ORIGINAL ARTICLE

SERO-PREVALENCE OF EPSTEIN-BARR VIRUS IN IRAQI INFLAMMATORY BOWEL DISEASE

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ABSTRACT

The aim: Determine the frequency of anti-viral capsid antigen IgM, IgG and IgA in inflammatory bowel disease.

Materials and methods: Case controlled study conducted during involved 60 Crohn's disease, 60 ulcerative colitis and 60 subjects as a control group with negative gastrointestinal symptoms. Diagnosis and disease classification were established according to Montreal disease classification of inflammatory bowel diseases. Measurement of serum anti-VCA IgM, IgG and IgA was done, using ELISA method.

Results: The current results showed a higher frequency of EBV seropositivity among both Crohn's disease and ulcerative colitis 96.67% in comparison with controls 78.33. None statistical significance observed according to sex of patients. IgM were significantly associated with younger than 16 years 33.33%. IgA anti-VCA were significantly frequent within 17-40 years old comprising 100%. Patients with colonic and ileocolonic site of lesions were significantly have frequent anti-VCA IgA 96.43% and 96%. In ulcerative colitis IgM subtype of anti-VCA 35.71% frequent in extensive colitis. Anti-VCA IgG were statistically significant with moderate and severe ulcerative colitis cases 100%. Also, anti-VCA IgA associated with severity of ulcerative colitis 100% of mild cases, 96.43% of moderate cases and 100% of severe cases.

Conclusion: EBV seropositivity were detected among IBD cases, however viral infection might be associated with distinct and severe cases that requires anti-viral therapy.

KEY WORDS: anti-viral capsid antigen, inflammatory bowel disease, Epstein-Barr virus

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INTRODUCTION

Inflammatory bowel disease (IBD) is a complex multifactorial chronic inflammatory disease with unknown etiology, viral infections might modulate disease courses as a relapsing-remittent or progressive inflammatory condition that affects the gastrointestinal tract [1-2]. An original description of Epstein Barr virus was originally detected in 1971 by Grotsky, et al., [3]. Then a lot of literatures supports the association between Epstein-Barr virus (EBV) and ulcerative colitis and Crohn's disease (CD) [4]. EBV transmission through the oropharyngeal mucosal epithelium is crucial to the entrance of the virus into the body. Mucosa associated lymphoid tissue (MALT) is essential in EBV infection because viral particles colonize tongue, oropharyngeal mucosa and salivary glands, later, they infect B cells, establishing the latent infection [5]. It has been suggested that extirpation of MALT as tonsils and appendix could be related with CD, although there are many discrepancies about this theory. The association were based on detection of EBV protein or its nucleic acid using immunohistochemistry, in situ hybridization and polymerase chain reaction [6]. Studies suggested that EBV infection might occur in young age groups mostly with malignancy associated inflammatory bowel disease [7]. Very high viral DNA have been found in immunosuppressed patients undergoing treatment with biological drugs such as Inflaximab [8]. Furthermore, EBV might exacerbate colitis [9], or symptoms [10].

THE AIM

The aim of this work was the study of the sero-prevalence of anti-viral capsid antigen IgG, IgA and IgM antibodies in inflammatory bowel disease patients and their relation with different clinical classifications criteria.

MATERIALS AND METHODS

STUDY POPULATION

In this case-controlled study, the sampling was done during June, 2017 to February, 2019. 120 Serum samples were collected from inflammatory bowel disease patients attending Gastroenterology units in AL-Emamain AL-Kadhemain Medical City and Gastroenterology and hepatology hospital from 60 Crohn's disease and 60 ulcerative colitis patients and maintained at -20°C until later serological analysis. In addition to that, 60 age-sex matched controls were collected for comparison purpose. Diagnosis

Table I. Study groups demographics and clinical data

Study groups		Crohn's Disease (n=60)	Ulcerative Colitis (n=60)	Control (n=60)
Female (%) NS		42(70)	45(75)	45(75)
Age (year) #, NS		39.7±12.5	42.2±8.80	40.11±1.24
Smokers (%) NS		12(20)	9(15)	10(16.67)
Age at diagnosis				
A1: Younger than 16		6(10)		
A2: 17-40 years old		40(66.67)		
A3: Older than 40		14(23.33)		
Disease behavior				
B1: Inflammatory		22(36.67)		
B2: Stenosing		17(28.33)		
B3: Penetrating		21(35)		
Disease location (CD)				
L1: Ileal		7(11.67)		
L2: Colonic		28(46.67)		
L3: Ileocolonic		25(41.67)		
Presence of extra-intestinal manifestations				
No		32(53.33)		
Yes		28(46.67)		
Need for surgery				
No		36(60)		
Yes		24(40)		
Disease location (UC)				
E1: ulcerative proctitis			16(26.67)	
E2: Left sided			30(50)	
E3: Extensive colitis			14(23.33)	
Disease severity (UC)				
Clinical remission (S0)			4(6.67)	
Mild UC (S1)			10(16.67)	
Moderate UC (S2)			28(46.67)	
Severe UC (S3)			18(30)	
Anti-VCA serology				
IgM	Positive	4(6.67)	6(10)	0(0)
	Negative	56(93.33)	54(90)	60(100)
IgG	Positive	58(96.67)	58(96.67)	47(78.33)
	Negative	2(3.33)	2(3.33)	13(21.67)
IgA	Positive	56(93.33)	57(95)	42(70)
	Negative	4(6.67)	3(5)	18(30)

#: data presented as mean and standard deviation

and disease classification were established according to Montreal disease classification of inflammatory bowel diseases [11]. Those subjects either previously or newly diagnosed as directed to do colonoscopy for complete their examination or receiving treatments (Infliximab and/or anti-inflammatory drugs).

DETERMINATION OF VIRAL CAPSID ANTIGEN IGG, IGM AND IGA

Qualitative ELISA kits were purchased from Abnova®, Taiwan using specific IgG (KA2254), IgM (KA2255) and IgA (KA0963) antibodies against EBV viral capsid antigen (VCA). The procedure of detection was carried out on the

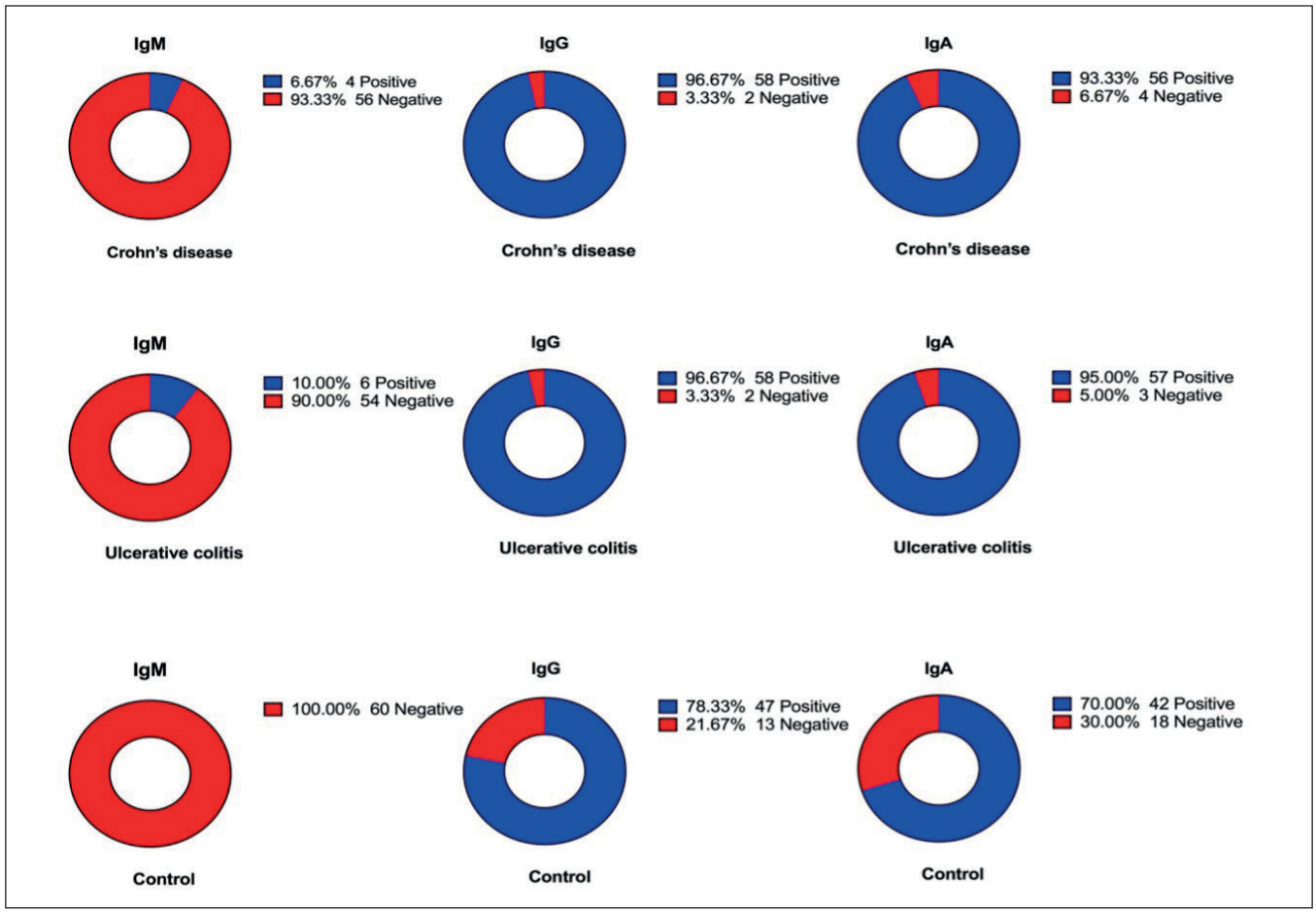

Fig. 1. Pie chart showing distribution of IgM, IgG and IgA anti-VCA serology among study groups.

serum of patients using ELISA test kits according to the manufacturer instructions.

STATISTICAL ANALYSIS

The statistical analysis of this case-controlled study performed with Graphpad Prism 7. Categorical data described as count and percentage. Chi-square or Fisher exact test used to describe the association of these data. Numerical data were described as mean, and standard deviation. Independent sample t-test used for comparison between two groups. The lower level of accepted statistically significant difference is bellow or equal to 0.05 [12].

RESULTS

DEMOGRAPHIC AND CLINICAL DATA

One hundred eighty samples were screened for IgM, IgG and IgA anti- viral capsid antigen of Epstein Barr virus in three equal subgroups including 60 patients with Crohn's disease, 60 patients with Ulcerative colitis and 60 healthy controls without gastrointestinal symptoms. Subject's demographics described in Table I and revealed none statistical significance among them in which females accounts 70% in Crohn's disease group and 75% in each of ulcerative colitis and control groups. Age of patients were relatively comparable among three groups accounts in Crohn's disease 39.7 years, 42.2 years in ulcerative colitis and 40.11 years for control group. 20% of Crohn's disease were smokers, 15% among ulcerative colitis and 16.67% were among controls. According to Montreal disease classification for Crohn's disease, the age at diagnosis showed 10% were younger than 16 years, 66.67% were ranged from 17 to 40 years and 23.33% were older than 0 years old. The disease behavior was 36.67% inflammatory, 28.33 were stenosing and 35 % penetrating behavior. The disease locations were classified into 11.67% with ileal lesions, 6.67% colonic and 1.67% were ileocolonic. 53.33% with extra-intestinal manifestations and 60% were on need for surgery. Ulcerative colitis was divided according to disease location, 26.67% were ulcerative proctitis, 50% were left sided, 23.33% were have had extensive colitis. Only 6.67% were get remission, 16.67% with mild symptoms, 6.67% with moderate symptoms and 30 % presented with severe symptoms.

HIGHER ANTI-VCA SEROLOGY FREQUENCY AMONG CROHN'S DISEASE AND ULCERATIVE COLITIS

The results of serological detection of anti-VCA IgM, IgG and IgA were summarized in table I and figure 1. The results indicated an increased frequency of IgM anti-VCA in

Table II. Association of IgM, IgG and IgA anti-VCA serology with Montreal disease classification of Crohn's disease.

Study groups	IgM				IgG				IgA			
	Positive		Negative		Positive		Negative		Positive		Negative	
Female [%]	3	7.14%	39	92.86%	41	97.62%	1	2.38%	40	95.24%	2	4.76%
Male [%]	1	5.56%	17	94.44%	17	94.44%	1	5.56%	16	88.89%	2	11.11%
P value	0.999[NS]				0.513[NS]				0.573[NS]			
Age at diagnosis												
A1: Younger than 16	2	33.33%	4	66.67%	6	100.00%	0	0.00%	5	83.33%	1	16.67%
A2: 17-40 years old	0	0.00%	40	100.00%	37	92.50%	3	7.50%	40	100.00%	0	0.00%
A3: Older than 40	2	14.29%	12	85.71%	13	92.86%	1	7.14%	11	78.57%	3	21.43%
P value	0.004*				0.787[NS]				0.013*			
Disease behavior												
B1: Inflammatory	2	9.09%	20	90.91%	22	100.00%	0	0.00%	21	95.45%	1	4.55%
B2: Stenosing	1	5.88%	16	94.12%	15	88.24%	2	11.76%	16	94.12%	1	5.88%
B3: Penetrating	1	4.76%	20	95.24%	19	90.48%	2	9.52%	19	90.48%	2	9.52%
P value	0.840[NS]				0.278[NS]				0.798[NS]			
Disease location (CD)												
L1: Ileal	0	0.00%	7	100.00%	6	85.71%	1	14.29%	5	71.43%	2	28.57%
L2: Colonic	3	10.71%	25	89.29%	27	96.43%	1	3.57%	27	96.43%	1	3.57%
L3: Ileocolonic	1	4.00%	24	96.00%	23	92.00%	2	8.00%	24	96.00%	1	4.00%
P value	0.460[NS]				0.561[NS]				0.047*			
Presence of extra-intestinal manifestations												
No	1	3.13%	31	96.88%	29	90.63%	3	9.38%	30	93.75%	2	6.25%
Yes	3	10.71%	25	89.29%	27	96.43%	1	3.57%	26	92.86%	2	7.14%
P value	0.239[NS]				0.368[NS]				0.890[NS]			
Need for surgery												
No	3	8.33%	33	91.67%	33	91.67%	3	8.33%	35	97.22%	1	2.78%
Yes	1	4.17%	23	95.83%	23	95.83%	1	4.17%	21	87.50%	3	12.50%
P value	0.526[NS]				0.526[NS]				0.139[NS]			

Data presented as count and percentage
NS: None statistical significance.
*: Statistical significance.
**: High statistical significance.

Crohn's disease 6.67%, ulcerative colitis 10% and none of controls 0%, while, IgG were 96.67% among Crohn's disease and ulcerative colitis and controls were 78.33%. IgA were positive in 93.33% among Crohn's disease. 95% were positive in ulcerative colitis and only 70% among controls.

ASSOCIATION OF ANTI-VCA SEROLOGY WITH MONTREAL DISEASE CLASSIFICATION OF CROHN'S DISEASE AND ULCERATIVE COLITIS

The results in table II showed the association of anti-VCA IgM, IgG and IgA serology with Montreal disease classifications criteria of Crohn's disease. The results showed IgM were significantly frequent in patients have younger than 16 years 2/6(33.33%), while IgG were none significantly associated with different ages at diagnosis. IgA serology were significantly frequent among all patients within 17-40 years old comprising 40(100%). Patients with colonic and ileocolonic site of lesions were significantly have frequent anti-VCA IgA 96.43% and 96% respectively than those with ileal disease location 71.43%, while IgM nor IgG didn't display significant association. Patients with ulcerative colitis have high statistical association of IgM subtype of anti-VCA (35.71%) among patients presented with extensive colitis. Anti-VCA IgG were statistically significant with more severe ulcerative colitis cases 28(100%) of moderate cases and 18(100%) of severe cases. Also, anti-VCA IgA subtype display high significant association with severity of ulcerative colitis 10 (100) of mild cases, 27(96.43%) of moderate cases and 18 (100%) of severe cases (Table III).

Table III. Association of IgM, IgG and IgA anti-VCA serology with Montreal disease classification of ulcerative colitis

Study groups	IgM				IgG				IgA			
	Positive		Negative		Positive		Negative		Positive		Negative	
Disease location (UC)												
E1: ulcerative proctitis	0	0.00%	16	100.00%	14	87.50%	2	12.50%	15	93.75%	1	6.25%
E2: Left sided	1	3.33%	29	96.67%	30	100.00%	0	0.00%	29	96.67%	1	3.33%
E3: Extensive colitis	5	35.71%	9	64.29%	14	100.00%	0	0.00%	13	92.86%	1	7.14%
P value	0.001**				0.058 NS				0.833 NS			
Disease severity (UC)												
Clinical remission (S0)	0	0.00%	4	100.00%	3	75.00%	1	25.00%	2	50.00%	2	50.00%
Mild UC (S1)	1	10.00%	9	90.00%	9	90.00%	1	10.00%	10	100.00%	0	0.00%
Moderate UC (S2)	2	7.14%	26	92.86%	28	100.00%	0	0.00%	27	96.43%	1	3.57%
Severe UC (S3)	3	16.67%	15	83.33%	18	100.00%	0	0.00%	18	100.00%	0	0.00%
P value	0.662 NS				0.032*				<0.001**			

Data presented as count and percentage
NS: None statistical significance
*: Statistical significance
**: High statistical significance

DISCUSSION

The link between Epstein Barr virus infection and inflammatory bowel disease have been assessed in this study through evaluation of serological values of anti-viral capsid antigen with IgM, IgG and IgA subtypes among Iraqi patients with Crohn's disease and ulcerative colitis. In the current study, the prevalence of EBV infection is 96.67% in both Crohn's disease and ulcerative colitis which is relatively higher than controls involved in this survey. Our records suggest no difference of seropositivity by sex among study groups. Younger ages display higher frequency of seropositivity as found by[13], IgA serology were detectable in higher frequency suggesting a strong interaction between immune response and viral entry at intestinal lining mucosa [5]. Even though studies have been describing that chronic active EBV infection of gastrointestinal tract display symptoms mimic the Crohn's disease [14]. Furthermore, our report found that EBV seropositivity associated with severity of ulcerative colitis cases. Since, majority of patients were under treatment of immunosuppressive treatment suggesting their impact on viral reactivation among IBD patients [15]. Even though, a rare case of EBV induce colitis in immunocompetent individuals [16]. The higher frequency of EBV infection among patients with inflamed intestinal mucosa have been reported with detection of EBV DNA in 55% of Crohn's disease and 64% of ulcerative colitis [17]. Our data describe association of colonic and ileocolonic site with anti-VCA IgA 96.43% and 96% respectively suggesting extensive mucosal immune responses for EBV. This occurs after virions entry to apical cells of mucosa and subsequent B cell entry in sub-epithelial zone [5]. However, mucosal immune response against EBV have potential protective mechanism since the anti-EBV sera were able to reduce virions entry to epithelial cells. In contrary, sub-epithelial T cells producing IL-5 and IL-6 were able to stimulate the proliferation of stimulated lymphocytes even infected with EBV [18-19]. EBV can escape from immune protective mechanisms have been reviewed [20]. EBV can produce IL-10 homologue that induce local immune regulation [21-22]. EBV infection could induce severe hemorrhagic diarrhea [23] and perforating colitis with IBD [24], other studies suggested causal association of EBV induce lymphoproliferative disease associated with inflammatory bowel disease such as: histiocytosis [25], malignant lymphoma [7]. Studies highlighted the presence of EBER1 associated latency of EBV in infected tissue [1]. The association of disease activity and EBV positivity have been reported [10], giving an advantage of using co- anti-viral therapy in addition to immunosuppressive treatment protocols for inflammatory bowel diseases [26].

CONCLUSION

EBV seropositivity were detected among IBD cases, however viral infection might be associated with distinct and severe cases that requires anti-viral therapy.

REFERENCES

1. 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.
2. Wu S, He C, Tang TY, Li YQ. A review on co-existent Epstein-Barr virus-induced complications in inflammatory bowel disease. Eur J Gastroenterol Hepatol. 2019;31(9):1085-1091
3. Grotsky HW, Hirshaut Y, Sorokin C, Sachar D, Janowitz HD, Glade PR. Epstein-barr virus and inflammatory bowel disease. Experientia. 1971;27(12):1474-1475.
4. Nissen LHC, Nagtegaal ID, de Jong DJ, et al. Epstein-Barr virus in inflammatory bowel disease: the spectrum of intestinal lymphoproliferative disorders. J Crohns Colitis. 2015;9(5):398-403.
5. Tugizov SM, Herrera R, Palefsky JM. Epstein-Barr Virus Transcytosis through Polarized Oral Epithelial Cells. J Virol. 2013;87(14):8179-8194.
6. Bertalot G, Villanacci V, Gramegna M, et al. Evidence of Epstein-Barr virus infection in ulcerative colitis. Dig Liver Dis. 2001;33(7):551-558.
7. Vos ACW, Bakkal N, Minnee RC, et al. Risk of malignant lymphoma in patients with inflammatory bowel diseases: A Dutch nationwide study. Inflamm Bowel Dis. 2011;17(9):1837-1845.
8. Magro F, Santos-Antunes J, Albuquerque A, et al. Epstein-barr virus in inflammatory bowel disease-correlation with different therapeutic regimens. Inflamm Bowel Dis. 2013;19(8):1710-1716.
9. Andari S, Hussein H, Fadlallah S, et al. Epstein–barr virus dna exacerbates colitis symptoms in a mouse model of inflammatory bowel disease. Viruses. 2021;13(7):1-14.
10. Dimitroulia E, Pitiriga 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.
11. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: Controversies, consensus, and implications. Gut. 2006;55(6)749-753.
12. Pearce N. What does the odds ratio estimate in a case-control study? Int J Epidemiol. 1993;22:1189-1192.
13. de Francisco R, Castaño-García A, Martínez-González S, et al. Impact of Epstein-Barr virus serological status on clinical outcomes in adult patients with inflammatory bowel disease. Aliment Pharmacol Ther. 2018;48(7):723-730.
14. Xu W, Jiang X, Chen J, et al. Chronic active Epstein-Barr virus infection involving gastrointestinal tract mimicking inflammatory bowel disease. BMC Gastroenterol. 2020;20(1):1-7.
15. Wagner J, Sim WH, Lee KJ, Kirkwood CD. Current knowledge and systematic review of viruses associated with Crohn's disease. Rev Med Virol. 2013;23(3):145-171.
16. Choi C, Yu Q, Deb PQ, Wang W. Rare case of EBV-induced colitis in an immunocompetent individual. BMJ Open Gastroenterol. 2020;7(1):1-3.
17. Ryan JL, Shen YJ, Morgan DR, et al. Epstein-Barr Virus Infection Is Common in Inflamed Gastrointestinal Mucosa. Dig Dis Sci. 2012;57(7):1887-1898.
18. Baumann MA, Paul CC. Interleukin-5 is an autocrine growth factor for Epstein-Barr virus- transformed B lymphocytes. Blood. 1992;79(7):1763-1767.
19. Tosato G, Tanner J, Jones KD, Revel M, Pike SE. Identification of interleukin-6 as an autocrine growth factor for Epstein-Barr virus-immortalized B cells. J Virol. 1990;64(6):3033-3041.
20. Loewendorf A, Benedict CA. Modulation of host innate and adaptive immune defenses by cytomegalovirus: Timing is everything: Symposium. J Intern Med. 2010;267(5):483-501
21. Burdin BN, Catherine P, Banchereau J, Rousset F. Lymphocytes to Produce Human Interleukin 10. J Exp Med. 1993;177(February):1-10
22. Slobedman B, Barry PA, Spencer JV, Avdic S, Abendroth A. Virus-Encoded Homologs of Cellular Interleukin-10 and Their Control of Host Immune Function. J Virol. 2009;83(19):9618-9629.
23. Denicola RP, Coben R, Katz L, McCue PA. Acute Gastrointestinal Hemorrhage due to Epstein-Barr Virus Colitis. ACG Case Reports J. 2019;6(10):e00238
24. Zhou JQ, Zeng L, Zhang Q, et al. Clinical features of Epstein-Barr virus in the intestinal mucosa and blood of patients with inflammatory bowel disease. Saudi J Gastroenterol. 2020;26(6):312-320.
25. Chapman S, El-Matary W. Screening for Epstein-Barr Virus Status and Risk of Hemophagocytic Lymphohistiocytosis in Children With Inflammatory Bowel Disease on Azathioprine. Gastroenterology. 2017;153(4):1167-1168.
26. Jones A, McCurdy JD, Loftus EV, et al. Effects of antiviral therapy for patients with inflammatory bowel disease and a positive intestinal biopsy forcytomegalovirus. Clin Gastroenterol Hepatol. 2015;13(5):949-955.

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Conflict of interest:

The Authors declare no conflict of interest.

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