colitis	
		IgM	IgG	IgM	IgG
Sexual distribution	Total	11.1%	88.9%	12.3%	89.2%
	Male	9.3%	88.4%	10.3%	89.7%
	Female	13.8%	89.7%	15.4%	88.5%
	P value	0.607	0.706	0.603	0.631
Involvement site	Left colitis	4.7%	90.7%	4.9%	90.2%
	Pancolitis	30.8%	100%	33.3%	100%
	Proctitis	0.0%	0.0%	0.0%	0.0%
	Proctocolitis	14.3%	85.7%	20.0%	90.0%
	P value	0.039	0.012	0.045	0.012
Medications	Aspirin	11.9%	89.6%	12.5%	90.6%
	NSAIDS	13.0%	95.7%	13.0%	95.7%

	Steroids	20.7%	86.2%	24.0%	88.0%
	Infliximab	0.0%	100%	0.0%	100%
	P value	0.079	0.123	0.076	0.097

Discussion

Given that the identification of risk factors for UC can be useful in the prevention and treatment of this disease, so the identification of infectious risk factors such as viral agents is very important. CMV has been identified among the viral infectious agents leading to recurrence of ulcerative colitis and its severe complications. However, there are a few studies about the frequency of cases of cytomegalovirus ulcerative colitis, especially in its recurrent type, so the relationship between the virus and the severity of gastrointestinal involvement in these patients has not been determined. In the present study, we evaluated the epidemioclinical features of ulcerative cytomegalovirus colitis in a selected Iranian population and also to evaluate the relationship between virus tracking and underlying characteristics such as site of involvement (severity of disease) and drugs used to treat the disease. In this evaluation, we used ELISA techniques (evaluation of anti-cytomegalovirus antibody levels) as well as PCR. The evaluation showed that the frequency of anti-CMV IgM and anti-CMV IgG in the patients with ulcerative colitis was 11.1% and 88.9%, and in patients with recurrent UC was 12.2% and 89.2%, respectively. First, a small number of the patients with ulcerative colitis have an acute and active form of cytomegalovirus, and in contrast, a significant proportion of patients have a history of exposure to the cytomegalovirus, in other words, exposure to the virus may be the initiator of pathophysiological onset in many patients with ulcerative colitis. Second, a small number of the patients with previous exposure to the virus had a positive PCR test showing the fact that a significant proportion of patients have had previous exposure to CMV (even as a pathophysiological agent of the disease). Also, the present study showed an acceptable agreement between ELISA and PCR techniques in tracking the acute stage of cytomegalovirus ulcerative colitis.

However, what is clear in comparing our findings with other studies is the significant difference in the rate of underlying infection with CMV in the setting of ulcerative colitis. In a study by Shin *et al* [20], the prevalence of CMV colitis was determined to be 14%, which was consistent with the present study. In a study by Henmi *et al* [21], 30.2% of the patients with colitis had positive PCR for CMV, but only 4 cases were reported for positive antigen testing, which was not consistent with our study. In a study by Weng *et al* [22], only 1.4% of the patients were diagnosed with CMV colitis. In a study by Levin *et al* [23], out of 28 cases of colitis, 46.4% were positive for CMV. In a study by Wada *et al* [24], the presence of CMV was confirmed in 34% of the patients. In a study by Lee *et al* [25], 33.6% of the patients were diagnosed with CMV colitis. Thus, a wide range of positive cases for CMV infection can be seen in the patients with ulcerative colitis, which may be due to various reasons such as stage and duration of the disease, extent of the disease, accuracy and calibration of diagnostic techniques as well as demographic and genetic characteristics. Another point to note is that the CMV tracking in the patients with ulcerative colitis was significantly related to the severity and extent of the disease. In other words, the highest rate of pancolic type was seen in the patients with ulcerative colitis, while in the isolated proctical type, no cases of CMV infection were detected indicating a relationship between the extent of UC and the frequency of CMV detection. However, there was no relationship between the disease treatment protocol and the rate of infection, so the use of drugs such as ASA, NSAIDs, or corticosteroids did not appear to affect the rate of CMV infection in ulcerative colitis.

Conclusion

The frequency of anti-CMV IgM and anti-CMV IgG cases in total patients with ulcerative colitis is 11.1% and 88.9% and in recurrent cases is 12.3% and 89.2% respectively. The rate of cytomegalovirus infection in patients with ulcerative colitis is directly related to the extent and severity of the disease but may not be affected by the treatment protocol.

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References

1. Powell RD, Warner NE, Levine RS, Kirsner JB. Cytomegalic inclusion disease and ulcerative colitis: report of a case in a young adult. Am J Med 1961; 30:334-340.
2. Kishore J, Ghoshal U, Ghoshal UC, *et al*. Infection with cytomegalovirus in patients with inflammatory bowel disease: prevalence, clinical significance and outcome. J Med Microbiol. 2004; 53(Pt 11):1155-1160.
3. Wada Y, Matsui T, Matake H, *et al*. Intractable ulcerative colitis caused by cytomegalovirus infection: a prospective study on prevalence, diagnosis, and treatment. Dis Colon Rectum 2003; 46(10):S59-S65.
4. Domenech E, Vega R, Ojanguren I, *et al*. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. Inflamm Bowel Dis 2008; 14:1373-1379.
5. Maconi G, Colombo E, Zerbi P, *et al*. Prevalence, detection rate and outcome of cytomegalovirus infection in ulcerative colitis patients requiring colonic resection. Dig Liver Dis 2005; 37:418-423.
6. Cottone M, Pietrosi G, Martorana G, *et al*. Prevalence of cytomegalovirus infection in severe refractory ulcerative and Crohn's colitis. Am J Gastroenterol 2001; 96:773-775.
7. Kambham N, Vij R, Cartwright CA, Longacre T. Cytomegalovirus infection in steroid-refractory ulcerative colitis: a case-control study. Am J Surg Pathol 2004; 28:365-373.
8. Matsumoto S, Yoshida Y. What are the factors that affect hospitalization and surgery for aggravation of ulcerative colitis? Eur J Gastroenterol Hepatol 2014; 26:282-287.
9. Park SH, Yang SK, Hong SM, *et al*. Severe disease activity and cytomegalovirus colitis are predictive of a nonresponse to infliximab in patients with ulcerative colitis. Dig Dis Sci 2013; 58:3592-3599.
10. Kim YS, Kim YH, Kim JS, *et al*. Long-term outcomes of cytomegalovirus reactivation in patients with

moderate to severe ulcerative colitis: a multicenter study. Gut Liver 2014; 8:643-647.
11. Matsuoka K, Iwao Y, Mori T, *et al*. Cytomegalovirus is frequently reactivated and disappears without antiviral agents in ulcerative colitis patients. Am J Gastroenterol 2007; 102:331-337.
12. Iida T, Ikeya K, Watanabe F, *et al*. Looking for endoscopic features of cytomegalovirus colitis: a study of 187 patients with active ulcerative colitis, positive and negative for cytomegalovirus. Inflamm Bowel Dis 2013; 19:1156-1163.
13. Suzuki H, Kato J, Kuriyama M, Hiraoka S, Kuwaki K, Yamamoto K. Specific endoscopic features of ulcerative colitis complicated by cytomegalovirus infection. World J Gastroenterol. 2010; 16:1245-1251.
14. Zidar N, Ferkolj I, Tepes K, *et al*. Diagnosing cytomegalovirus in patients with inflammatory bowel disease: by immunohistochemistry or polymerase chain reaction? Virchows Arch. 2015; 466:533-539.
15. McCurdy JD, Enders FT, Jones A, *et al*. Detection of cytomegalovirus in patients with inflammatory bowel disease: where to biopsy and how many biopsies? Inflamm Bowel Dis. 2015; 21:2833-2838.
16. De la Hoz RE, Stephens G, Sherlock C. Diagnosis and treatment approaches of CMV infections in adult patients. J Clin Virol 2002; 25(2):S1-S12.
17. Kornbluth A, Sachar DB. Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College Of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol 2010; 105:501-523.
18. Choi CH, Moon W, Kim YS, *et al*. Second Korean guideline for the management of ulcerative colitis. Korean J Gastroenterol 2017; 69:1-28.
19. Kishore J, Ghoshal U, Ghoshal UC, Krishnani N, Kumar S, Singh M, *et al*. Infection with cytomegalovirus in patients with inflammatory bowel disease: Prevalence, clinical significance and outcome. J Med Microbiol. 2004; 53:1155-60.
20. Shin Ju Oh 1, Chang Kyun Lee 1, Youn-Wha Kim 2, Su Jin Jeong 3, Yoo Min Park 1, Chi Hyuk Oh 1, *et al*. True cytomegalovirus colitis is a poor prognostic indicator in patients with ulcerative colitis flares: the 10-year experience of an academic referral inflammatory bowel disease center. Scand J Gastroenterol. 2019; 54(8):976-983. Doi: 10.1080/00365521.2019.1646798. Epub 2019 Jul 29.
21. Yujiro Henmi 1, Kazuki Kakimoto 1, Takuya Inoue 1, Kei Nakazawa 1, Minori Kubota 1, Azusa Hara 1, *et al*. Cytomegalovirus infection in ulcerative colitis assessed by quantitative polymerase chain reaction: risk factors and effects of immunosuppressants. J Clin Biochem Nutr. 2018; 63(3):246-251. Doi: 10.3164/jcbn.18-14. Epub 2018 Jun 8.
22. Meng-Tzu Weng, Chien-Chih Tung, Yi-Shuan Lee, Yew-Loong Leong, Ming-Jium Shieh, Chia-Tung Shun, *et al*. Cytomegalovirus colitis in hospitalized inflammatory bowel disease patients in Taiwan: a referral center study. BMC Gastroenterol. 2017; 17(1):28. doi: 10.1186/s12876-017-0586-9.
23. Avi Levin, Shaul Yaari, Ronen Stoff, Orit Caplan, Dana G Wolf, Eran Israeli. Diagnosis of Cytomegalovirus Infection during Exacerbation of Ulcerative Colitis. Digestion. 2017; 96(3):142-148. doi: 10.1159/000479865. Epub 2017 Aug 26.
24. Yoko Wada, Toshiyuki Matsui, Hiroaki Matake, Toshihiro Sakurai, Junya Yamamoto, Yosuke Kikuchi, *et al*. Intractable ulcerative colitis caused by cytomegalovirus infection: a prospective study on prevalence, diagnosis, and treatment. Dis Colon Rectum. 2003; 46(10):S59-65. doi: 10.1097/01.DCR.0000087486.21981.C6.
25. Ho-Su Lee, Sang Hyoung Park, Sung-Han Kim, Jihun Kim, Jene Choi, Hyo Jeong Lee, *et al*. Risk Factors and Clinical Outcomes Associated with Cytomegalovirus Colitis in Patients with Acute Severe Ulcerative Colitis. Inflamm Bowel Dis. 2016; 22(4):912-8. doi: 10.1097/MIB.0000000000000675.