Original Article

Epstein-Barr Virus Infection in Inflammatory Bowel Disease Patients: Its Relationship with Immunosuppressive Therapy, Liver Dysfunction, and Oncogenic Risk Markers in Kirkuk City, Iraq

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Abstract

Background: Epstein-Barr Virus (EBV) is the first identified human oncogenic virus and has been increasingly linked to the pathogenesis of inflammatory bowel disease (IBD), particularly in patients undergoing immunosuppressive therapy. The virus is believed to contribute to disease worsening and liver dysfunction in this vulnerable population. **Objective:** This study aimed to assess the prevalence of EBV infection among patients with IBD and to explore its potential association with immunosuppressive treatments and liver enzyme abnormalities. **Methods:** Case-control study was conducted involving 100 IBD patients (56 with ulcerative colitis and 44 with Crohn's disease) and 100 healthy controls. Serum samples were analyzed for EBV VCA IgM, VCA IgG, and EBNA-1 IgG using ELISA. Liver function tests (ALT, AST, TSB, ALP, and GGT) were performed. EBV DNA was detected using real-time polymerase chain reaction (PCR). **Results:** EBV seropositivity was significantly higher in IBD patients (UC: 76.7%; CD: 70.4%) compared to controls (6%) ($p < 0.0001$). EBV DNA was detected in 22% of IBD patients. The highest rates of positivity were observed among those receiving azathioprine (UC: 94%, CD: 90%). Abnormal liver enzyme levels were strongly associated with EBV positivity, particularly elevated ALT, AST, and TSB in both UC and CD groups. **Conclusion:** These findings suggest a possible link between EBV infection, immunosuppressive therapy, and hepatic dysfunction in IBD patients. Screening for EBV before initiating immunosuppressive treatment may be beneficial in managing potential complications.

Keywords: Epstein-Barr Virus, Immunosuppressive Therapy, Inflammatory Bowel Disease, Liver Enzymes, Real-time PCR.

Introduction

Inflammatory bowel disease (IBD) is a chronic, immune-mediated condition that includes two main clinical entities: Crohn's disease (CD), which can affect any part of the gastrointestinal tract, and ulcerative colitis (UC), which is limited to the colonic mucosa. Despite ongoing research, the exact etiology of IBD remains unclear, but it is believed to arise from complex interactions among genetic susceptibility, environmental triggers, intestinal microbiota, and dysregulated immune responses.[1-4]

Liver involvement is a well-documented extraintestinal manifestation of IBD, with elevated liver enzymes (ELE) reported in up to one-third of patients.[2] Several factors may contribute to hepatic dysfunction in IBD, including primary sclerosing cholangitis, drug-induced hepatotoxicity, and viral infections. Among these, herpesviruses—particularly Epstein-Barr virus (EBV)—have gained attention for their potential role in immune modulation and liver injury.[5,6]

EBV is a ubiquitous gamma herpesvirus that infects

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over 90% of the global population. While primary infection is usually asymptomatic or presents as infectious mononucleosis, EBV can persist in a latent state within B cells and reactivate under conditions of immunosuppression or chronic inflammation.[7,8] Such reactivation has been associated with hepatitis ranging from mild transaminase elevation to fulminant liver failure, particularly in immunocompromised hosts.

Furthermore, EBV has been proposed as a potential cofactor in the development of hepatocellular carcinoma (HCC), especially in patients with chronic liver disease or co-infection with hepatitis B or C viruses.[9] In the context of IBD, EBV reactivation may exacerbate mucosal inflammation and contribute to disease complications. Elevated liver enzymes in EBV infection may reflect either hepatocellular or cholestatic patterns, including increased levels of ALT, AST, ALP, and GGT, and can occasionally progress to severe outcomes such as cholestatic hepatitis or liver failure.[10-12]

Given the growing use of immunosuppressive therapies in IBD management, especially thiopurines and biologic agents, it is critical to explore the implications of latent viral reactivation. In particular, the interaction between EBV and these therapies may influence disease course and hepatic outcomes. However, data on EBV prevalence and its clinical consequences in IBD patients from the Middle East are limited.

This study, therefore, aims to investigate the prevalence of EBV infection in patients with IBD and assess its possible association with immunosuppressive treatment and hepatic dysfunction in a cohort from Kirkuk, Iraq.

Materials and Methods

Study Design

This study included 100 patients diagnosed with inflammatory bowel disease (IBD), comprising 56 with ulcerative colitis (UC) and 44 with Crohn's disease (CD). All participants were recruited from Azadi Teaching Hospital in Kirkuk, Iraq, between September and November 2024. A comparison group of 100 healthy individuals matched for age and sex wPatient Selection and Inclusion Criteria

Patient Selection and Inclusion Criteria

All patients were clinically and endoscopically confirmed cases of IBD, aged between 8 and 62 years, and all were under active treatment with at least one of the following: aminosalicylates (5-ASA), immunosuppressive agents (azathioprine or methotrexate), or biologic therapies (infliximab or adalimumab). None had received antiviral therapy in the previous six months. Written informed consent was obtained from all participants or their legal guardians.

Exclusion Criteria

Individuals were excluded if they had any of the following: coexisting chronic liver diseases (such as hepatitis A, B, or C; autoimmune hepatitis; or alcoholic liver disease), known immunodeficiency (e.g., HIV infection), prior organ transplantation, or current use of antiviral agents. Patients with incomplete clinical records or who declined to participate were also excluded.

Sample Collection and Laboratory Procedures

Five milliliters of peripheral blood were drawn from each participant. Samples were centrifuged to separate serum, which was then stored at –80°C for subsequent analyses.

Serological Testing

EBV-specific antibodies, including viral capsid antigen (VCA) IgM and IgG, and Epstein-Barr nuclear antigen-1 (EBNA-1) IgG, were detected using a commercial ELISA kit (Sunlong Biotech, China), following the manufacturer's protocol.

Liver Function Tests (LFTs)

Biochemical parameters were measured using a standard clinical chemistry analyzer and Biolab reagent kits (France), assessing levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin (TSB), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT).

Molecular Detection of EBV DNA

DNA was extracted from blood samples using the QIAamp DNA Mini Kit (Qiagen, Germany). EBV DNA was amplified using a commercial real-time PCR kit (DNA-Technology Research & Production LLC, Russia), which targets a conserved sequence in the EBNA-1 gene. Amplification was monitored using fluorescent dyes and a probe specific to the target sequence, allowing real-time quantification without reopening the reaction tubes.

Statistical Analysis

Data were analyzed using SPSS software. Chi-square tests were employed to assess associations between categorical variables. A p-value of less than 0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was approved by the Director of Health in Kirkuk. Participant information was collected in accordance with ethical guidelines, and the study protocol, including data handling and On Septembert 10, 2024, the consent form was examined and accepted by the local ethics committee (698).

Study Samples and Methods

Five milliliters of blood were extracted from every individual. An automated pipette was used to separate the serum from the packed red blood cells (RBCs) after the blood sample had been allowed to coagulate and centrifuged for 15 minutes at 3,000 rpm. Following that, the serum was kept in sterile Eppendorf tubes at -80°C for ensuing genetic and serological examinations. Following the manufacturer's instructions (Sunlong, China), Epstein-Barr virus (EBV) VCA IgM, IgG, and EBNA-1 IgG antibodies were detected in all serum samples using enzyme-linked immunosorbent assays (ELISA), with

optical density (OD) assessed at 450 nm. Furthermore, the levels of liver enzymes such as gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total serum bilirubin (TSB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured using (Biolab kit/France)

DNA extraction for genetic detection of EBV

For viral DNA isolation, the procedure was extracted using the QIAamp DNA Mini Kit (Qiagen/Germany).

Real-time PCR for EBV Identification

EBV DNA was found using (DNA-Technology Research & Production LLC, Russia Kit) by real-time polymerase chain reaction (RT-PCR), which amplifies a conserved sequence in the single-copy gene that codes for Epstein-Barr nuclear antigen 1 (EBNA-1). Fluorescent dyes were employed in real-time PCR to track the amplification process. A dye linked to a probe, which binds precisely to the amplified target sequence, is used in thermoselective amplification. Because fluorescence intensity was evaluated in real-time, the accumulation of the amplified product could be continually detected without the need to reopen reaction tubes after each cycle.

The amplification curve shown in Figure 1 was directly generated from patient samples processed in our laboratory during the current study.

Calculation of the PCR value

The instrument software analyzes the data based on the fluorescence curve crossing the threshold line.

Editorial Assistance

To improve the academic clarity of this manuscript, the authors used ChatGPT (OpenAI, 2025) to assist with language editing. The tool was only used to enhance the readability and grammar of the text and was not involved in the scientific design, data analysis, or interpretation of results

Result

Table (I) shows The prevalence of EBV infection was significantly higher among patients with IBD compared to the control group. Specifically, 76.7% of patients with UC and 70.4% of those with CD tested positive for EBV serologically, whereas only 6% of the control group showed EBV positivity. This difference was statistically significant ($p < 0.0001$), indicating a strong association between EBV infection and IBD status.

Table 1: Prevalence of EBV Ab among Ulcerative Colitis (UC), Crohn's Disease (CD) Patients and Control Group.

Group	EBV Positive	EBV Negative	Total	p-value
UC Patients	43 (76.7%)	13 (23.3%)	56	
CD Patients	31 (70.4%)	13 (29.5%)	44	<0.0001
Controls	6 (6.0%)	94 (94.0%)	100	

Table (2) shows, Among patients with ulcerative colitis (UC), 5.3% tested positive for VCA IgM, 12.5% for VCA IgG, and 58.9% for EBNA-1 IgG. In Crohn's disease (CD) patients, the corresponding rates were 2.3% for VCA IgM, 6.8% for VCA IgG, and 61.3% for EBNA-1 IgG. In contrast, none of the healthy controls tested positive for VCA IgM or VCA IgG, and only 6% were positive for EBNA-1 IgG. Statistically significant differences were observed for VCA IgG ($p = 0.002$) and EBNA-1 IgG ($p < 0.0001$), but not for VCA IgM ($p = 0.071$). These findings suggest a significantly higher seroprevalence of past EBV exposure among IBD patients compared to healthy individuals.

Table 2: Prevalence of EBV Antibodies (VCA IgM, VCA IgG, EBNA-1 IgG) among UC, CD, and Control Groups.

Anti-EBV Ab		UC Patients N=56	CD Patients N=44	Control Group N=100	p-value
Anti-VCA	IgM +	3(5.3%)	1(2.3%)	0(0%)	0.071
	IgM -	53 (95%)	43(98%)	100(100%)	
Anti-VCA	IgG +	7 (12.5%)	3(6.8%)	0(0%)	0.002**
	IgG -	49 (87%)	41 (93%)	100(100%)	
Anti-EBNA-1	IgG +	33(58.9%)	27(61.3%)	6 (6%)	<0.0001***
	IgG -	23 (41%)	17(39%)	94(94%)	

*All patients who tested positive for VCA IgM also have VCA IgG and EBNA-1 IgG.
* **Abbreviations:** VCA: Viral Capsid Antigen; EBNA-1: Epstein-Barr Nuclear Antigen 1; UC: Ulcerative Colitis; CD: Crohn's Disease.
***Statistical test:** $p < 0.05$ considered significant.
*VCA: viral capsid Antigen, EBNA-1: Epstein-Barr Nuclear Antigen 1

Table 3: Correlation between EBV Serology Result and Treatment in UC Patients.

Drug used	VCA IgM		VCA IgG		EBNA-1 IgG		Total of	
	+VE	-VE	+VE	-VE	+ VE	-VE	EBV+	Patient
AZA	1(6.2%)	15(94%)	2(12.5%)	14(87%)	12(75%)	4(25%)	15(94%)	16
MTX	0(0%)	4(100%)	0(0%)	4(100%)	2(50%)	2(50%)	2(50%)	4
5-ASA	0(0%)	7(100%)	0(0%)	7(100%)	2(29%)	5(71%)	2(29%)	7
IFX	1(9%)	10(91%)	4(27%)	7(73%)	5(55%)	6(45%)	10(91%)	11
AdA	0(0%)	5(100%)	0(0%)	5(100%)	3(60%)	2(40%)	3(60%)	5
IFX+AZA	1(7.6%)	12(92%)	1(7.6%)	12(92%)	9(69%)	4(31%)	11(85%)	13
P-value	0.927		0.141		0.316		-	

5-ASA: Aminosalicylates, AZA: Azathioprine, MTX: Methotrexate, IFX: Infliximab, AdA: Adalinumab

Table (3) shows, Among ulcerative colitis patients, EBV seropositivity was most prevalent in those receiving azathioprine (94%) and infliximab (91%), followed closely by combination therapy with infliximab and azathioprine

(85%). Lower rates were observed in patients treated with adalimumab (60%), methotrexate (50%), and 5-ASA alone (29%). Despite these numerical differences, the associations between EBV seropositivity and treatment type did not reach statistical significance for any antibody marker (VCA IgM p = 0.927; VCA IgG p = 0.141; EBNA-1 IgG p = 0.316).

Table (4) shows, in patients with CD, the highest EBV seropositivity rates were observed among those receiving azathioprine (90%), infliximab (89%), and combination therapy with IFX and AZA (83%). In contrast, lower positivity rates were seen in patients on adalimumab (67%), 5-ASA (50%) and methotrexate (17%). Despite these differences, none of the associations reached statistical significance. The p-values for VCA IgM, VCA IgG, and EBNA-1 IgG were 0.262, 0.7422, and 0.146, respectively.

Table 4: Correlation between EBV Serology Result and Treatment in CD Patients.

Treatment	VCA IgM		VCA IgG		EBNA-1 IgG		Total of	
	+VE	-VE	+VE	-VE	+ VE	-VE	EBV+	Patient
AZA	0(0%)	10(100%)	1(10%)	9(90%)	8(80%)	2(20%)	9(90%)	10
MTX	0(0%)	6(100%)	0(0%)	6(100%)	1(17%)	5(83%)	1(17%)	6
5-ASA	0(0%)	4(100%)	0(0%)	4(100%)	2(50%)	2(50%)	2(50%)	4
IFX	0(9%)	9(100%)	1(11%)	8(89%)	7(78%)	2(22%)	8(89%)	9
AdA	0(0%)	9(100%)	0(0%)	9(100%)	6(67%)	2(33%)	6(67%)	9
IFX+AZA	1(17%)	5(83%)	1(17%)	5(17%)	3(50%)	3(50%)	5(83%)	6
P-value	0.262		0.742		0.146		-	

5-ASA: Aminosalicylates, AZA: Azathioprine, MTX: Methotrexate, IFX: Infliximab, AdA: Adalinumab

Table (5) real-time PCR-based detection of EBV DNA revealed that 22% of the total IBD patients tested positive for the virus, while 78% were negative. Among UC patients, the detection rate was 23%, slightly higher than the 20% observed in CD patients. However, this difference was not statistically significant (p = 0.93).

Table 5: Molecular Detection of EBV among Inflammatory Bowel Disease Patients.

Study Group	PCR Result		Total of Patients	P Value
	+VE	-VE		
UC patients	13 (23%)	43 (77%)	56 (100%)	0.93
CD patients	9 (20%)	35 (80%)	44 (100%)	
Total	22 (22%)	78 (78%)	100 (100%)	-

***PCR:** polymerase chain reaction

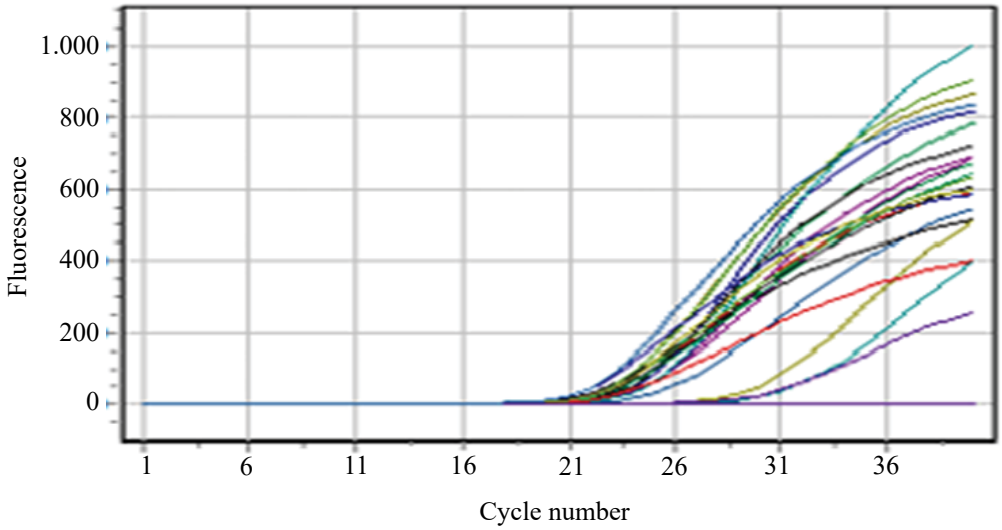

Figure 1: Real-time Polymerase Chain Reaction, FAM Channel Fluorescence Dependence on Cycle Number.

Table (6) A higher frequency of EBV DNA positivity was observed among IBD patients receiving azathioprine alone (38%) and those on combined azathioprine and infliximab therapy (37%), compared to lower rates in IFX (15%) and AdA (14%) monotherapy groups. In terms of disease duration, 24% of patients with more than one year of illness tested PCR-positive versus 18% of those with shorter disease duration. Furthermore, EBV DNA was detected in 26% of patients who had experienced more than five relapses, compared to 19% in those with fewer than five

Table 6: Distribution of EBV (PCR+) among IBD Patients based on to Type of Treatment, Disease Duration, and Number of Relapses.

Clinical and Therapeutic Variables		PCR Result		Total of Patients
		+ VE	-VE	
Disease duration	< 1 years	5 (18%)	23 (82%)	28 (100%)
	> 1 years	17 (24%)	55 (76%)	72 (100%)
	Total	22 (22%)	78 (78%)	100 (100%)
No. of Relapses	< 5 relapses	3 (19%)	13 (81%)	16 (100%)
	> 5 relapses	19 (26%)	55 (74%)	74 (100%)
	Total	22 (24%)	68 (76%)	90 (100)
Type of treatment	AZA	10 (38%)	16 (62%)	26 (100%)
	AZA+IFX	7 (37%)	12 (63%)	19 (100%)
	IFX	3 (15%)	17 (85%)	20 (100%)
	AdA	2 (14%)	12 (86%)	14 (100%)
	Total	22 (28%)	57 (72%)	79 (100%)

Table (7) Among UC patients with abnormal liver function, EBV infection was detected in 90% of those who had simultaneous elevation of ALT, AST, and TSB, and in all patients (100%) who had elevation across all measured liver enzymes. Those with elevated ALP and GGT showed a slightly lower EBV positivity rate of 75%. Despite these apparent trends, the association between EBV infection and liver enzyme abnormalities was not statistically significant (p = 0.629).

Table 7: Association of Abnormal Liver Enzyme Elevation with EBV Infection in UC Patients.

Abnormal Liver Enzyme Elevation	EBV+VE	EBV-VE	Total	P-value
Elevated AST, ALT, TSB	19 (90%)	2 (10%)	21 (100%)	0.629
Elevated ALP, GGT	3 (75%)	1 (25%)	4 (100%)	
Elevated all liver enzyme	5 (100%)	0 (0%)	5 (100%)	
Total	27 (90%)	3 (10%)	30 (100%)	

*AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSB: Total serum bilirubin; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase.

Table (8) In Crohn's disease patients, EBV infection was detected in 90% of those with elevated AST, ALT, and TSB, and in 80% of patients who had elevation across all measured liver enzymes. A lower EBV positivity rate (60%) was noted in patients with isolated ALP and GGT elevation. Although these findings suggest a possible trend linking EBV infection with hepatocellular injury, the association did not reach statistical significance (p = 0.507).

Table 8: Association between Abnormal Liver Enzyme Elevation and EBV Infection in CD Patients.

Abnormal Liver Enzyme Elevation	EBV+VE	EBV-VE	Total of Patients	P-value
Elevated AST, ALT, TSB	9 (90%)	1 (10%)	10 (100%)	0.507
Elevated ALP, GGT	3 (60%)	2 (40%)	5 (100%)	
Elevated all liver enzymes	4 (80%)	1 (20%)	5 (100%)	
Total	16 (80%)	4 (20%)	20 (100%)	

DISCUSSION

Epstein–Barr virus (EBV) has been implicated in the pathogenesis of various malignancies. Among its latent proteins, EBNA-1 is consistently expressed across all EBV-associated tumors and is considered a potential therapeutic target, particularly in immunocompromised hosts.[13,14] This highlights the importance of vigilant EBV monitoring in inflammatory bowel disease (IBD) patients, especially those receiving immunosuppressive therapy.

In the present study, the seroprevalence of EBV was investigated among IBD patients. Notably, VCA IgM positivity was detected in 4% of IBD cases, suggestive of either a primary acute infection or viral reactivation, while none of the controls tested positive. These findings are in agreement with the data reported by Ghazi *et al.*[15], who documented VCA IgM positivity in 10% of ulcerative colitis (UC) cases, 6.67% of Crohn's disease (CD) patients, and 0% of controls. The observed discrepancy in IgM levels between UC and CD patients may be attributed to distinct immunological mechanisms underlying each condition. CD is typically associated with a Th1/Th17-dominant immune response that confers stronger antiviral activity, while UC exhibits a Th2-skewed profile, which may be less efficient in suppressing viral replication.[16-18]

Serological analysis further revealed that 10% of patients were VCA IgG positive, while 60% exhibited simultaneous positivity for EBNA-1 and VCA IgG. According to the serological interpretation framework outlined by Ekşi *et al.*[19], the detection of all three antibodies (VCA IgM, IgG, and EBNA-1 IgG) is indicative of either viral reactivation or a late primary infection. The presence of VCA IgG alone may correspond to past or acute EBV infection, whereas the

co-detection of EBNA-1 IgG and VCA IgG typically signifies past infection and long-term immunological memory. The statistically significant elevation of EBNA-1 + VCA IgG positivity among IBD patients compared to controls (6%) underscores the persistent nature of EBV infection in the context of IBD.

These findings are consistent with Baran *et al.*[20], who identified EBNA-1 IgG and VCA IgG positivity in 74% and 67% of pediatric IBD cases, respectively, despite a younger cohort (mean age: 11 years). Rodríguez-Lago *et al.*[21] reported an even higher prevalence, with 97% of adult IBD patients testing positive for both antibodies. Furthermore, 94% of cases exhibited simultaneous VCA and EBNA-1 IgG positivity, reinforcing the notion of long-term viral persistence in the IBD population.

Due to the known limitations of serological testing in immunocompromised and pediatric patients, EBV DNA was assessed through real-time PCR as a complementary diagnostic approach.[22,23] EBV DNA was identified in 23% of UC and 20% of CD patients via real-time PCR, suggesting a role for the virus in a subset of IBD cases. This finding corroborates the observations of Kornitzer *et al.*[24], who reported EBV viremia in pediatric IBD cases, particularly after initiation of immunosuppressive agents.

Xu *et al.*[25] emphasized that chronic active EBV (CAEBV) infection can mimic IBD, with overlapping gastrointestinal manifestations and elevated blood EBV DNA levels. These findings underscore the clinical utility of quantitative viral load assessment to differentiate CAEBV from EBV-associated IBD. Additionally, the immunomodulatory impact of EBV in IBD patients under immunosuppressive treatment has been noted, with several studies demonstrating associations between EBV seropositivity and therapies such as thiopurines and anti-TNF agents.[26,27]

Immunosuppressive regimens, particularly azathioprine (AZA), have been implicated in increasing the risk of EBV reactivation and virus-associated complications. In this study, EBV seropositivity was observed in 94% of UC and 90% of CD patients receiving AZA monotherapy. Combination therapy with AZA and infliximab (IFX) was associated with similarly high positivity rates (85% in UC and 83% in CD). Notably, IgM positivity—suggestive of active or reactivating infection—was detected in 18%, 12.5%, and 7.6% of UC patients receiving IFX, AZA+IFX, and AZA, respectively, and in 17% of CD patients treated with AZA+IFX.

PCR testing supported these findings, revealing EBV DNA in 38% and 37% of patients on AZA and AZA+IFX, respectively. Comparatively lower positivity rates were recorded among IFX- (15%) and adalimumab (AdA)-treated patients (14%). These observations are aligned with Espinheira *et al.*[28], who found that 76% of pediatric IBD patients had previous EBV exposure, and with Bachmann *et al.*[29], who reported EBV seroconversion during thiopurine therapy.

The immunosuppressive effect of AZA on natural killer (NK) cells may reduce viral surveillance and contribute to severe outcomes.[30] Honkila *et al.*[31] described a case of life-threatening EBV infection in a pediatric UC patient on AZA, which resolved following AZA discontinuation and NK cell recovery. Similarly, Zhang *et al.*[32] and Levhar *et al.*[33] reported IFX-associated pulmonary EBV infection and B-cell proliferation with increased EBV DNA, respectively. Kato *et al.*[34] documented a case of hemophagocytic syndrome in an AdA-treated patient, emphasizing the potential severity of EBV reactivation during biologic therapy.

Although 5-ASA and methotrexate (MTX) demonstrated minimal influence on EBV markers in the present study, other reports suggest that mesalazine may modulate immune responses and warrant further investigation.[35] The current findings substantiate recommendations for baseline EBV screening prior to initiating thiopurines or biologic agents.[36]

In addition, EBV DNA positivity was more frequently observed among patients with prolonged disease duration (>1 year: 24%) and higher relapse frequency (>5 relapses: 26%). These trends are supported by Nunez Ortiz *et al.*[37], who linked mucosal EBV presence to heightened disease activity, increased endoscopic and histological severity, and elevated hospitalization rates. Similarly, Wang *et al.*[38] demonstrated that EBV prevalence increased with disease severity, ranging from 53.93% in mild to 94.9% in severe cases.

Histological inflammation was also significantly more pronounced among EBV-positive individuals (52%) compared to EBV-negative counterparts (17.2%) ($p=0.007$), as demonstrated by Núñez Ortiz *et al.*[39]. Zhou *et al.*[40] found a positive correlation between EBER-1 cell density and mucosal inflammation, identifying AZA use and advanced age as risk factors for EBV infection.

The hepatotropic potential of EBV is well documented, with manifestations ranging from transient enzyme elevations to fulminant hepatitis and hepatobiliary neoplasms.[10,11] Given that one-third of IBD patients exhibit elevated liver enzymes,[2] the current study evaluated the relationship between EBV infection and liver dysfunction, excluding cases positive for hepatitis A, B, or C viruses.

Among patients with elevated ALT, AST, and TSB, EBV was detected in 90% of both UC and CD cases. Moreover, EBV positivity was recorded in 100% of UC and 80% of CD patients with elevation in all measured liver enzymes. In contrast, only 10% (UC) and 20% (CD) of enzyme-elevated patients were EBV-negative. These findings support a link between EBV infection and hepatocellular injury in IBD.

Adelodun *et al.*[41] described a case of EBV-induced hepatitis with ALT and AST elevation, consistent with our observations. Persistent inflammation in IBD may compromise immune control of latent EBV, contributing to hepatic dysfunction.[42] While cholestatic patterns (ALP and GGT elevation) were less strongly associated with EBV, earlier studies have linked the virus to bile duct inflammation and cholestatic hepatitis.[12,43]

The clinical relevance of these findings lies in avoiding misdiagnosis of EBV-related hepatitis as autoimmune hepatitis, which may lead to unnecessary immunosuppression and further liver injury.[42] Singh *et al.*[44] emphasized that EBV hepatitis may lack classic symptoms, underscoring the need to include EBV in the differential diagnosis of liver dysfunction, particularly in immunosuppressed IBD patients.

Conclusion

This study highlights the potential involvement of EBV in the immunopathogenesis of IBD, particularly among patients receiving immunosuppressive therapies. EBV seropositivity and viremia were more prevalent in patients on thiopurines and anti-TNF agents, in those with prolonged disease duration, frequent relapses, and elevated liver enzymes. Given the virus's association with disease activity, hepatic dysfunction, and possibly malignancy, these findings support the incorporation of EBV screening into routine clinical assessment of IBD patients, especially prior to initiation of immunosuppressive therapy. Early detection and tailored monitoring may help prevent EBV-related complications and improve long-term clinical outcomes.

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