Original Article

Cytomegalovirus Reactivation in Ulcerative Colitis Patients: Early Indicators

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Abstract

Background: The association of cytomegalovirus (CMV) infection with ulcerative colitis (UC) still remains a controversial topic for the clinicians. **Aim:** In this study, we aimed to elucidate the CMV infection related parameters in the exacerbation of UC. **Material and Methods:** In this study, 812 UC patients who have admitted to our institution between June 2008 and November 2020 were analyzed retrospectively. CMV infection was diagnosed by the detection of CMV DNA with polymerase chain reaction (PCR) in tissue biopsies with presence of clinical colitis symptoms. CMV negative UC patient group was defined as UC activation group with negative PCR results. **Result:** A total of 153 patients met the inclusion criteria during the study period, with a median age of 41.8 years. CMV PCR positivity had been detected in tissue biopsy in 43 (28.1%) UC patients. CMV-positive patients had a statistically significant higher frequency of steroid resistance, treatment with azathioprine, longer disease duration, longer remission, and hospitalization day. The mean C-reactive protein (CRP) level, platelet to lymphocyte ratio (PLR) were higher, and mean albumin level was lower in CMV positive patients, with statistically significance. Also, colectomy and anti-tumor necrosis factor-α (TNF-α) therapy were more frequent in CMV-reactivated group in long-term follow-up. In a multivariable model, steroid resistance, treatment with azathioprine, long disease duration, low albumin value was independently associated with colonic CMV infection. **Conclusion:** Steroid resistance, treatment with azathioprine, long disease duration, low albumin levels were significant risk factors for CMV colitis, among patients with UC activation.

Keywords: *Cytomegalovirus, inflammatory bowel disease, ulcerative colitis*

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) with symptoms of bloody diarrhea, fever, and abdominal pain. The incidence has been increasing especially in the industrialized countries during last decades.[1,2] Although its etiology is not clearly known, UC can be defined as a multifactorial, complex disease where genetic factors, environmental factors, mucosal barrier dysfunction, intestinal microbiota, and infectious agents play a major role.[3] Tumor necrosis factor-α (TNF-α) and calcineurin inhibitors, immunomodulators, amino-salicylates, and corticosteroids, have been widely used for the treatment of UC.[4]

Cytomegalovirus (CMV) is a member of β-herpes virus family that infects majority of humans. After primary infection, CMV persists as a latent virus in lymphocytes, endothelial vascular tissue, renal epithelial cells, and smooth muscle. Immuno-compromised cases such as individuals receiving immune-suppressive therapy, patients with T-cell deficiency, solid organ transplantation, and malnourished subjects may have reactivation of CMV.[5] Similar risk factors are often

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present in patients with UC, hence, CMV is often more detectable in their intestinal tissue samples.[6]

There are several ways to detect CMV infection such as serological examination, blood viral load (determined by antigenemia), or quantitative real-time polymerase chain reaction (RT – PCR), hematoxylin and eosin (H and E) staining, specific immune-histochemistry (IHC), and tissue PCR.[7] In many cases, tissue biopsy is recommended as diagnostic blood tests are unreliable.[8] Activated CMV inclusion bodies could be found in biopsy specimens from colon with inflamed and ulcerated mucosa via H and E staining. Sensitivity of H and E staining ranges from 10% to 87%, resulting in high rate of false negative biopsy.[9,10] IHC methods using monoclonal antibodies for CMV early antigen accumulation is highly sensitive (93%), and specific (ranges between 92% and 100%).[11] Tissue PCR can also be utilized for CMV infection in immune-modulatory refractory cases of IBD, with a sensitivity of 65% to 100% and a specificity of 40% to 100%.[11,12] A recent systematic review demonstrated the poor sensitivity of blood tests, and histology compared with IHC and tissue PCR for colonic CMV reactivation.[13] International guidelines recommend IHC or tissue PCR for the detection of CMV infection in IBD patients.[11,12]

In this study, the relationship between CMV infection and UC was investigated via examining the clinical features and long-term disease course among CMV positive and CMV negative patients with activated UC.

Materials and Method

Study design

In this study, 812 UC patients who were admitted to our institution between June 2008 and November 2020 were analyzed retrospectively. The Ethics Committee approval was granted on 11/02/2022 and protocol number: 55 – 2022.

Patient selection

All patients were diagnosed with UC via standard clinical and endoscopic protocols. Numerous biopsies were obtained from severe inflammatory areas including the base of ulcers. Severity of UC was described by Truelove and Witts' Score, and by Mayo Endoscopic Score.[14] The manifestation of UC was determined according to the Montreal Classification System.[15]

CMV infection was diagnosed by the detection of CMV DNA with PCR (GenXpert Ultra, Cepheid) in tissue biopsies with presence of clinical colitis symptoms.[11,12] CMV negative ulcerative colitis patient group was defined as UC activation group with negative PCR results.

Patients with active UC >18 years of age, with available long-term follow-up information, and no recurrent hospital admissions were included in the study. Patients <18 years of age with less than one-year follow-up or insufficient long-term follow-up information and recurrent hospital admission were excluded from the study. The active disease (UC) group consisted of 110 of CMV negative and 43 CMV positive patients [Figure 1].

The demographic characteristics, comorbid diseases, Mayo Score, Montreal Score, treatment before disease activation, duration of disease, laboratory parameters such as white blood cell count, lymphocyte count, hemoglobin count, platelet count, C-reactive protein (CRP) value, albumin value, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and long-term follow-up outcomes were recorded.

Statistical analysis

Patient data were analyzed with the SPSS 25.0 (Statistical Package for Social Sciences) program. Descriptive statistical methods (median, IQR, frequency, percentage) were used while evaluating the study data. The conformity of the quantitative data to the normal distribution was tested with the Kolmogorov–Smirnov test. For non-normally distributed quantitative data, two-group comparisons were made with Mann–Whitney U test. Chi-square test was utilized in the evaluation of qualitative data. Regression analysis was performed to analyze the effect of independent variables on the dependent variable. The statistical significance level (*P* value) was accepted as <0.05.

Results

Among the 153 patients included in this study, 101 (66%) were male, and 52 (34%) were female. The

Table 1: Baseline Demographics of the patients

Parameters	*N* (mean) (%) (STD)
Age (year)	41.8±15.28
Female	52 (34.0%)
Male	101 (66.0%)
Comorbid Diseases	
Hypertension	31 (20.3%)
Diabetes Mellitus	16 (10.5%)
Rheumatic Disease	16 (10.5%)
Coronary Artery Disease	6 (3.9%)
Renal Disease	6 (3.9%)
Malignancy	4 (2.6%)
Other Diseases	17 (11.1%)
CMV colitis patients	43 (28.1%)
Total Follow-up Time (month)	57.5±31.25
Alcohol consumption	40 (26.1%)
Tobacco use	20 (13.1%)

CMV: Cytomegalovirus

Table 2: Analysis of predictive factors on CMV colitis and long-term results

Parameters	CMV Colitis (*n*: 43) *n* (%) - Median (IQR)	Without CMV Colitis (*n*: 110) *n* (%) - Median (IQR)	*P*
Age	43.0 (24.0)	37.5 (23.0)	0.080
Female	17 (39.5%)	35 (31.8%)	0.013
Alcohol consumption	8 (18.6%)	12 (10.9%)	0.204
Tobacco use	11 (25.6%)	29 (26.4%)	0.921
Before Activation			
Steroid Resistance	12 (27.9%)	13 (11.8%)	0.016
Azathioprine	22 (51.2%)	16 (14.5%)	0.000
Anti-TNF-α Therapy	4 (9.3%)	3 (2.7%)	0.080
Disease Duration (month)	36.0 – 48.0	0-31.0	0.000
Hospitalization			
Montreal Score			
Rectal infection	0 (0.0)	0 (0.0)	0.470
Sigmoiditis	0 (0.0)	0 (0.0)	
Colitis	17 (39.5)	52 (47.3)	
Pan-colitis	26 (60.5)	58 (52.7)	
MAYO			
2	4 (9.3)	23 (20.9)	0.103
3	39 (90.7)	87 (79.1)	
Bloody Stool	10,0 (8.0)	12.0 (5.0)	0.194
Fever	8 (18.6)	13 (11.8)	0.273
Pulse	78,0 (12.0)	78.0 (12.0)	0.966
Laboratory			
Leukocyte (u/L)	8.100 (4.440)	8.695 (3.660)	0.390
Hemoglobin (g/dL)	11,0 (2.7)	10.7 (2.4)	0.932
Platelet (u/L)	266,0 (116.0)	268.5 (114.0)	0.792
NLR	4,0 (3.5)	2.9 (3.0)	0.777
PLR	0,2 (0.2)	0.1 (0.1)	0.007
CRP (mg/L)	3,3 (9.7)	4.8 (2.9)	0.026
Albumin (g/dL)	3,3 (0.8)	3.6 (0.6)	0.000
CRP/Albumin ratio	1,1 (3.0)	1.3 (1.0)	0.092
Remission time (day)	8,0 (6.0)	6.0 (3.0)	0.001
Hospitalization (day)	15,0 (16.0)	8.0 (3.0)	0.000
Long-term Follow-up			
Follow-up Time (month)	44,0 (28.0)	55.5 (55.0)	0.006
Anti-TNF Therapy	15 (34.9)	19 (17.3)	0.019
Colectomy	6 (14.0)	3 (2.7)	0.008

Anti-TNF-α=Anti-tumor necrosis factor-α, CRP=C-reactive protein, NLR: neutrophil to lymphocyte ratio, PLR=platelet to lymphocyte ratio

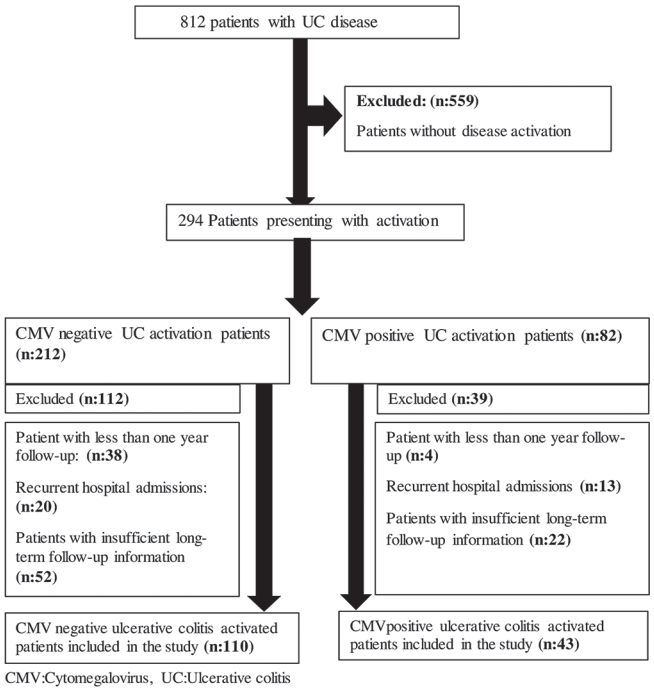
Table 3: Multivariate logistic regression analysis

	P	ODDS	95% CI	
			Lower	Upper
Gender	0.329	0.447	0.637	3.44
Steroid Resistance	0.026	1.213	1.154	9.805
Azathioprine	0.005	1.469	1.573	11.998
Disease Duration (days)	0.032	0.015	1.001	1.030
CRP	0.279	-0.060	0.846	1.050
Albumin	0.000	-10764	0.065	0.454
PLR	0.558	0.594	0.248	13.202

-2 Log likelihood: 131,306. CRP=C-reactive protein, PLR=platelet to lymphocyte ratio

mean age was 41.8 ± 15.28 years old. The demographic characteristics of the patients were given in Table 1.

Steroid resistance (27.9% versus 11.8%, $P < 0.016$), treatment with azathioprine (51.2% versus 14,5%, $P < 0.001$), longer disease duration (36 – 48 months versus 0 – 31 months, $P < 0.001$) longer remission period (6 days versus 3 days, $P < 0.000$), and duration of hospital stay (16 days versus 3 days, $P < 0.001$) were significantly more common in CMV—positive patients. The mean C-reactive protein (CRP) level, platelet to lymphocyte ratio (PLR) were higher, and mean albumin level was lower in CMV positive patients, with statistical significance. Colectomy and anti-tumor necrosis factor-α (TNF-α) therapy were more frequent in CMV-reactivated group in the long-term follow-up. Clinical features of CMV positive and CMV negative



CMV:Cytomegalovirus, UC:Ulcerative colitis

Figure 1: Inclusion and exclusion criteria

individuals with activated UC were compared in Table 2.

A logistic regression model identified 4 factors associated with CMV colitis: steroid resistance (odds ratio [OR], 1.21; 95% confidence interval [CI], 1.15 to 9.8), treatment with azathioprine (OR, 1.46; 95% CI, 1.57 to 11.9), disease duration (OR, 0.01; 95% CI, 1 to 1.03), and low albumin value (OR, 1.7; 95% CI, 0.06 to 0.45) [Table 3].

Discussion

The association of CMV and UC has been recognized for the past 60 years, especially in patients with steroid resistance. Recent studies demonstrated that females, and individuals older than 30 years of age were more frequently associated with CMV colonic disease[16,17] and additionally UC patients with CMV reactivation have worse outcomes.

A systematic meta-analysis stated that UC onset at older age was a risk factor for CMV reactivation in UC patients.[18] However, in our study we did not find any correlation with age. Female gender was higher in the CMV positive group ($P < 0.013$), but statistical significance was not achieved in multivariate analysis.

Roblin *et al*. found that CMV DNA positivity in intestinal tissue was associated with steroid resistance.[7] A meta-analysis suggested that CMV infection was an independent risk factor for steroid resistance in IBD patients.[19] In our study, we have shown that one of the factors for predicting CMV positivity was steroid resistance (OR, 1.21; 95% CI, 1.15 to 9.8), similar with the literature. This situation may be related to immune system dysfunction due to steroids.

Albumin is a nutritional and inflammatory marker that indicates the potential for infection or disease activity. In previous literature, it was elaborated that CMV was associated with severe UC activation.[10,20] Hypo-albuminemia can be observed during severe inflammatory processes, and malnutrition in activated UC patients with CMV. Levin *et al*. found that mean serum albumin levels were significantly lower in the CMV positive group.[8] Also, a recent study reported that lower serum albumin levels were associated with CMV infection in UC patients.[21] Our findings supported this data, and additionally, the impact of albumin replacement treatment should be evaluated in CMV colitis.

Azathioprine is an immune-modulatory drug which can be utilized for the maintenance treatment of UC. Mc Curdy *et al*. found that immuno-modulators have been independently associated with CMV disease in IBD patients probably due to leukopenia.[17] A meta-analysis showed that immune-suppressive therapy increased the occurrence of CMV reactivation, and the results of the subgroup analysis demonstrated that treatment with azathioprine was a risk factor for CMV reactivation (OR, 1.444; 95% CI, 1.012 to 2.061, $P = 0.043$).[18] Similarly, we found that azathioprine was an independent risk factor for CMV colitis (OR, 1.46; 95% CI, 1.57 to 11.9, $P = 0.05$).

Non-invasive markers of inflammation such as CRP and erythrocyte sedimentation rate (ESR) are used to predict UC disease activity in clinical practice. Studies on PLR and NLR (inflammatory markers) were generally conducted in individuals with malignancy[22] and only a few studies have investigated the association between NLR, PLR, and UC activation. Akpinar *et al.* found that higher NLR and PLR values were independent predictors of UC exacerbation.[23] In another study, it was demonstrated that elevated NLR and PLR can differentiate patients with UC from healthy controls.[24]

This was the first study evaluating PLR and NLR in CMV-activated UC patients and we found that PLR was significantly higher in patients with CMV activation ($P < 0,007$). On the other hand, NLR and PLR did not exhibit a predictive value for CMV activation.

Published evidence for the correlation between duration of UC and CMV colitis are controversial. Gauss A. *et al*. found that IBD patients with a disease duration shorter than 60 months had higher risk of CMV infection than patients with longer disease duration.[16] Similarly, a

recent study elaborated that the risk for CMV colitis decreased over time.[20] On the contrary, Nowcki *et al*. demonstrated that CMV reactivated patients had significantly longer disease duration compared to CMV negative reactivated patients.[25] In previous studies, longer disease duration has been associated with CMV reactivation in UC patients.

In this research, no correlation was found between anti-TNF-α therapy and CMV reactivation. In accordance with our findings, TNF-α antibodies were not found to reactivate latent CMV in IBD patients.[17,26] This was probably due to the fact that TNF-α facilitates viral replication *in vitro*. This mechanism of action leads to the utilization of TNF-α antagonists to suppress CMV reactivation.[17]

In this study, long-term outcomes were more favorable in the CMV negative group. Colectomy and anti-TNF-α therapy rate were statistically higher in the CMV positive group. These findings were consistent with the literature in UC patients with CMV reactivation.[27,28]

There were several limitations of this research. First, it was a single-center retrospective, observational study. Second, semi-quantitative PCR was used for the detection method of CMV reactivation and IHC was not performed. Additionally, the results of antiviral treatment were not included in our analysis. Therefore, prospective randomized trials, comparing the outcome of patients treated with antiviral therapy versus no antiviral treatment are warranted.

Conclusion

In conclusion, steroid resistance, azathioprine use, long disease duration, and low albumin levels were independent factors associated with CMV infection. Regarding the results of this research and published studies, one can say that it is crucial to closely monitor the risk factors for CMV reactivation in UC patients and initiate empirical therapy in a timely manner.

Ethical declaration

The ethics committee approval has been granted on 12/01/2022 and protocol number: E1-22-2293. The study complied with the Declaration of Helsinki and informed consent has been obtained from all participants.

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Conflicts of interest

There are no conflicts of interest.

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