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Steroid-refractory inflammatory bowel disease is a risk factor for CMV infection

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Abstract. – OBJECTIVE: Patients with inflammatory bowel disease (IBD) show increased the prevalence of cytomegalovirus (CMV) infection due to the severity of the disease and the immunosuppressive treatments they receive. The aim of this study was to determine the prevalence of CMV infection in IBD patients and identify the risk factors for CMV infection with different demographic characteristics in IBD patients.

PATIENTS AND METHODS: We enrolled 85 patients diagnosed with IBD (43 with ulcerative colitis (UC) and 42 with Crohn's disease (CD)) in this prospective study. The clinical disease activities of UC and CD were assessed using Truelove-Witts and Crohn's disease activity index (CDAI). CMV infection was assessed by detection of DNA using real-time polymerase chain reaction (PCR) in blood samples and quantitative PCR in colonic biopsy specimens and by detection of inclusion bodies using hematoxylin-eosin staining.

RESULTS: Thirteen patients with IBD exhibited concomitant CMV infection. CMV infection was not detected in any of the patients in remission. Viral loads measured in the colonic mucosa of infected patients ranged from 800–7000 genome copies/mL total extracted DNA. The mean serum CMV DNA level was 1694 ± 910 copies/mL (range: 800-3800). The rate of steroid resistance in CMV-positive cases was significantly higher than that in CMV-negative cases ($p = 0.001$). CD with acute exacerbation was a risk factor for CMV disease ($p = 0.04$). All of the CMV-positive patients received immunosuppressive treatments.

CONCLUSIONS: CMV infection should be suspected in steroid-resistant UC and CD. Antiviral treatment improved the clinical outcome in steroid-resistant IBD cases with serum CMV DNA levels above 1000 copies/mL.

Key Words:
Cytomegalovirus, Inflammatory bowel disease, Ulcerative colitis, Crohn's disease.

Introduction

Inflammatory bowel disease (IBD) is a group of chronic diseases with no specific etiology that includes ulcerative colitis (UC) and Crohn's disease (CD) and is characterized by alternating phases of exacerbation and remission. Use of non-steroid anti-inflammatory drugs (NSAIDs) and antibiotics can exacerbate IBD, as can viral or bacterial infections[1]. Cytomegalovirus (CMV) infection is one prominent cause of exacerbation. CMV belongs to the herpesviridae family. It enters a latent period after initial symptomatic infection and remains throughout the lifetime of the host[2]. Patients with IBD exhibit increased the occurrence of infection due to the severity of the disease and immunosuppressive treatments they receive[3,4]. Whether the CMV infection seen in immunosuppressed IBD patients is caused by activation of CMV infection remains controversial.

Previous studies recommended that CMV infection be suspected in IBD patients with resistance to steroid treatment; however, international guidelines state that CMV should be excluded in patients with acute steroid-resistant colitis[5-7].

CMV infection is diagnosed by histological and serological examinations, CMV antibody tests, and the presence of CMV DNA in serum and tissue[8]. However, there is no gold standard diagnostic test for CMV[9]. Thus, the relative effi-

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cacy of tests for detecting CMV infection in the colon and signifying the need for treatment remains unknown. Current European Crohn's and Colitis Organisation (ECCO) guidelines[7] recommend the combination of histology and hematoxylin-eosin (H&E) staining with intranuclear inclusion body detection for CMV infection diagnosis in patients with a UC flare-up. Polymerase chain reaction (PCR) has emerged as the most sensitive method for viral infection diagnosis, including for CMV[10]. The clinical significance of the detection of the CMV genome in bowel tissue or titration of the CMV genome within tissue is debated. Some studies defined a cut-off value for both tissue and serum CMV DNA loads to diagnose CMV infection for treatment initiation in patients with acute IBD[11-13]. Roblin et al[11] reported that a level of CMV DNA above 250 copies/mL in tissues was a predictor for steroid resistance. In contrast, Leveque et al[14] found no significant relationship between CMV viral load and disease severity in patients with active IBD. Management of the disease following diagnosis and the effect of antiviral treatment on outcomes remain of broad and current interest. Clinical improvement may be achieved in some patients only with antiviral therapy and steroid reduction[15].

The aim of this study was to determine the prevalence of CMV infection in patients with IBD and identify the risk factors for CMV infection with different demographic characteristics in IBD patients.

Patients and Methods

Patients

The present study was conducted by the Istanbul University, Istanbul Medical Faculty and was approved by the local Ethics Committee. Informed consent was obtained from each included patient. Eighty-five patients with IBD (43 UC and 42 CD) who were admitted to the Gastroenterology Department at Istanbul University between 2011-2012 were enrolled in this prospective study. Diagnosis of IBD was based on clinical, endoscopic, radiologic, and histologic parameters. After recording the clinical and demographic properties, clinical disease activity of UC and CD was assessed using Truelove-Witts[16] and Crohn's disease activity index (CDAI)[17]. The age, sex, clinical activity, and laboratory parameters of all patients were determined. As a routine procedure, stool microscopy, stool culture, and *Clostridium difficile* toxin A-B detection tests were performed for IBD patients with acute activation. Remission diagnosis was made based on clinical, laboratory, and endoscopic results. Current treatments and previously received immunosuppressant treatments were also assessed. Current treatment and drugs as follows: mesalamine (Salofalk, Ali Raif Medical, Istanbul, Turkey), methylprednisolone (prednol, Mustafa Nevzat Medical, Istanbul Turkey), azathioprine (Imuran, GlaxoSmithKline, Boronia Victoria, Australia), Anti tumor necrosis factor therapies (infliximab [Remicade, Janssen Biotech, Inc., Titusville, NJ, USA], adalimumab [Humira, Abbott Biotechnology Deutschland GmbH, Wiesbaden, Germany]), cyclosporine (Sandimmune, Novartis, East Hanover, NJ, USA).

The exclusion criteria were pregnancy, detected immunodeficiency (Human immunodeficiency virus infection, hypogammaglobinemia), severe malnutrition, proctitis, and positive *Clostridium difficile* infection in stool culture. Corticosteroid resistance was defined as lack of improvement even with prednisone administration at a daily dose ≥30 mg for at least 2 weeks. Patients with active IBD were taken to the inpatient ward of the Gastroenterology Department, and patients undergoing remission were treated on an outpatient basis. Colonoscopy was performed in all patients to identify the affected bowel area and to outline the disease activity. Involvement up to the proximal hepatic flexure was defined as pancolitis. Involvement up to the splenic flexure was defined as left colonic involvement.

Methods for Detecting CMV Infection in Serum-Biopsy Samples

Quantitative assessment of CMV DNA was performed in plasma and biopsy specimens using COBAS Ampliprep/COBAS TaqMan CMV Test (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. This system consisted of two integrated platforms: COBAS Ampliprep (an automated nucleic acid extraction platform) and COBAS Taqman (a real-time PCR platform utilizing TaqMan probe technology). Plasma samples were used directly. DNA was extracted from biopsy samples by combining 500 μL MagNA Pure 96 DNA Tissue Lysis Buffer (Roche Diagnostics GmbH, Mannheim, Germany), 100 μL Proteinase K

(Roche Diagnostics GmbH, Mannheim, Germany), and 20 mg biopsy samples in a sterile microcentrifuge tube and incubating the mixture at 56 °C for 2 h. CMV DNA was quantitatively measured from 500 μL plasma or biopsy lysate (Roche Diagnostics GmbH, Mannheim, Germany) by amplifying a fragment of the CMV UL54 region. The dynamic range of quantitation of COBAS Ampliprep/COBAS TaqMan CMV Test was 150-10,000,000 copies/mL in plasma.

A large number of biopsies from the ulcerative area were taken to assess the inflammatory activity of the disease. H&E-stained sections of formalin-fixed, paraffin-embedded colonoscopic tissues were evaluated for CMV inclusions in endothelial and stromal cells. In suspicious cases for CMV infection, immunohistochemistry was performed.

Diagnosis and Treatment Protocol

The diagnosis criteria for CMV infection were positive results in one or more of these tests (positive CMV DNA in plasma or tissue and/or inclusion body in H&E-stained sections). We defined the cut-off value for serum CMV DNA as 1000 copies/mL for our treatment protocol.

The treatment protocol for CMV infection cases was initiation of antiviral treatment (ganciclovir [Cymevene, Roche Products Limited, Welwyn Garden City, UK]) for two weeks followed by 4 weeks of oral valganciclovir administration (Valcyte, Roche Products Limited, Basel, Switzerland), stepwise termination of steroid use, discontinuation of anti-TNF if used, and no interruption in other treatments for patients with serum CMV DNA levels above 1000 copies/mL.

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, UT, USA) was used for statistical analysis. During the assessment of study data, descriptive statistical parameters were determined (mean, standard deviation, median, frequency, rate, min-max), and the Mann-Whitney U-test was used to compare quantitative variables that did not show a normal distribution. Qualitative data were compared using Fisher-Freeman-Halton test, Fisher's Exact test, and Chi-squared test. Spearman's correlation analysis was performed to assess the interactions between variables. Significance levels were assessed for $p < 0.01$ and $p < 0.05$.

Results

Eighty-five patients diagnosed with IBD (42 with CD and 43 with UC) were enrolled in this prospective study. At the time of assessment, the mean age was 38.4 ± 12.1 (range: 19-69) years. Seventy patients (82.4%) applied to our department for exacerbation, and 15 patients (17.6%) were in remission and came for follow-up examinations. The mean disease duration was 55.9 ± 24.5 (range: 12-144) months. Thirteen patients (15.4%) were resistant to steroids. The demographic and treatment data for the patients are given in Table I.

CMV Infection in Patients with IBD

Thirteen (15.4%) of the 85 IBD patients had CMV infection (5/42 with CD and 8/43 with UC). CMV infection was not detected in any of the patients in remission. The CMV infection incidence was not significantly different between the two types of IBD. CMV DNA was detected in six patient tissue samples (mean: 2766.6 ± 2151.8 copies/mL; range: 800-7000 copies/mL) and in 12 patient serum samples (mean: 1694 ± 910 copies/mL; range: 800-3800 copies/mL). Only one patient exhibited a positive CMV inclusion body. The demographic and clinical profiles of IBD patients with CMV infection are provided in Table II.

Risk Factors Associated with CMV Infection

There was no significant difference in terms of age, disease duration period, or sex between CMV-positive and CMV-negative patients ($p > 0.05$). In patients with CD, there was a significant relationship between CMV infection and disease activity ($p = 0.048$). There was no significant difference in the frequency of CMV infection with respect to the involvement sites of IBD ($p > 0.05$) although all of the UC patients with CMV infection had pancolonic involvement.

Of the 13 CMV-positive cases, six were receiving immunomodulatory. mesalamine and corticosteroid treatments; three were receiving corticosteroid and mesalamine treatments; three were receiving mesalamine and immunomodulatory treatments; one was receiving anti-TNF treatment. The rate of receiving corticosteroids with mesalamine treatment was significantly higher than for CMV-negative patients ($p = 0.024$). There was a significant relationship between CMV infection and immunosuppressive treatment in patients with UC ($p < 0.05$). All CD patients with CMV infections were receiving im-

Table I. Demographic and clinical profiles of patients with inflammatory bowel disease (IBD).

	Crohn's disease (n = 42)	Ulcerative colitis (n = 43)	IBD (n = 85)
Male/female	11/31	23/20	34/51
Mean age (years) ± SD	34.9 ± 11.1	41.8 ± 12	38.46 ± 12.15
Duration of disease (month)	54.9 ± 19.2	56.8 ± 29	55.9 ± 24.5
Disease extent			
Distal colitis		1 (2.3%)	
Left colonic involvement		1 (2.3%)	
Pancolitis		41 (95.3%)	
Colonic	4 (9.5%)		
Ileocolonic	38 (90.5%)		
Disease severity			
Remission	4 (9.5%)	11 (25.2%)	
Mild	12 (28.6%)	3 (7%)	
Moderate	23 (54.8%)	19 (44.2%)	
Severe	3 (7.1%)	10 (23.3%)	
Treatment			
MES		14 (32.6%)	
MES-CS		5 (11.6%)	
MES-CS-immunomodulatory	15 (35.6%)	11 (25.6%)	
MES-immunomodulatory	23 (54.8%)	10 (23.3%)	
Anti-TNF	2 (4.8%)	2 (4.6%)	
Anti-TNF-immunomodulatory	2 (4.8%)		
Cyclosporine		1 (2.3%)	

CS, corticosteroids; MES, mesalamine; Anti-TNF, anti-tumor necrosis factor.

munosuppressive treatment, so they were not included in this assessment.

Eight of the 13 CMV-positive cases showed steroid resistance. The rate of steroid resistance in CMV-positive cases was significantly higher than that in CMV-negative cases. CD patients with CMV showed increased steroid resistance (Odds ratio: 17, 95% CI 1.9-145) compared with

Table II. Demographic and clinical profiles of cytomegalovirus (CMV)-positive patients with inflammatory bowel disease.

		CMV		
		(-) n (%)	(+) n (%)	*p*-value
Disease	Crohn's disease	37 (51.4)	5 (38.5)	[a]0.578
	Ulcerative colitis	35 (48.6)	8 (61.5)	
Duration of the disease (months)	Mean ± SD	55.71 ± 24.95	57.15 ± 23.01	[b]0.583
	Min-max (median)	12-144 (48.0)	24-96 (60.0)	
Gender	Female	45 (62.5)	6 (46.2)	[c]0.424
	Male	27 (37.5)	7 (53.8)	
Age	Mean ± SD	37.81 ± 12.07	42.08 ± 12.45	[c]0.231
	Min-max (median)	19-69 (36.0)	19-69 (45.0)	
Clinical index Truelove-Witts	Remission	11 (31.5)	0 (0.0)	[d]0.132
	Mild	3 (8.5)	0	
	Moderate	14 (46)	5 (62.5)	
	Severe	7 (20)	3 (37.5)	
Clinical index CDAI activity	Remission	4 (10.8)	0 (0.0)	[d]0.048*
	Mild	12 (32.4)	0 (0.0)	
	Moderate	20 (54.1)	3 (60.0)	
	Severe	1 (2.7)	2 (40.0)	

[a]Yates Continuity Correction Test, [b]Mann-Whitney U-Test, [c]Fisher's Exact Test, [d]Fisher-Freeman-Halton Test *$p < 0.05$, CDAI: Crohn's Disease Activity Index.

those without CMV, as did UC patients with CMV (Odds ratio: 27.5, 95% CI 3.6-207). Risk factors for CMV infection with IBD are listed in Table III.

Relationship Between Viral Load in Colonic Biopsies and Serum and Clinical Management of Exacerbation

The 11 patients with serum CMV DNA ≥1000 copies/mL received 5 mg/kg ganciclovir treatment. Two patients with serum CMV DNA below 1000 copies/mL received high-dose immunosuppressive and immunomodulatory treatment, and remission was subsequently achieved. Steroid and anti-TNF treatments were all stopped in CMV-positive patients before ganciclovir treatment. We made an exception for one patient with a high tissue CMV DNA titer and low serum CMV DNA level (800 copies/mL) and provided treated with ganciclovir for ethical reasons; remission was achieved in this patient. Since this was a clinically susceptible situation, we consecutively checked serum CMV DNA levels and initiated ganciclovir treatment after detecting titers above 1000 copies/mL.

Out of 13 patients, 11 patients were received intravenous ganciclovir for two weeks followed by 4 weeks of oral valganciclovir administration. Tissue and serum CMV DNA levels were monitored, and treatment was terminated after determining two negative CMV DNA (serum) results. A summary of the 13 IBD patients with CMV infection is given in Table IV.

Outcome of Ganciclovir Treatment in Patients with CMV Infection

After the treatment ended, patients were evaluated for disease activity at week 2. Only one patient received colectomy (1/13) because remission could not be obtained despite ganciclovir treatment. All other patients went into a remission period following ganciclovir treatment.

Table III. Risk factors for cytomegalovirus (CMV) infection with inflammatory bowel disease.

		CMV infection (+/-)					
		CD (5/37)			UC (8/35)		
		n	p	OR (95% CI)	n	p	OR (95% CI)
Age	≤ 50	4/33	[a]0.48	0.485 (0.043-5.478)	8/25	[a]0.165	0.758 (0.625-0.919)
	> 50	1/4			0/10		
Gender	Female	4/27	[a]1.000	1.481 (0.147-14.900)	2/18	[a]0.250	0.315 (0.056-1.780)
	Male	1/10			6/17		
Disease extent	Pancolitis				8/33	[a]1.000	0.800 (0.685-0.934)
	Left side				0/1	[a]1.000	1.235 (1.067-1.430)
	Distal				0/1	[a]1.000	1.235 (1.067-1.430)
	Ileocolonic	4/34	[a]0.637	0.635 (0.093-4.342)			
	Colonic	1/3	[a]0.410	2.833 (0.235-34.141)			
Steroid resistance	(+)	3/3	[a]0.015*	17.000 (1.993-145.000)	5/2	[a]0.001**	27.500 (3.643-207.587)
	(-)	2/34			3/33		
Immunosuppressive treatment	(+)	5/37	[a]1.000	0.878 (0.783-0.984)	8/21	[a]0.039*	0.724 (0.578-0.907)
	(-)	0/0			0/14		
Treatment	MES				0/14	[a]0.099	1.381 (1.103-1.729)
	MES-CS				3/2	[a]0.037*	9.900 (1.311-74.731)
	MES-CS immunomodulatory	2/13	[a]1.000	1.231 (0.182-8.330)	4/7	[a]0.172	4.000 (0.796-20.102)
	MES-immunomodulatory	3/20	[a]1.000	1.275 (0.190-8.545)	0/10	[a]0.090	1.333 (1.092-1.629)
	Anti-TNF (infliximab)				1/1	[a]1.000	1.235 (1.067-1.430)
	Anti-TNF (infliximab)-immunomodulatory	0/2	[a]1.000	1.139 (1.016-1.277)			
	Cyclosporine				0/1	[a]0.186	6.000 (3.051-11.799)
	Anti-TNF (adalimumab)	0/2	[a]1.000	1.139 (1.016-1.277)			

[a]Fisher's Exact Test *$p < 0.05$, **$p < 0.01$. Anti-TNF: anti-tumor necrosis factor; CD, Crohn's disease; CS, corticosteroids; MES, mesalamine; UC, ulcerative colitis.

Table IV. Summary of the 13 cytomegalovirus (CMV)-positive patients with inflammatory bowel disease.

	Disease	Severity	CMV DNA (copies/mL)	Tissue CMV (copies/mL)	Inclusion body	Ganciclovir	Treatment	Exacerbation outcome
1	UC	Moderate	1100	-		+	MES-AZA- CS	Remission
2	UC	Moderate	1100	-		+	MES-CS	Remission
3	UC	Severe	1000	7000		+	MES-AZA-CS	Remission
4	UC	Moderate	800	2200		+	MES-CS	Remission
5	CD	Severe	1560	2400	+	+	MES-AZA-CS	Remission
6	UC	Moderate	1200	-		+	MES-AZA- CS	Remission
7	CD	Moderate	540	-		-	MES-AZA- CS	Remission
8	UC	Severe	2400	-		+	MES-AZA- CS	Remission
9	UC	Severe	2300	2000		+	Infliximab	Colectomy
10	CD	Severe	3800	2200		+	MES-AZA	Remission
11	UC	Moderate	1080	-		+	MES-CS	Remission
12	CD	Moderate	2300	-		+	MES-AZA	Remission
13	CD	Moderate	-	800		-	MES-AZA	Remission

AZA, azathioprine; CD, Crohn's disease; CS, corticosteroids; MES, mesalamine; UC, ulcerative colitis.

Discussion

The relationship between CMV and IBD was first defined in 1961[18]. Since then, the effect of CMV infection on IBD activation has been of broad and current interest amongst clinicians. CMV infections can be seen in patients receiving immunosuppressive treatments as a reactivation of latent CMV infections[19]. However, it is also possible that CMV is simply an "innocent bystander," detected in the background of the colonic mucosa during an active period of IBD. For this reason, routine tests for detecting CMV infection are not recommended in the remission state. However, in cases of steroid-resistant colitis or acute exacerbations managed by steroid treatment, PCR or immunohistochemical methods should be used to assess CMV infections.

In the literature, most CMV studies have been performed on patients with UC. In the IBD spectrum, there is no definitive information on the frequency of this situation. Therefore, we included both UC and CD patients with severe, mild, moderate, and remission forms in our study. We observed a significant relationship between CMV infection and patients with active CD. In our remission group, we did not detect CMV infection in serum/tissue samples or immunohistochemical analysis.

Unfortunately, there is significant heterogeneity in the definition of CMV in the literature. In this study, CMV infection was defined as positive detection of CMV DNA in tissues or serum or the presence of CMV inclusion bodies in immunohistochemical analysis. Thirteen (15.4%) of 85 IBD patients were thus identified as CMV-positive in our study group.

In patients with IBD, steroid resistance has an incidence of 16-30% in patients with UC and 16-20% in patients with CD[20-23]. The mechanism that causes steroid resistance is still unknown. Steroid resistance during IBD was thought to be secondary to CMV infection in many previous studies. The latest meta-analysis also reported significantly higher steroid resistance in CMV-positive cases than in CMV-negative cases[24]. In our IBD patients, the steroid resistance rate was 15.4%. Consistent with previous results, the steroid resistance in patients with UC and CD was significantly higher in CMV-positive cases than in others ($p < 0.01$).

In the literature, there are no pre-defined cut-off values for tissue or serum CMV DNA levels for CMV infection diagnosis. Therefore, we defined the cut-off value for serum CMV DNA as 1000 copies/mL for our treatment protocol. Two patients below 1000 copies/mL did not receive ganciclovir treatment. These two patients continued receiving immunosuppressive and immunomodulatory treatment and showed clinical improvement without additional ganciclovir treatment. We initiated ganciclovir treatment in patients above 1000 copies/mL serum CMV DNA. Only one patient with < 1000 copies/mL serum CMV DNA received ganciclovir treatment because he had a high tissue CMV DNA level. We initiated treatment in this case since consecutive serum CMV DNA levels were also above the threshold.

The diagnostic value of inclusion bodies is very high, but they are quite difficult to detect. CMV inclusion bodies are most frequently found around mucosal vessels or at the base of ulcers in large numbers; therefore, looking for CMV inclusion bodies in superficial biopsies is not recommended[25]. In CMV-positive patients, CMV inclusion bodies were detected only in one patient since acquiring a biopsy from the ulcerative location is necessary but challenging even for an endoscopy specialist in daily routine practice. Therefore, we used serum and tissue CMV positivity for assessment of patients during daily routine practice.

Many studies reported an increased risk of CMV infection with immunosuppressive treatments[8,10,26]. We detected an increased CMV infection risk in patients receiving immunosuppressant treatments. A prospective study by Pillet et al[27] investigated the relationship between CMV and anti-TNF treatment and reported that anti-TNF treatments did not increase the risk of colonic CMV infection in patients with moderate-to-severe UC. In our study, only one patient receiving anti-TNF treatment developed CMV infection. Therefore, we are unable to comment on this subject based on our study results.

Conclusions

This prospective, single-center study demonstrated that CMV infection should be suspected in steroid-resistant UC and CD. Ganciclovir treatment improved the clinical outcome in steroid-resistant IBD cases with serum CMV DNA levels above 1000 copies/mL. Thus, treatment with ganciclovir without interrupting immunomodulatory treatment in patients with serum CMV DNA levels over 1000 copies/mL appears to be an appropriate approach.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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