

Prospective Study

Evaluation of a multiplex PCR assay for detection of cytomegalovirus in stool samples from patients with ulcerative colitis

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Abstract

AIM: To evaluate a multiplex PCR assay for the detection of bacterial and viral enteropathogens in stool samples from patients with ulcerative colitis (UC).

METHODS: We prospectively analyzed 300 individuals, including immunocompetent patients, immunocompromised patients, and patients with UC. Stool samples were collected from the recto-sigmoid region of the colon by endoscopy. The samples were qualitatively analyzed for bacterial and viral enteropathogens with a multiplex PCR assay using a Seeplex[®] Kit. Additional clinical and laboratory data were collected from the medical records.

RESULTS: A multiplex PCR assay detected 397 pathogens (191 bacteria and 206 viruses) in 215 samples (71.7%). The most frequently detected bacteria were *Escherichia coli* H7, 85 (28.3%); followed by *Aeromonas* spp., 43 (14.3%); and *Clostridium perfringens*, 36 (12.0%) samples. The most prevalent viruses were Epstein-Barr virus (EBV), 90 (30.0%); followed by human herpes virus-6 (HHV-6), 53 (17.7%); and cytomegalovirus (CMV), 37 (12.3%) samples. The prevalence rate of CMV infection was significantly higher in the immunocompromised group than in the immunocompetent group ($P < 0.01$). CMV infection was more common in patients with UC (26/71; 36.6%)

than in the immunocompetent patients excluding UC (6/188; 3.2%) ($P < 0.01$). CMV infection was more prevalent in UC active patients (25/58; 43.1%) than in UC inactive patients (1/13; 7.7%) ($P < 0.05$). Among 4 groups which defined by the UC activity and immunosuppressive drugs, the prevalence rate of CMV infection was highest in the UC active patients with immunosuppressive drugs (19/34; 55.8%). Epstein-Barr virus (EBV) infection was more common in the immunocompromised patients excluding UC (18/41; 43.9%) than in the immunocompetent patients excluding UC (47/188; 25.0%) ($P < 0.05$). The simultaneous presence of CMV and EBV and/or HHV6 in UC active patients (14/58; 24.1%) was greater than in immunocompromised patients excluding UC (5/41; 12.2%) ($P < 0.05$).

CONCLUSION: The multiplex PCR assay that was used to analyze the stool samples in this study may serve as a non-invasive approach that can be used to exclude the possibility of CMV infection in patients with active UC who are treated with immunosuppressive therapy.

Key words: Polymerase chain reaction; Ulcerative colitis; Cytomegalovirus; immunosuppressive drugs; Epstein-Barr virus

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Core tip: Infection with cytomegalovirus (CMV) can cause exacerbation of ulcerative colitis (UC). Thus, early diagnosis of CMV infection is important. Although endoscopic biopsy is the best approach for the diagnosis of CMV infection, this procedure may be invasive for patients and damaging to the inflamed intestine. Our prospective study on the use of the qualitative multiplex PCR assay in stool samples revealed that CMV infection is significantly more prevalent in UC active patients with immunosuppressive drugs. The multiplex PCR assay for stool samples may prove useful, non-invasive method to exclude the presence of CMV infection in patients with active UC who are treated with immunosuppressive drugs.

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INTRODUCTION

Cytomegalovirus (CMV), a double-stranded enveloped DNA virus and a member of β -herpesviridae family, commonly infects 40%-100% of adult populations^[1]

and 15.8%-34% of patients with inflammatory bowel disease (IBD) who are treated with steroids and/or other immunosuppressive drugs^[2]. The eyes, lungs, central nervous system, liver and intestine are the primary target organs for CMV infection. Typically, individuals who are infected with CMV remain asymptomatic, but the infection may manifest with mild mononucleosis-like symptoms. CMV, like other herpes viruses, persists in a life-long latency coupled with a risk of intermittent reactivation in some situations, such as the following: recipients of solid organ transplants, patients undergoing hemodialysis, patients with HIV, and patients who are treated with steroids and other immunosuppressive drugs.

Typically, enteric infections are self-limiting and acute, but serious illness can occur in immunocompromised patients. The number of immunocompromised patients has been increasing dramatically in recent years due to the increased number of organ transplants, increased numbers of patients on hemodialysis, or infected with HIV, and widespread use of immunosuppressive drugs and steroids. Due to defective or altered cellular and humoral immunity, immunocompromised patients are more susceptible to infections. Any infection has the possibility to cause an overwhelming disease in these populations^[3,4].

Patients with IBD such as ulcerative colitis (UC) and Crohn's disease (CD) are often immunosuppressed. Patients with severe, steroid-refractory or steroid-dependent states, as well as those treated with other biologic therapies undergo even more intensive immunosuppression. Together with the disease activity, these factors may contribute to the increased risk of colonic reactivation of latent CMV or CMV reinfection in patients with UC^[5]. CMV can cause the exacerbation of UC, particularly in those with steroid-dependent/steroid-refractory diseases^[2,6-8]. Histopathology and the identification of CMV DNA in colonic tissue by PCR or immunohistochemistry were recommended as the gold standard diagnostic tool for the diagnosis of CMV infection in immunosuppressed groups by the European Crohn's and Colitis Organization in 2009^[9]. Although histopathology may be the most specific diagnostic approach, a biopsy is an invasive procedure that requires an endoscopic examination. In patients with UC, the inflamed colonic tissue is friable, which leads to an increased risk of hemorrhage or perforation during invasive procedures^[10]. In addition, an extended length of time is required to obtain results of a histopathological analysis, and during this time, a given patient's condition may deteriorate clinically^[11].

Molecular diagnostic tools based on the PCR method, have been developed to improve the detection of enteropathogens^[12-14]. Of late, additional advances have been made to simultaneously identify enteropathogens using multiplex real-time PCR^[15-18], but these methods can be low-throughput and expensive. Therefore, the implementation of a rapid, sensitive, powerful, and non-invasive molecular tool is necessary to determine

the etiology of diarrhea in UC patients. Only then will it be possible to provide early and specific interventions for the prevention and control of infections in both individuals and the community.

The aim of this prospective study was to evaluate the feasibility of a qualitative multiplex PCR assay of stool for the simultaneous detection of bacterial and viral enteropathogens, focusing on CMV infection for adult patients, including patients with UC, immunocompetent patients, and immunocompromised patients.

MATERIALS AND METHODS

Study population and specimens

We prospectively analyzed 300 patients who underwent colonoscopy at the Department of Endoscopy at the University of the Ryukyus Hospital from August 2014 to January 2015. Stool samples were collected endoscopically from the recto-sigmoid region of the colon and were transported to the laboratory. Stool samples were immediately processed for the multiplex PCR. Additional clinical and laboratory data were collected from medical records. UC activity was assessed using the Mayo scoring system^[19].

DNA extraction

DNA was extracted using Ribospin™ vRD kit (GeneAll Biotechnology, Seoul, South Korea) according to manufacturer's instructions. Briefly, 300 μ L of endoscopically collected stool was transferred into a 1.5-mL microcentrifuge tube followed by the addition of 500 μ L of buffer to lyse the fecal matter. After an incubation of 10-15 min at room temperature, 700 μ L of nucleic binding buffer was added; the solution was then vortexed and mixed. The resultant solution was transferred to a mini spin column and was centrifuged at 10000 *g* for 30-60 s. Total DNA was bound to the glass fiber membrane while the remaining impurities on the membrane were removed by two successive wash buffers. Pure DNA was eluted to a final volume of 50 μ L of nuclease-free water. All procedures were performed at room temperature. The extracted DNA was stored at 4 °C for immediate use or at -20 °C for long-term use.

PCR amplification

The multiplex PCR was performed according to the manufacturer's instructions using a Seeplex® Meningitis V1, Diarrhea B1 and Diarrhea B2 ACE detection kits (Seegene, Seoul, South Korea). In regards to the Meningitis V1 ACE Detection assay, multiplex PCR was performed in a 20- μ L total volume, which included 2 μ L of each 10 \times MV1 PM and 8-MOP solution, 1 μ L of the Meningitis ACE internal control, 10 μ L of the 2 \times multiplex master mix and 5 μ L of nucleic acid template. Negative and positive control samples were included in every PCR procedure and

contained 5 μ L of the Meningitis ACE NC and PC, respectively. DNA amplification was performed in an Eppendorf Mastercycler under the following conditions: an initial denaturation at 94 °C for 15 min, 94 °C for 30 s followed by 40 cycles at 63 °C for 1.5 min, 72 °C for 1.5 min with a final cycle at 72 °C for 10 min and then a hold at 4 °C.

In regards to the Diarrhea B1/B2 ACE Detection assay, the multiplex PCR was performed in a 20- μ L volume that contained 4 μ L of 5 \times DB1 PM, 3 μ L of 8-MOP solution, 10 μ L of 2 \times master mix and 3 μ L of nucleic acid template. Negative and positive controls samples were included in each PCR reaction and contained 3 μ L of DB1 NC and DB1 PC, respectively, instead of nucleic acid.

All multiplex PCR mixtures underwent the same amplification conditions shown above. The Seeplex® Meningitis V1 kit is able to detect five pathogens in a single reaction tube including CMV, human herpes virus-6 (HHV-6), Epstein-Barr virus (EBV), herpes simplex virus type 1 (HSV-1), HSV-2, and varicella zoster virus (VZV). The Seeplex® Diarrhea B1 & B2 ACE detection assay permits the simultaneous amplification of the target DNA of the following: *Salmonella* spp. (*S. bongori* and *S. enterica*), *Shigella* spp. (*S. flexneri*, *S. boydii*, *S. sonnei*, and *S. dysenteriae*), *Vibrio* spp. (*V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*), *Clostridium difficile* toxin B, *Campylobacter* spp. (*C. jejuni* and *C. coli*), *Clostridium perfringens* toxin, *Yersinia enterocolitica*, *Aeromonas* spp. (*A. salmonicida*, *A. sobria*, *A. bivalvium*, and *A. hydrophila*), *Escherichia coli* O157: H7, and Verotoxin-producing *E. coli* (VTEC). In total, we were able to test for 25 pathogens simultaneously.

Analysis of PCR product

PCR products were analyzed by microchip electrophoresis system using the DNA-1000 Reagent kit in the MCE-202 MultiNA machine (Shimadzu, Japan). The data was analyzed using MultiNA viewer software (Shimadzu, Japan).

Ethical considerations

This prospective study was approved by the institutional review board of the University of the Ryukyus, and written informed consent was obtained from all patients prior to their inclusion in the study.

Statistical analysis

Statistical comparisons were conducted by two-tailed χ^2 test with Yates' correction using SPSS (version 21.0, SPSS Inc., Chicago, IL, United States). A *P* value < 0.05 was considered statistically significant.

RESULTS

Study population

The clinical characteristics of the included patients

Table 1 Demographic and clinical characteristics of the patients n (%)

Characteristics	Number of patients 300 (100)
Gender	
Male	177 (59)
Female	123 (41)
Age in years	59.04 (16-86)
Underlying disease	
Immunocompromised	41 (13.7)
Ulcerative colitis	71 (23.7)
One or more bacteria/viruses detected	215 (71.7)
One or more bacteria detected	146 (48.7)
Number of bacteria detected	
1	108 (36.0)
2	33 (11.0)
3	5 (1.67)
One or more viruses detected	148 (49.3)
Number of viruses detected	
1	96 (32.0)
2	45 (15.0)
3	7 (2.3)
Bacteria detected	
<i>Clostridium difficile</i> toxin B	15 (5.0)
<i>Salmonella</i> spp.	4 (1.3)
<i>Campylobacter</i> spp.	3 (1.0)
<i>Vibrio</i> spp.	1 (0.3)
<i>Clostridium perfringens</i>	36 (12.0)
<i>E. coli</i> H7	85 (28.3)
<i>E. coli</i> O157	1 (0.3)
VTEC	1 (0.3)
<i>Aeromonas</i> spp.	43 (14.3)
Virus detected	
CMV	37 (12.3)
HHV6	53 (17.7)
EBV	91 (30.3)
HSV1	14 (4.7)
HSV2	6 (2.0)
VZV	6 (2.0)

E. coli: *Escherichia coli*; VTEC: Verotoxin-producing *E. coli*; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV6: Human herpes virus-6; VZV: Varicella zoster virus.

are summarized in Table 1. Three hundred patients were enrolled, including 41 immunocompromised patients and 71 patients with UC. The average age of the patients was 59.04 years (range 16-86 years), and there were more males (59%) than females. All of the UC active patients had diarrhea. A total of 300 samples collected by colonoscopy were examined. Among the 300 samples, multiplex PCR showed a positive reaction in 215 samples (71.7%) (Table 1). Among the 300 samples, one or more bacteria and viruses were identified in 146 (48.7%) and 148 (49.3%) samples, respectively. One bacterium was detected in 108 (36.0%) samples. Two and three other types of bacteria were detected in 33 (11.0%) and 5 (1.67%) samples, respectively. Ninety six (32.0%) had a single viral infection while 45 (15.0%) and 7 (2.3%) had 2 and 3 viruses, respectively. The most frequently detected bacteria were *E. coli* H7, 85 (28.3%); followed by *Aeromonas* spp., 43 (14.3%); *C. perfringens*, 36 (12.0%); and *C. difficile* Toxin B, 15 (5.0%). *Shigella* spp. and *Y. enterocolitica* were not

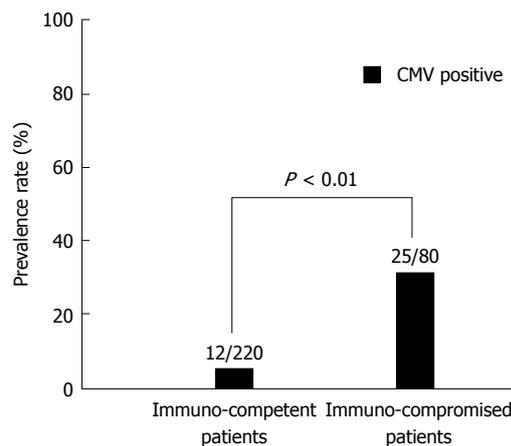


Figure 1 Comparison of prevalence rate of cytomegalovirus infection. The prevalence rate was significantly higher in the immunocompromised patients than in the immunocompetent patients. CMV: Cytomegalovirus.

detected. The most prevalent viruses were EBV, 91 (30.3%); followed by HHV-6, 53 (17.7%); and CMV, 37 (12.3%). The least prevalent viruses were HSV2, 6 (2.0%) and VZV, 6 (2.0%). Internal control was positive for all samples.

Patients who were treated with immunosuppressive drugs (e.g., anticancer drugs, T-cell inhibitors, steroids), HIV patients, and patients on hemodialysis for chronic kidney disease were included in the immunocompromised group (80; 26.7%). Patients who were not treated with immunosuppressive drugs were incorporated into the immunocompetent group (220; 73.3%). The prevalence rate of CMV infection was significantly higher in the immunocompromised group than in the immunocompetent group ($P < 0.01$) (Figure 1). CMV infection was more common in patients with UC (26/71; 36.6%) than in the immunocompetent patients excluding UC (6/188; 3.2%) ($P < 0.01$) (Figure 2).

In UC patients, CMV infection was more prevalent in UC active patients (25/58; 43.1%) than in UC inactive patients (1/13; 7.7%) ($P < 0.05$) (Figure 3). UC patients were further categorized into 4 groups based on the UC activity and the administration of immunosuppressive drugs. The prevalence rate of CMV infection was highest among individuals in the active UC group who were treated with immunosuppressive drugs compared with individuals in the other 3 groups (Figure 4). Among the 58 patients with active UC, immunosuppressive drugs were prescribed for 34 patients. The CMV infection rate was significantly higher in those who were treated with immunosuppressive drugs (19/34; 55.8%) compared with those who were not treated with immunosuppressive drugs (6/24; 25.0%) ($P < 0.05$).

As for EBV infection, EBV infection was more common in the immunocompromised patients excluding UC (18/41; 43.9%) than in the immunocompetent patients excluding UC (47/188; 25.0%) ($P < 0.05$) (Figure 5). Figure 6 shows no significant differences

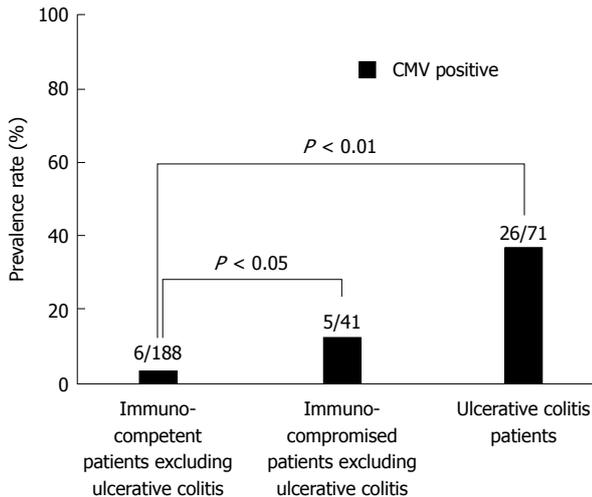


Figure 2 Comparison of prevalence rate of cytomegalovirus infection in 3 groups, including the immunocompetent patients excluding ulcerative colitis, the immunocompromised patients excluding ulcerative colitis, and ulcerative colitis patients. CMV: Cytomegalovirus.

of the prevalence rate of HHV6 infection among the groups. The overlapping prevalence patterns for CMV, EBV, and HHV6 are shown for UC active patients (Figure 7) and the immunocompromised patients excluding UC (Figure 8). The simultaneous presence of CMV and EBV and/or HHV6 in UC active patients (14/58; 24.1%) was greater than in immunocompromised patients excluding UC (5/41; 12.2%) ($P < 0.05$). No patients in our cohort were simultaneously infected with all three viruses.

DISCUSSION

Multiplex PCR has been widely applied to the diagnosis of gastrointestinal infectious diseases^[18]. Notably, the Seeplex[®] Diarrhea ACE Detection assay has shown itself to be a rapid, sensitive, specific, and reliable diagnostic tool for the direct detection of the most common enteropathogens in stool samples^[20]. Other multiplex PCR assays (*e.g.*, the FilmArray gastrointestinal panel and Luminex xTag gastrointestinal pathogen panel) have also yielded an increased percent positive rate compared with routine testing^[21]. Based on these recent developments, we have conducted this prospective assay with the Seeplex[®] Diarrhea and Meningitis Detection method. We have analyzed (1) the results of a multiplex PCR assay as a rapid and non-invasive molecular diagnostic tool for the early diagnosis of CMV infection in patients with UC; (2) the prevalence rate of CMV infection in UC in comparison with control populations; and (3) the relationship of CMV infection with immunosuppressive drugs and disease activity. We have found that CMV infection is significantly more prevalent in the active UC group compared with the immunocompetent group and the immunocompromised group ($P < 0.05$), which further indicates the strong association of CMV

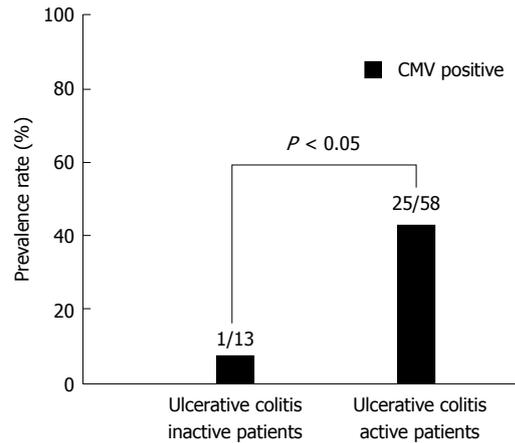


Figure 3 Comparison of prevalence rate of cytomegalovirus infection. The prevalence rate was significantly higher in ulcerative colitis active patients than in ulcerative colitis inactive patients. CMV: Cytomegalovirus.

infection in patients with active UC.

In addition, CMV infection is significantly correlated with the administration of immunosuppressive drugs among the patients with active UC ($P < 0.05$). UC patients who were treated with immunosuppressive drugs such as corticosteroids, tacrolimus, azathioprine, 6-mercaptopurine, or cyclosporine A, and those with existing inflammation are considered to be at an increased risk for the development of CMV infection^[22-24]. Although some studies have suggested that CMV infection may be an occasional finding or that CMV may be an inactive participant in UC^[22,25,26], CMV itself can be a major cause of exacerbation of UC. As a result, CMV subsequently leads to the worst clinical conditions seen among patients with UC^[27]. Therefore, an early diagnosis of CMV infection in UC is crucial, and several modalities have been developed for the diagnosis of CMV infection.

Currently, the guidelines of the European Colitis and Crohn's Organization recommend the combined use of hematoxylin-eosin (HE) staining, immunohistochemical (IHC) staining with a CMV-specific monoclonal antibody and PCR for CMV in colonic tissue for the detection of colonic CMV infection in patients with UC^[9]. IHC staining has a higher sensitivity than conventional histology (78%-93%) for the detection of CMV in colonic tissue^[28].

Several studies have reported that the quantitative real-time PCR technique is a highly sensitive method for the diagnosis of CMV infection in inflamed colonic tissue compared with non-inflamed colonic tissue in the setting of UC^[29]. The detection of CMV DNA in mucosal biopsies by PCR analysis has been regarded as the most sensitive assay for the diagnosis of CMV infection of the intestinal tract^[30]. However, this technique requires invasive procedures such as sigmoidoscopy or colonoscopy to collect biopsy material and in some instances a physician may not be able to perform a colonoscopy. Furthermore, inflamed colonic tissue in cases of UC is mostly friable, edematous, and eroded

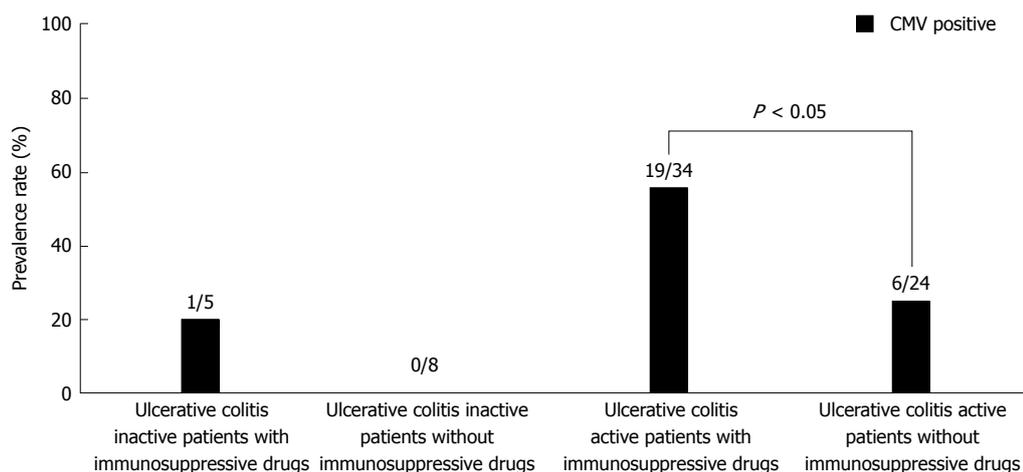


Figure 4 Comparison of prevalence rate of cytomegalovirus infection in 4 groups, including, ulcerative colitis inactive patients with immunosuppressive drugs, ulcerative colitis inactive patients without immunosuppressive drugs, ulcerative colitis active patients with immunosuppressive drugs, and ulcerative colitis active patients without immunosuppressive drugs. CMV: Cytomegalovirus.

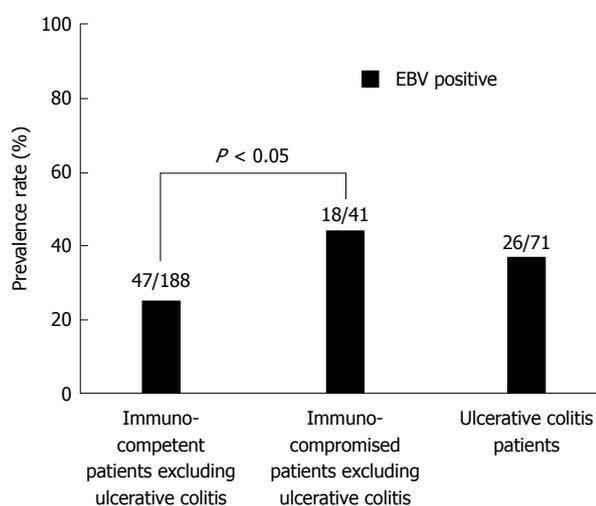


Figure 5 Comparison of prevalence rate of Epstein-Barr virus infection in 3 groups, including the immunocompetent patients excluding ulcerative colitis, the immunocompromised patients excluding ulcerative colitis, and ulcerative colitis patients. EBV: Epstein-Barr virus.

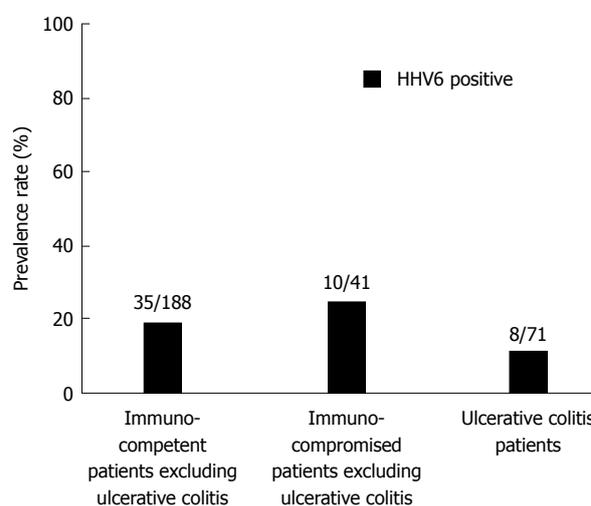


Figure 6 Comparison of prevalence rate of human herpes virus-6 infection in 3 groups, including the immunocompetent patients excluding ulcerative colitis, the immunocompromised patients excluding ulcerative colitis, and ulcerative colitis patients. HHV6: Human herpes virus-6.

and bleeds when touched. As a result, there is a high risk of bleeding during the collection of tissues. Additionally, endoscopic biopsy may lead to false-negative results due to sampling bias. To reduce sampling error, tissues in different locations along the colon are needed. Therefore, serology, a CMV antigenemia assay and PCR of blood samples for CMV are more convenient than invasive procedures^[11,31]. Serological tests, such as the detection of CMV-specific IgM antibodies, have been shown to have a 100% sensitivity and a 99% specificity^[32]. However, the presence of IgM antibodies can only reveal acute infection, and as such, cases of reactivation are often undiagnosed. Therefore, serology also has limited value in the diagnosis of CMV infection in patients with UC.

A recent study^[11] evaluated the diagnostic performance of a CMV antigenemia assay and PCR of

blood for the detection of CMV in patients with UC and showed lower sensitivities for the diagnosis of CMV infection compared with a previous study^[10]. The presence of a CMV infection in the intestine cannot be ruled out in the case of a negative CMV antigenemia assay. In many cases, additional testing will be required to reach the diagnosis of CMV infection. The CMV antigenemia assay also has limited clinical value in the prediction of the reactivation of CMV infection in the gastrointestinal tract^[33] and is therefore ineffective at preventing the development of CMV colitis. The CMV antigenemia assay is also relatively time consuming and demands expert pathologists to reduce subjective bias during interpretation of the slides. As the pp⁶⁵ antigen for the CMV antigenemia assay is examined in blood leukocytes, it may also reveal false-negative results in leucopenic patients^[27]. The diagnostic accuracy of CMV antigenemia may depend

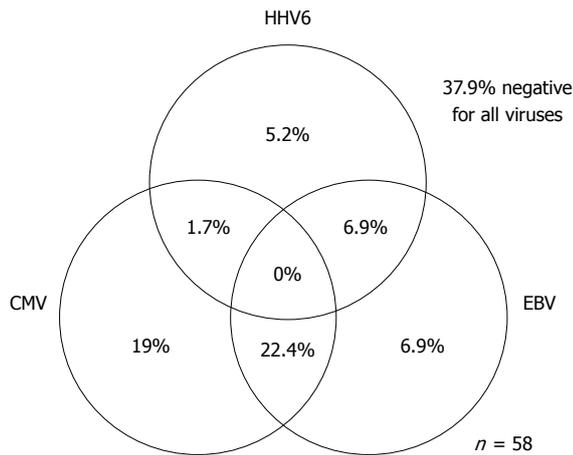


Figure 7 Overlapping prevalence patterns for cytomegalovirus, Epstein-Barr virus, and human herpes virus-6 infection in active ulcerative colitis patients. CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV6: Human herpes virus-6.

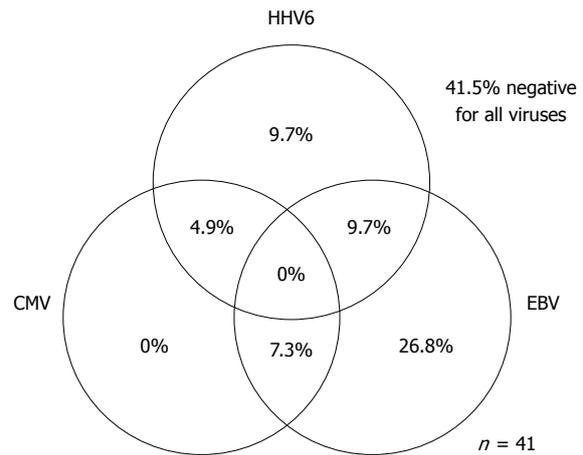


Figure 8 Overlapping prevalence patterns for cytomegalovirus, Epstein-Barr virus, and human herpes virus-6 infection in the immunocompromised patients excluding ulcerative colitis. CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HHV6: Human herpes virus-6.

on site of organ/tissue involvement^[34]. Under these circumstances, the testing of stool samples by PCR was chosen as an alternative to these techniques.

We have found advantages in the detection of CMV DNA in stool samples by the multiplex PCR technique. Although this prospective study was designed so that fresh samples would be prepared from stool samples obtained by endoscopy, stool analysis is traditionally a non-invasive procedure. The procurement of tissues by biopsy may have potential risks when the intestine is inflamed. In addition, the stool may better reflect the CMV enterocolitis that is located in the proximal colon and small intestine. We are planning to analyse stool samples which are collected by the patients at home.

Several studies have evaluated CMV DNA in stool samples^[26,35,36]. Ganzenmueller *et al.*^[37] have retrospectively evaluated quantitative real-time PCR in 66 fecal samples and intestinal biopsies for the diagnosis of CMV intestinal disease. Their study also compared CMV DNA in stool and colonic biopsies with the results of histopathology and the CMV antigenemia assay. In their study, the sensitivity and specificity of stool CMV DNA for the diagnosis of CMV intestinal disease are 67% and 96%, respectively. Therefore, CMV DNA in the stool is more sensitive and specific than the gold standard method (histopathology and IHC) for the diagnosis of CMV in inflamed colonic tissue.

Additional studies have evaluated the sensitivity, specificity and reliability of the multiplex PCR assay as a rapid molecular diagnostic tool for the investigation of the etiology of enteric infections in pediatric patients^[20]. However, the diagnostic significance of the multiplex PCR assay in stool samples for the diagnosis of CMV infection in patients with UC has not yet been evaluated. In our prospective study, we evaluated the ability of a commercially available multiplex PCR assay to diagnose CMV infection in UC using stool samples. To our knowledge, this is the first large-scale

prospective study that assesses the multiplex PCR assay as a diagnostic tool for the diagnosis of CMV in patients with active UC, and in both immunocompetent and immunocompromised patients. As for EBV and HHV6, this study has showed a possible synergistic role for these viruses in the pathogenesis in active UC activation, as suggested by the prior reports^[38,39].

Our study has some limitations. First, we did not quantify the CMV DNA. However, in patients with UC, any positive result with respect to CMV is clinically significant without quantification of the viral load. Quantification of CMV in stool specimens is not feasible during specimen processing (*e.g.*, diluted versus non-diluted)^[40], and positive results should be carefully considered, especially in patients with UC who are treated with immunosuppressive drugs^[37]. Furthermore, this prospective study was not designed to compare the different modalities for the detection of CMV, including histopathology, CMV antigenemia assay, serology, and tissue CMV PCR. Herfarth *et al.*^[36] have shown that the sensitivity and specificity of the stool PCR analysis compared with PCR in mucosal biopsies were 83% and 93%, respectively. They have noted that it is not clear whether CMV DNA detected in the stool samples was due to leakage from the blood compartment into the intestinal tract, or if it was derived from intestinal CMV infection.

In conclusion, this prospective study proposes multiplex PCR as a successful, non-invasive diagnostic technique for rapid detection of CMV infection among UC patients in clinical in-patient and out-patient settings. Additionally, we present a new protocol for the broad analysis of the enteropathogens in stool samples in adult populations. This method will also help predict CMV infection prior to the development of intestinal symptoms, which is important for the prevention of exacerbation of UC by CMV reactivation. Positive PCR results may help to rapidly diagnose patients at a high risk for CMV infection. Further

studies are needed to confirm these results. Future studies that involve colonic biopsies will be needed to confirm the reliability of the multiplex PCR test results.

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COMMENTS

Background

Infection with cytomegalovirus (CMV) can cause exacerbation of ulcerative colitis (UC). Thus, early diagnosis of CMV infection is important. Although endoscopic biopsy is the best approach for the diagnosis of CMV infection, this procedure may be invasive for patients and damaging to the inflamed intestine.

Research frontiers

Molecular diagnostic advances have been made to simultaneously identify enteropathogens using multiplex polymerase chain reaction (PCR). The aim of this prospective study was to evaluate the feasibility of a qualitative multiplex PCR assay of stool for the simultaneous detection of bacterial and viral enteropathogens, focusing on CMV infection for adult patients, including patients with UC, immunocompetent patients, and immunocompromised patients.

Innovations and breakthroughs

This is the first large-scale prospective study that assesses the multiplex PCR assay in stool samples as a diagnostic tool for the diagnosis of CMV in patients with UC. This assay may prove useful, non-invasive method to exclude the presence of CMV infection in patients with active UC who are treated with immunosuppressive drugs.

Applications

This multiplex PCR as a successful, non-invasive diagnostic technique will help predict CMV infection prior to the development of intestinal symptoms, which is important for the prevention of exacerbation of UC by CMV reactivation.

Terminology

Multiplex PCR assay: A rapid, sensitive, specific, and reliable diagnostic tool for the simultaneous detection of bacterial and viral enteropathogens by PCR.

Peer-review

In this study, the authors detected CMV and other enteric pathogens using multiplex PCR in stool samples collected from 300 patients who underwent colonoscopy including patients with UC.

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