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Original Article

Analysis ulcerative colitis for presence Epstein-Barr virus DNA sequences by polymerase chain reaction technique

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Abstract

Introduction: Ulcerative colitis (UC) is one type of inflammatory bowel disease (IBD). The purpose of this study is to explore the prevalence of Epstein–Barr virus (EBV) in UC patients in comparison with healthy subjects using the polymerase chain reaction (PCR) method.

Methods: In this case-control study, five biopsies of patients with UC and 30 healthy people as controls were selected. Sampling was performed by endoscopic biopsy operation. After DNA extraction, PCR was used to determine EBV genome by specific primers. Statistical analysis was performed using the chi-square test.

Results: The results of PCR indicated that EBV genome was detected in 60.0% of samples in the case group, and 36.7% of samples in the control group were positive for EBV. Thus, no significant association was observed between the prevalence of EBV and incidence of UC in comparison with the control group (P = 0.36).

Conclusion: The findings presented herein demonstrate no direct molecular evidence to support an association of EBV with UC. These results, do not exclude the possibility oncogenic role of EBV to infect the different colon cell.

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Introduction

Epstein–Barr virus (EBV) is a ubiquitous herpes virus that infects and establishes a persistent infection in the host. Clinically, its primary infection ranges from a mild self-limited illness in children to infectious mononucleosis in adolescents and adults.[1,2] EBV is associated with a number of human malignancies, including Burkitt lymphoma and nasopharyngeal carcinoma, etc. Inflammatory bowel disease (IBD) represents a group of idiopathic chronic inflammatory intestinal conditions. Ulcerative colitis (UC) is a form of IBD that causes inflammation and ulcers in the colon. UC is slightly a more common in women than in men. The exact etiology of UC is unknown, but the disease appears to be multifactorial, and polygenic proposed causes include environmental factors, immune dysfunction, and a likely genetic predisposition. Recently, involvement of EBV has been demonstrated in IBD.[3-9] In these patients, EBV has been recognized in colonoscopic biopsy specimens obtained during evaluation and management of IBD or diagnosed after pathologic examination of the colon.

Magro et al.[10] showed IBD is a risk factor

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for the presence of EBV DNA in blood. However, prospective studies examining the prevalence of EBV genome in patients with IBD in comparison with a control population are limited. EBV-infection in IBD patients often makes clinical diagnosis and treatment complex. Patients with IBD treated with immunosuppressive agents including corticosteroids, cyclosporine, azathioprine, and methotrexate, either alone or in combination. Juffermans et al.[11] used antiviral drugs for the treatment of EBV-positive colitis and showed azathioprine is an important drug in IBD patient. 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 present study to investigate the prevalence of EBV in patients with UC in comparison with healthy subjects using polymerase chain reaction (PCR) technique conducted.

Methods

In this 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. We enrolled five patients with UC and 30 patients without malignancy as the controls. Sampling was performed by endoscopic biopsy, and a tissue sample size of 25 mg was calculated for each patient. All collected tissues were kept frozen under -20 °C until analysis.

DNA extraction

DNA was extracted using the KiaSpin® Tissue Kit (Kiagen CA, Iran) according to the manufacturer's instructions. The concentration of the sample absorbance at a wavelength to 260 nm was determined by BioPhotometer system (Eppendrof, Germany). Absorbances at 280/260 and 230/260 nm were used to determine sample purity.

PCR

We performed PCR amplification of the human β-globulin gene (Table 1) to determine the quality of extracted DNA.[12] The PCR reaction mixture contained 5 µl DNA template, 10 µl prime Taq premix (2×; Kiagen CA, Iran), 3 µl of sterile distilled water, 1 µl of forward and reverse primers (TAG Copenhagen, Denmark). The PCR reaction was carried out as follows: an initial incubation for 5 minutes at 95 °C, the samples were subjected to 35 amplification cycles at 95 °C for 50 seconds, 55 °C for 45 seconds, 72 °C for 40 seconds and a final elongation at 72 °C for 5 minutes.

We used specific primers to reproduce the EBV genome from the samples (Table 1).[12] The PCR reaction mixture contained 5 µl DNA template, 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). The PCR reaction was carried out as follows: initial incubation for 5 minutes at 95 °C, the samples were subjected to 35 amplification cycles at 95 °C for 40 seconds, 65 °C for 40 seconds, 72 °C for 40 seconds, and a final elongation at 72 °C for 5 minutes. At the end of amplification, 5 µl of the PCR products was analyzed on a 1.5% agarose gels.

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

Results

EBV DNA was found in 60.0% 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 with the control group ($P = 0.36$). We observed the highest prevalence of EBV in UC patients older than 55 years (60.0%) of age and in the normal control participants who were 35-55 years of age (16.6%). In terms of gender, the highest prevalence of EBV was observed in male UC patients (60.0%) and in normal control group female (26.6%) ($P > 0.05$) (Table 2).

Table 1. Primers sequences and base pair (bp) length

Primer	Sequence (5´-3´)	Size (bp)	References
b_2-F	TCCAACATCAACATCTTGGT	106	12
b_2-R	TCCCCCAAATTCTAAGCAGA	102	12
EBV-F	GTGTGCGTCGTGCCGGGGCAGCCAC		
EBV-R	ACCTGGGAGGGCCATCGCAAGCTCC		

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 EBV-specific primers was performed where we observed 102 bp bands that represented the replication (Figure 2).

Table 2. Clinical and pathological features of the ulcerative colitis and control group patients related to the presence of Epstein-Barr virus

Patients	EBV DNA		P	Total [n (%)]
	Positive [n (%)]	Negative [n (%)]		
UC				
Age groups			> 0.05	
Under 35 years	0 (0)	0 (0)		0 (0)
35-55 years	0 (0)	2 (40.0)		2 (40.0)
Over 55 years	3 (60.0)	0 (0)		3 (60.0)
Gender			> 0.05	
Male	3 (60.0)	0 (0)		3 (60.0)
Female	0 (0)	2 (40.0)		2 (40.0)
Total	3 (60.0)	2 (40.0)		5 (100)
Control group				
Age groups			> 0.05	
Under 35 years	4 (13.4)	5 (16.6)		9 (30.0)
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 (30.0)		12 (40.1)
Female	8 (26.6)	10 (33.3)		18 (59.9)
Total	11 (36.7)	19 (63.3)		30 (100)

UC: Ulcerative colitis; EBV: Epstein-Barr virus

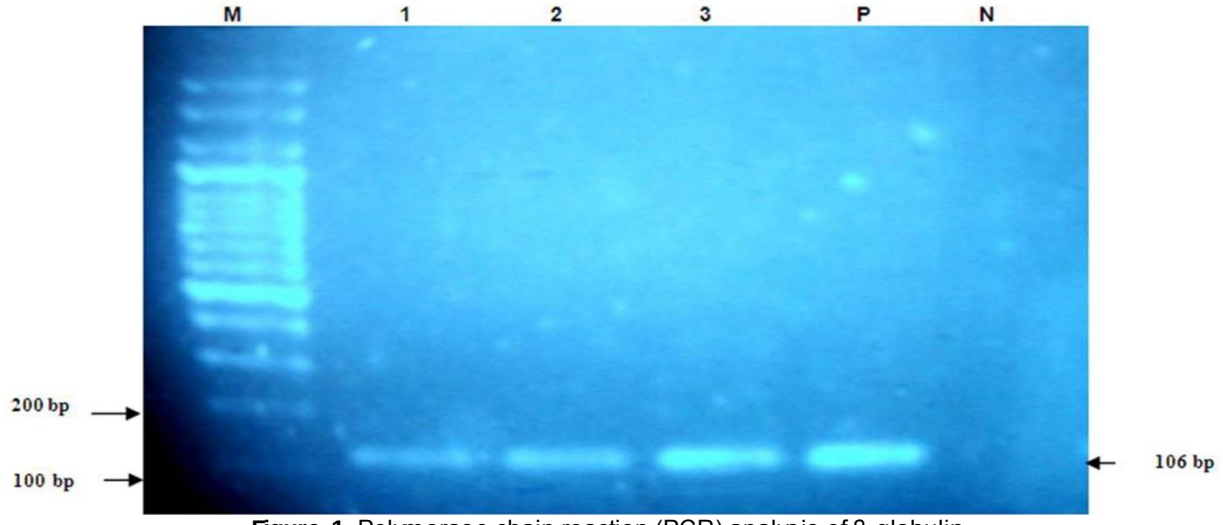

Figure 1. Polymerase chain reaction (PCR) analysis of β-globulin

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)

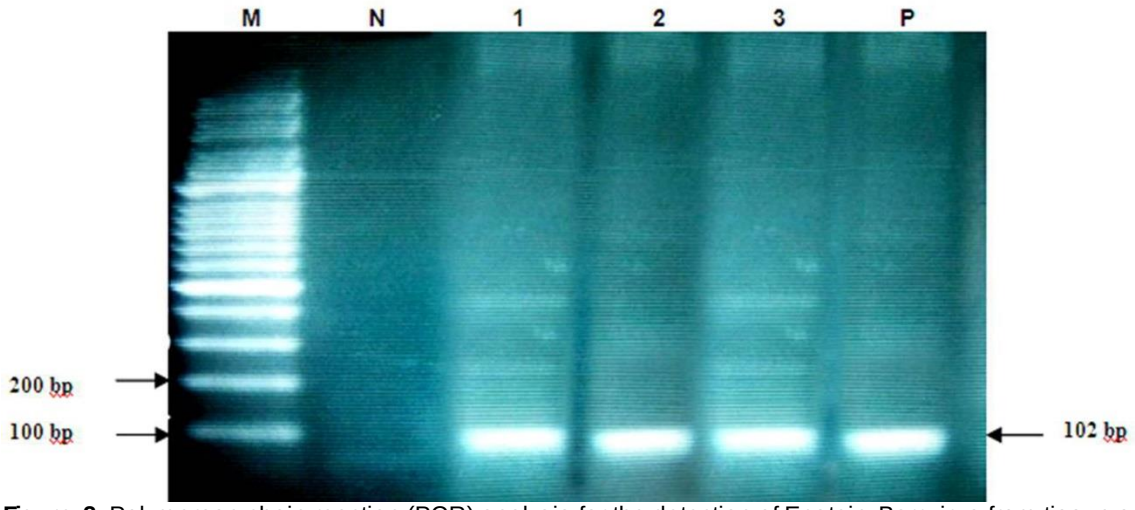

Figure 2. Polymerase chain reaction (PCR) analysis for the detection of Epstein-Barr virus from tissue samples
DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 102 bp.
Positive control (P); negative control (N); clinical samples, lanes 1, 2 and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

Discussion

We investigated UC and healthy tissues for the presence of EBV DNA by the PCR method. In UC patients, we detected EBV DNA in 60.0% of samples. In the healthy control group, 36.7% had EBV DNA. There was no association between EBV DNA presence and occurrence of UC as compared with control group tissue samples.

EBV is an opportunistic pathogenic microorganism. During recent years, a clear association between complicated courses of UC and the presence of EBV has been established. The exact pathogenic role of EBV in these patients remains unclear despite a great number of published reports. However, the role of EBV in UC patients has not been reported in the literature in Iran until now. This is the first study to investigate the prevalence of EBV in UC patients in Iran. The importance of EBV as an exacerbating factor of UC is neglected by many clinicians, but the coincidental diagnosis of UC and EBV colitis has also been reported.[9] EBV-infection in UC patients, especially in those who are immune-compromised by steroid therapy, can produce sever systemic disease and often leads to colectomy. 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 EBV.[4-7,13]

In the current study, has shown the presence of EBV sequences in UC and healthy tissues by PCR method reflects the ability of the virus to infect of the different colon cells. Although our results confirmed the results of Takeda et al.[14] 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.[3-9] Yanai et al.[4] found that EBV was detected in 63.3% of Crohn's disease (CD) cases and 60.0% of UC cases using in situ hybridization for EBV-encoded small RNA 1, indicating that EBV-infection may be related to IBD colonic diseases. Gehlert et al.[6] 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.[8] detected EBV DNA in 55.0% of CD and 64.0% of UC tissues, with mean viral loads significantly higher in these lesions than in normal colon tissues. Dimitroulia et al.[9] 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.[5] showed a possible role of EBV in infection of UC patients. Kangro et al.[3] reported EBV-

infection was associated with UC. Furthermore, Spieker and Herbst[7] 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 indicating that the IBD exacerbation associated with EBV-infection.[3-9]

Conclusion

The findings presented herein demonstrate no direct molecular evidence to support an association of EBV with UC. These results do not exclude the possibility of an oncogenic role for EBV to infect various colon cells. The carcinogenesis mechanism needs to be clarified further.

Conflict of Interests

Authors have no conflict of interest.

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