Detection of Herpes simplex virus, Cytomegalovirus and Epstein-Barr virus in Ulcerative Colitis patients by PCR method

Sahar Mehrabani Khasraghi*[,1], Mitra Ameli[2], Farzad Khalily[3]

[1]Department of Microbiology, Tonekabon Branch, Islamic Azad University Tonekabon, Mazandaran, Iran.
[2]Department of Medicine, Tonekabon Branch, Islamic Azad University Tonekabon, Mazandaran, Iran.
[3]Gastroenterology and Hepatology Research Center, Karaj University of Medical Sciences, Alborz, Iran.

*Corresponding Author: email address: saharmehrabani1@gmail.com (S. Mehrabani Khasraghi)

ABSTRACT

Ulcerative Colitis (UC) is one type of inflammatory bowel disease (IBD). Herpes viruses have been suggested as possible etiological agents of UC and Crohn's disease (CD). The aim of this study was to investigate the prevalence of Herpes Simplex virus (HSV), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) in patients with UC in comparison with healthy subjects by using PCR technique. In this analytical case-control study, five patients with UC and thirty healthy patients as controls were selected. Sampling was performed by endoscopic biopsy operation. Biopsy specimens were frozen under sterile conditions at -20˚C until analysis. After DNA extraction, analysis of PCR to detect HSV, CMV and EBV DNA in tissue samples was performed. Statistical analysis was performed with the χ^2 test. We observed HSV DNA in 80% of UC samples (4 of 5) and 10% from the normal control group (3 of 30). CMV DNA was found in 80% of UC samples (4 of 5) and 30% from the normal control group (9 of 30). Also, EBV DNA was found in 60% of UC samples (3 of 5) and 36.7% from the normal control group (11 of 30). Statistical analysis showed a significant association between the prevalence of HSV and CMV and the incidence of UC compared with the control group. The results of the present study has shown UC patients have a predisposition to HSV and CMV infections as compared to healthy individuals. Also, the results demonstrate a lack of direct molecular evidence to support an association between HSV and CMV with UC.

Keywords: Ulcerative Colitis; Herpes simplex virus; Cytomegalovirus; Epstein-Barr virus; PCR.

INTRODUCTION

Inflammatory bowel disease (IBD) represents a group of idiopathic chronic inflammatory intestinal conditions. Patients with IBD are occasionally hospitalized due to fever, abdominal pain, and diarrhea, which are commonly attributed to an exacerbation of their underlying disease. Ulcerative colitis (UC) is a form of IBD that causes inflammation and ulcers in the colon. UC has an incidence of 1 to 20 cases per 100.000 individuals per year, and a prevalence of 8 to 246 per 100.000 individuals [1]. In recent years, cases of IBD associated with Herpes viruses infections have occasionally been reported [2-5]. Herpes Simplex virus (HSV), Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) are ubiquitous Herpes viruses and establishes persistent infections in the host. HSV, CMV and EBV are transmitted through close personal contact with body fluids, including blood, urine, saliva, and also HSV, CMV and EBV infections can occur in immunocompromised patients such as recipients of organ transplants, patients undergoing hemodialysis, patients receiving immunosuppressive drugs, and patients with acquired immunedeficiency syndrome. In IBD patients, Herpes viruses have been recognized in colonoscopic biopsy specimens obtained during evaluation and management of IBD or diagnosed after pathologic examination of colon. However prospective studies examining prevalence of HSV, CMV and EBV genome in patients with IBD in comparison with a control population are limited. Patients with IBD treated with immunosuppressive agents including corticosteroids, cyclosporine, azathioprine and methotrexate, either alone or in combination. De Saussure et al. [6] treated 3 CMV-positive IBD cases with antiviral therapy, and only 1 patient got remission. Kandiel et al. [7] used antiviral drugs for the treatment of CMV positive colitis, and achieved a remission rate of 67-100%. Juffermans et al. [8] used antiviral drugs for the treatment of EBV positive colitis and showed

azathioprine is an important drug in IBD patients. Give the importance of IBD as the unknown etiology and detection of an infectious agent in these patients might have important implications in treatment and prevention. The aim of the present study was to investigate the prevalence of HSV, CMV and EBV in patients with UC in comparison with healthy subjects by using PCR technique conducted.

MATERIALS AND METHODS

Patients

In this analytical case-control study, informed consent was received from all patients admitted to the Endoscopy clinic of Toos and Firoozgar hospital in Tehran, Iran between January 2013 and June 2013. In this study, five patients with UC and thirty healthy patients as controls were sampled. Sampling was performed by endoscopic biopsy and a tissue sample size of 25-50 mg was calculated for each patient. All collected tissues were kept frozen at -20ºC until analysis.

DNA extraction

The DNA was extracted using the KiaSpin®Tissue Kit (Kiagen CA, Iran) according to the manufacture's instructions. In order to determine the concentration of the sample absorbance at a wavelength to 260 nm was performed by biophotometer system (Eppendrof, Germany). In addition, to determine the purity of the sample wavelength of 280/260 and 230/260 was also examined.

PCR

We performed PCR amplification of the human β-globulin gene (Table 1) to determine the quality of extracted DNA [9]. The PCR amplification was performed in a 20 µL reaction volume contained 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: an initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 50 sec, 55ºC for 45 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min.

Specific primers listed in Table 1 were used to reproduce the HSV genome of the samples [9]. For the reaction, the PCR mixture for a reaction volume of 20 µL consisted of 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: an initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 50 sec, 64ºC for 45 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min.

Specific primers listed in Table 1 were used to reproduce the CMV genome of the samples [9]. For the reaction, the PCR mixture for a reaction volume of 20 µL consisted of 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: an initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 50 sec, 60ºC for 45 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min.

We used specific primers to reproduce the EBV genome from the samples (Table 1) [9]. The mixture reaction PCR for a reaction volume of 20 µL contained 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 40 sec, 65ºC for 40 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min. Then, 5 µL of the PCR product was analyzed on a 1.5% agarose gel.

Statistical analysis

Statistical analysis was performed using the SPSS-20 (SPSS, Inc., Chicago, IL, USA) software package. We used the t- and $\chi 2$ tests to analyze the relationship between the prevalence of HSV, CMV and EBV and occurrence of UC in addition to a comparison with control group tissue samples. Statistical significance was accepted at the 0.05 level.

Table1. Primers sequences and base pair (bp) length

Primer	Sequence (5´-3´)	Size (bp)
b_2- F	TCCAACATCAACATCTTGGT	106
b_2- R	TCCCCCAAATTCTAAGCAGA	
HSV-1/2F	CAGTACGGCCCCGAGTTCGTGA	465
HSV-1/2R	TTGTAGTGGGCGTGGTAGATG	
CMV-F	GTCACCAAGGCCACGACGTT	167
CMV-R	TCTGCCAGGACATCTTTCTC	
EBV- F	GTGTGCGTCGTGCCGGGGCAGCCAC	102
EBV- R	ACCTGGGAGGGCCATCGCAAGCTCC	

RESULTS

HSV DNA was found in 80% of UC samples (4 of 5) and 10% from the normal control group (3 of 30). Statistical analysis showed a significant association between the prevalence of HSV and the incidence of UC compared to the control group (P= 0.003).

CMV DNA was found in 80% of UC cases (4 of 5) and 30% from the normal control group (9 of 30). Statistical analysis showed a significant association between the prevalence of CMV and the incidence of UC compared to the control group (P= 0.03). EBV DNA was found in 60% of UC patients (3 of 5) and 36.7% from the normal control group (11 of 30). Statistical analysis showed no significant association between the prevalence of EBV and the incidence of UC compared to the control group (P= 0.36). We observed the highest prevalence of HSV in UC patients older than 55 years (60%) of age and in the normal control participants who were 35-55 years of age (6.7%) (Table 2).

The highest prevalence of CMV was observed in older than 55 years (60%) of age in UC patients and in two groups of normal control participants, those 35-55 years (13.3%) and under 35 years (13.3%) of age (Table 3).

The highest prevalence of EBV was observed in older than 55 years (60%) of age in UC patients and in 35-55 years (16.6%) of age in the normal control group (Table 4). Statistical analysis showed no significant association between the prevalence of HSV, CMV and EBV in terms of age in patients with UC compared to the control group (*P*>0.05; Tables 2, 3 and 4).

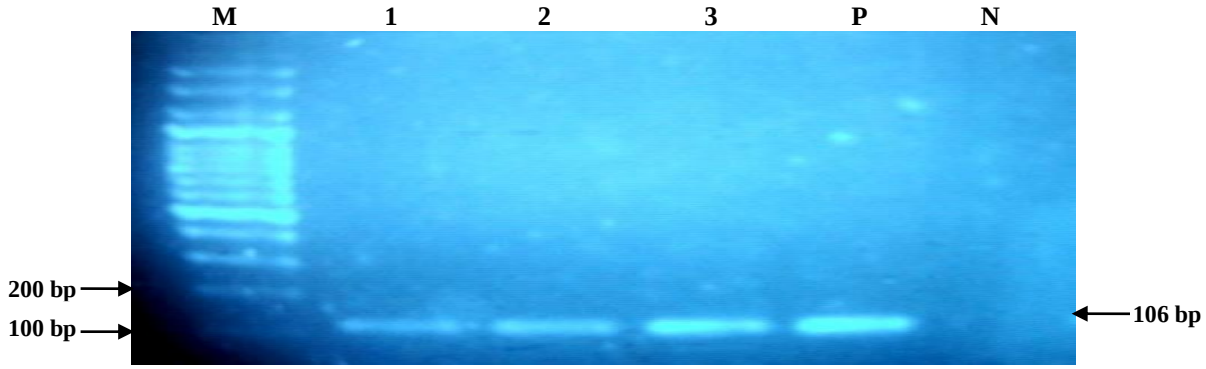

Figure 1. PCR analysis of β-globulin. DNA extracted from tissue samples was amplified for β-globulin gene using primers described in Materials and Methods. Amplification yielded a band of 106 bp. As positive control (P), we used human DNA from fresh tissue; the negative control (N) was PCR master mix without DNA. Clinical samples, lanes 1-3. DNA molecular weight marker, M.

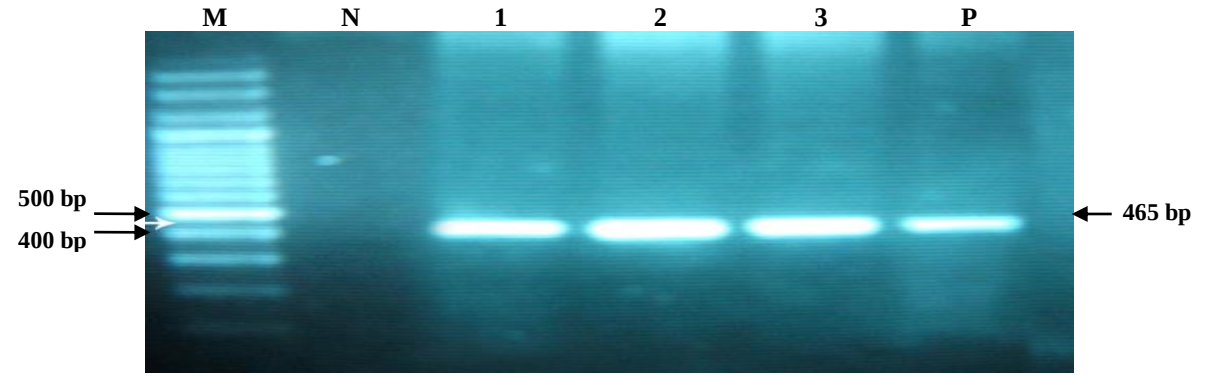

Figure 2. PCR analysis for the detection of herpes simplex virus (HSV) from tissue samples. DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 465 bp. Positive control (P); negative control (N); clinical samples, lanes 1, 2 and 3; DNA molecular weight marker, M.

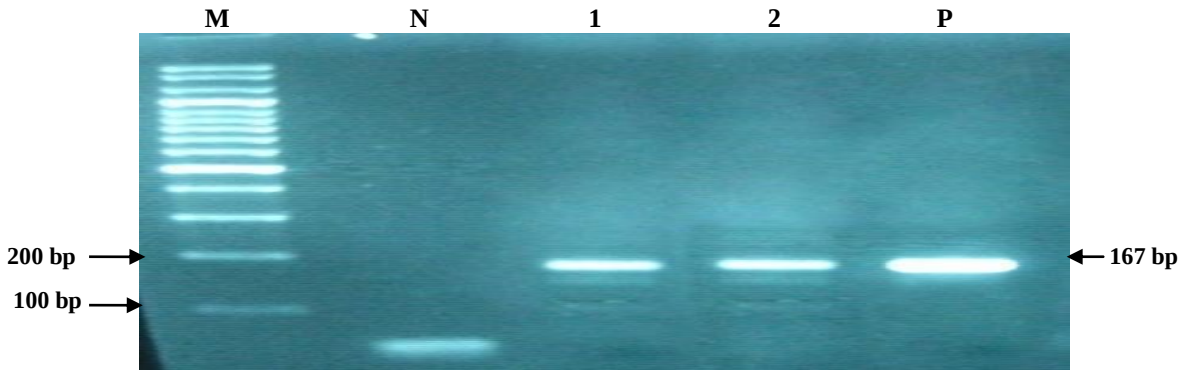

Figure 3. PCR analysis for the detection of Cytomegalovirus (CMV) from tissue samples. DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 167 bp. Positive control (P); negative control (N); clinical samples, lanes 1 and 2; DNA molecular weight marker, M.

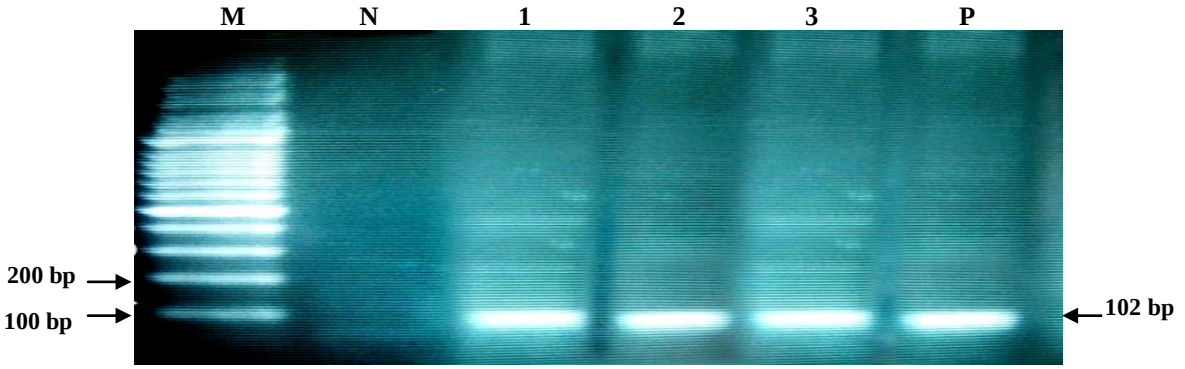

Figure 4. PCR analysis for the detection of Epstein-Barr virus (EBV) from tissue samples. DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 102 bp. Positive control (P); negative control (N); clinical samples, lanes 1, 2 and 3; DNA molecular weight marker, M.

Table 2. Clinical and pathological features of the UC and control group patients related to the presence of HSV

Patients		**HSV DNA**		***P* value**	**Total**
	Positive		**Negative**		
UC					
Age groups				>0.05	
Under 35 years	1 (20%)		1 (20%)		2 (40%)
35-55 years	0 (0%)		0 (0%)		0 (0%)
Over 55 years	3 (60%)		0 (0%)		3 (60%)
Gender				>0.05	
Male	2 (40%)		1 (20%)		3 (60%)
Female	2 (40%)		0 (0%)		2 (40%)
Total	4 (80%)		1 (20%)		5 (100%)
Control group					
Age groups				>0.05	
Under 35 years	1 (3.3%)		6 (20%)		7 (23.3%)
35-55 years	2 (6.7%)		11 (37%)		13 (43.7%)
Over 55 years	0 (0%)		10 (33%)		10 (33%)
Gender				>0.05	
Male	1 (3.3%)		11 (37%)		12 (40.3%)
Female	2 (6.7%)		16 (53%)		18 (59.7%)
Total	3 (10%)		27 (90%)		30 (100%)

Table 3. Clinical and pathological features of the UC and control group patients related to the presence of CMV

Patients		**CMV DNA**		***P* value**	**Total**
	Positive		**Negative**		
UC					
Age groups				>0.05	
Under 35 years	1 (20%)		1 (20%)		2 (40%)
35-55 years	0 (0%)		0 (0%)		0 (0%)
Over 55 years	3 (60%)		0 (0%)		3 (60%)
Gender				>0.05	
Male	2(40%)		1 (20%)		3 (60%)
Female	2 (40%)		0 (0%)		2 (40%)
Total	4 (80%)		1 (20%)		5 (100%)
Control group					
Age groups				>0.05	
Under 35 years	4 (13.3%)		3(10%)		7 (23.3%)
35-55 years	4 (13.3%)		9 (30%)		13 (43.3%)
Over 55 years	1 (3.4%)		9 (30%)		10 (33.4%)
Gender				>0.05	
Male	4 (13.4%)		8 (26.7%)		12 (40.1%)
Female	5 (16.6%)		13 (43. 3%)		18 (59.9%)
Total	9 (30%)		21 (70%)		30 (100%)

Table 4. Clinical and pathological features of the UC and control group patients related to the presence of EBV

Patients		**EBV DNA**		***P* value**	**Total**
	Positive		**Negative**		
UC					
Age groups				>0.05	
Under 35 years	0 (0%)		0 (0%)		0 (0%)
35-55 years	0 (0%)		2 (40%)		2 (40%)
Over 55 years	3 (60%)		0 (0%)		3 (60%)
Gender				>0.05	
Male	3 (60%)		0 (0%)		3 (60%)
Female	0 (0%)		2 (40%)		2 (40%)
Total	3 (60%)		2 (40%)		5 (100%)
Control group					
Age groups				>0.05	
Under 35 years	4 (13.4%)		5 (16.6%)		9 (30%)
35-55 years	5 (16.6%)		6 (20.1%)		11 (36.7%)
Over 55 years	2 (6.7%)		8 (26.6%)		10 (33.3%)
Gender				>0.05	
Male	3 (10.1%)		9 (30%)		12 (40.1%)
Female	8 (26.6%)		10 (33.3%)		18 (59.9%)
Total	11 (36.7%)		19 (63.3%)		30 (100%)

In terms of gender, the highest prevalence of HSV was observed in both of two gender male (40%) and female (40%) in UC patients and in control group female (6.7%) (Table 2). Also, we observed the highest prevalence of CMV in both of two gender male (40%) and female (40%) in UC patients and in control group female (16.6%) (Table 3). We observed the highest prevalence of EBV in male UC patients (60%) and in normal control group female (26.6%) (Table 4). Statistical analysis showed no significant association between the prevalence of HSV, CMV and EBV and gender in UC patients compared to the control group (P>0.05; Tables 2, 3 and 4). In all tissue samples, we observed a 106 bp band that represented amplification of the human β-globulin gene (Figure 1). Due to the quality and reliability of DNA extracted, PCR analysis with HSV specific primers was performed where we observed 465 bp bands that represented the replication (Figure 2). PCR analysis with CMV specific primers was performed where we observed 167 bp bands that represented the replication (Figure 3). PCR analysis with EBV specific primers was performed. A total of 102 bp bands that represented the replication were observed (Figure 4).

DISCUSSION

In this study, we investigated UC and healthy tissues for the presence of HSV, CMV and EBV DNA by PCR method. In UC patients we detected HSV DNA in 80% of samples, CMV DNA in 80% of samples, and EBV DNA was detected in 60% of samples. In the control group, 10% had HSV DNA, 30% had CMV DNA and 36.7% had EBV DNA. There was an association between HSV and CMV presence and occurrence of UC compared to control group tissue.

During recent years, a clear association between complicated courses of UC and the presence of Herpes viruses has been established. The exact pathogenic role of Herpes viruses in these patients remains unclear despite a great number of published reports. Powell et al. [10] first reported the association of CMV with UC in 1961. However, the role of HSV, CMV and EBV in UC patients has not been reported in the literature in Iran till now. This is the first study to investigate the prevalence of HSV, CMV and EBV in UC patients in Iran. Herpes viruses infection in UC patients, especially in those who are immune-compromised by steroid therapy, can produce severe systemic disease and often leads to colectomy, but the coincidental diagnosis of UC and Herpes virus colitis have also been reported [11-13]. However, the importance of Herpes viruses as an exacerbating factor of UC is neglected by many clinicians. PCR has emerged as the most sensitive laboratorial method and immunohistochemistry or in situ hybridization has been reported for diagnosis of viral infection including that with Herpes viruses [2, 14-18]. In the current study has shown the presence of HSV, CMV and EBV in UC tissues and non-malignant by PCR method reflects the ability of the virus to infect of the different colon cells. In the study by Dimitroulia et al. [13] in the intestinal tissue CMV genome was detected in 32.9% of the IBD patients and only in 2.4% of the controls, also a significant association was detected between CMV intestinal infection and either UC or CD, although the association was even stronger for patients with UC. Hommes et al. [19] evaluated the pathogenicity of CMV in IBD; their results showed CMV causes significant clinical morbidity in IBD patients.

Kishore et al. [14] investigated infection with CMV in patients with IBD, sixty-three patients with IBD (both UC and CD) were selected, results showed CMV infection in patients with IBD may be common, this has definite clinical significance and therefore should not be ignored. Although our results confirmed the results of Dimitroulia et al. [13], Hommes et al. [19] and Kishore et al. [14] indicating that there is association between CMV infection and progression of IBD. However, in other studies, no evidence of a direct association between IBD and CMV infection was found [12, 20].

In the study by Yi et al. [12] on the prevalence and risk factors of CMV infection in IBD in Wuhan, central China, two hundred and twenty six IBD patients (189 UC and 37 patients with CD) were selected, CMV DNA was detected by nested PCR, their results showed no risk factor was found to be significantly correlated with CMV infection in risk factors analysis. Also, Leveque et al. [20] found no relationship between CMV viral load and disease severity in patients with active IBD. Although our results confirmed the results of Takeda et al. [21] indicating that EBV was found in biopsy specimens in patients with UC. However, our results are in stark contrast to other author's data results that EBV and other herpes family viruses have been implicated in the pathogenesis of IBD [2, 3, 13, 15-17, 22]. Yanai et al. [17] found that EBV was detected in 63.3% of CD cases and 60% of UC cases using in situ hybridization for EBV-encoded small RNA 1 (EBER-1), indicating that EBV infection may be related to IBD colonic diseases. Gehlert et al. [15] detected a large number of EBV infection in UC and CD patients tissue as compared to non-malignant cases of patients tissue. Ryan et al. [22] detected EBV DNA in 55% of CD and 64% of UC tissues, with mean viral loads significantly higher in these lesions than in normal colon tissues. Dimitroulia et al. [13] showed the prevalence of EBV was significantly higher in IBD patients than the controls tissue, the results showed EBV is associated with IBD.

Bertalot et al. [2] showed a possible role of EBV in infection of UC patients. Kangro et al. [3] reported EBV infection was associated with UC. Also, Spieker and Herbst [16] have shown that EBV-positive lymphocytes accumulate in UC, suggesting the colon as a potential site for EBV replication and transmission in IBD patients. Although these results confirmed the other author's results [2, 3, 13, 15-17, 22], indicating that the IBD exacerbation associated with EBV infection.

CONCLUSION

The results of the present study has shown UC patients have a predisposition to HSV and CMV infections as compared to healthy individuals.

Also, the results demonstrate a lack of direct molecular evidence to support an association between HSV and CMV with UC.

ACKNOWLEDGEMENTS

This paper has been extracted from proposal no. 23796 (Performer: Miss Sahar Mehrabani-Khasraghi). We wish to acknowledge the very kind help of Dr. Gholamreza Hemasi to sample collection. We would like to express thanks of Miss Sara Mehrabani Khasraghi for her kind help, and all patients who participated in the study.

REFERENCES

1. Danese S, Fiocci C. Ulcerative colitis. The New England Journal of Medicine. 2011; 365: 1713-1725.

2. Bertalot G, Villanacci V, Gramegna M, Orvieto E, Negrini R, Saleri A, Terraroli C, Ravelli P, Cestari R, Viale G. Evidence of Epstein-Barr virus infection in ulcerative colitis. Dig Liver Dis. 2001; 33(7): 551-558.

3. Kangro HO, Chong SK, Hardiman A, Heath RB, Walker Smith JA. A prospective study of viral and mycoplasma infections in chronic inflammatory bowel disease. Gastroenterology. 1990; 98: 549-553.

4. Vega R, Bertran X, Menacho M, et al. Cytomegalovirus infection inpatients with inflammatory bowel disease. Am J Gastroenterol. 1999; 94: 1053-1056.

5. Hamlin PJ, Shah MN, Scott N, et al. Systemic cytomegalovirus infection complicating ulcerative colitis: a case report and review of the literature. Postgrad Med J. 2004; 80: 233-235.

6. De Saussure P, Lavergne-Slove A, Mazeron MC, Alain S, Matuchansky C, Bouhnik Y. A prospective assessment of cytomegalovirus infection in active inflammatory bowel disease. Aliment Pharmacol Ther. 2004; 20: 1323-1327.

7. Kandiel A, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. Am J Gastroenterol. 2006; 101: 857-2865.

8. Juffermans NP, Jager A, Kersten MJ, Van Oers MH, Hommes DW. Epstein-Barr virus-related lymphomas in patients with inflammatory bowel disease. Ned Tijdschr Geneeskd. 2005; 149(33): 1859-1863.

9. Zaravinos A, Bizakis J, Spandidos DA. Prevalence of human papilloma virus and human herpes virus type 1-7 in human nasal polyposis. J Med Virol. 2009; 81(9): 1613-1619.

10. Powell RD, Warner NE, Levine RS, Kirsner JB. Cytomegalovirus inclusion disease and ulcerative colitis: report of a case in a young adult. Am J Med. 1961; 30: 334-340.

11. Orvar K, Murray J, Carmen G, Conklin J. Cytomegalovirus infection associated with onset of inflammatory bowel disease. Dig Dis Sci. 1993; 38: 2307-2310.

12. Yi F, Zhao J, Luckheeram RV, Lei Y, Wang C, Huang S, Song L, Wang W, Xia B. The prevalence and risk factors of cytomegalovirus infection in inflammatory bowel disease in Wuhan, central China. Virology Journal. 2013; 43: 1-10.

13. Dimitroulia E, Pitirga VC, Piperaki ET, Spanakis NE, Tsakris A. Inflammatory bowel disease exacerbation associated with Epstein-Barr virus infection. Dis Colon Rectum. 2013; 56(3): 322-327.

14. 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: 1155-1160.

15. Gehlert T, Devergne O, Niedobitek G. Epstein-Barr virus (EBV) infection and expression of the interleukin-12 family member EBV-induced gene 3 (EBI3) in chronic inflammatory bowel disease. J Med Virol. 2004; 73(3): 432-438.

16. Spieker T, Herbst H. Distribution and phenotype of Epstein-Barr virus-infected cells in inflammatory bowel disease. Am J Pathol. 2000; 157(1): 51-57.

17. Yanai H, Shimizu N, Nagasaki S, Mitani N, Okita K. Epstein-Barr virus infection of the colon with inflammatory bowel disease. Am J Gastroenterol. 1999; 94(6): 1582-1586.

18. Wakefield AJ, Fox JD, Sawyerr AM, Taylor JE, Sweenie CH, Smith M, Emery VC, Hudson M, Tedder RS, Pounder RE. Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and crohn's disease using the nested polymerase chain

reaction. J Med Virol. 1992; 38: 183-190.
19. Hommes DW, Sterringa G, Van Deventer SJ. The pathogenicity of cytomegalovirus in inflammatory bowel disease: a systematic review and evidence-based recommendations for future research. Inflamm Bowel Dis. 2004; 10: 245-250.
20. Leveque N, Brixi-Benmansour H, Reig T, Renois F, Talmud D, Brodard V, Coste JF, et al. Low frequenty of cytomegalovirus infection during exacerbation of inflammatory bowel disease. J Med Virol. 2010; 82: 1694-1700.
21. Takeda Y, Takeda K, Togashi H, Takeda H, Sakano M, Osada Y, Shinzawa H, Takahashi T. Demonstration of Epstein-Barr virus localized in the colonic and ileal mucosa of a patient with ulcerative colitis. Gastrointest Endosc. 2000; 51: 205-209.
22. Ryan JL, Shen YJ, Morgen DR, Thorne LB, Kenney SC, Dominguez RL, Gulley ML. Epstein-Barr virus infection is common in inflamed gastrointestinal mucosa. Dig Dis Sci. 2012; 57(7): 1887-1898.