Academic Journal of International University of Erbil

Journal Homepage: https://ojs.cihanrtv.com/index.php/public

PRINT-ISSN: 2519-6928

Online-ISSN: 3080-7174

Serological Evidence of Epstein-Barr Virus Infection and Disease Activity Assessment in Rheumatoid Arthritis: A Case-Control Study in Erbil, Iraq

Kasya Aswad Othman[1*], Katan Sabr Ali[2]

[1,2] College of Health Sciences, Hawler Medical University, Erbil, IRAQ

DOI: https://doi.org/10.63841/iue31670

Received 20 Jul 2025; Accepted 18 Aug 2025; Available online 20 Jan 2026

ABSTRACT

Epstein-Barr virus (EBV) has been implicated in the pathogenesis of rheumatoid arthritis (RA) through molecular mimicry and immune dysregulation mechanisms. This study aimed to investigate the association between EBV infection markers and RA in patients from Erbil, Iraq, and to explore the relationships between viral antibody levels and disease activity. A case-control study was conducted involving 66 RA patients diagnosed according to ACR-EULAR 2010 criteria and 25 healthy controls from Rizgary Teaching Hospital, Erbil, Iraq. Serum levels of Epstein-Barr Nuclear Antigen-IgG and Viral Capsid Antigen-IgM were measured using Enzyme-Linked Immunosorbent Assay. Disease activity was assessed using the Disease Activity Score 28 (DAS28) scores. RA patients showed significantly higher VCA-IgM seropositivity compared to controls (75.76% vs 20%, $p<0.05$), which suggests increased viral reactivation. EBNA-IgG levels did not differ significantly between groups ($p=0.61$). EBV antibody levels were significantly higher in RA patients compared to healthy controls, indicating increased viral reactivation or altered immune responses to EBV antigens in the RA group. Moreover, a significant positive correlation was found between EBNA-IgG and VCA-IgM levels ($r=0.329$, $p<0.01$), indicating concurrent antibody responses in RA patients compared to healthy controls. RA patients exhibit altered EBV immune responses characterized by elevated VCA-IgM levels, supporting viral reactivation patterns in autoimmune disease. These findings contribute to understanding EBV-RA relationships and suggest potential clinical utility for EBV serological testing in RA diagnosis and monitoring.

Keywords: Rheumatoid arthritis, Epstein-Barr virus, Correlation, EBNA-IgG, VCA-IgM.

1 INTRODUCTION

Rheumatoid arthritis (RA) represents one of the most challenging autoimmune diseases clinicians face today. The chronic condition affects roughly 1% of people worldwide, with women experiencing the disease two to four times more frequently than men [1]. RA is particularly devastating in its relentless progression, persistent inflammation in the joints leads to irreversible damage and significant disability over time [2]. Among the many environmental suspects researchers have investigated, viral infections have emerged as particularly intriguing culprits. The Epstein-Barr virus stands out in this regard, not just because nearly everyone encounters it at some point in their lives, but because of its unique ability to manipulate our immune system in ways that other viruses simply cannot, Epstein-Barr Virus (EBV) is concerning from an autoimmune perspective because of its remarkable ability to transform B cells regardless of what those cells were initially designed to recognize. This transformation can potentially create antibody-producing cells that might turn against the body's tissues [3]. Recent studies have shown that people with RA are much more likely to show signs of active or recent EBV infection. Additionally, RA patients consistently demonstrate higher levels of specific EBV antibodies, particularly those against the capsid antigen and early antigens, suggesting their immune systems are either failing to control the virus effectively or experiencing more frequent viral reactivations [1]. EBNA-1 IgG antibodies appear to have genuine clinical relevance beyond just indicating past infection. A study has found that RA patients with lower levels of these antibodies at the start of treatment tend to achieve better disease control, particularly those receiving newer biological therapies [4]. The presence of VCA-IgM antibodies in established RA cases may indicate episodic viral reactivation triggered by immunosuppressive therapy or disease-related immune dysfunction rather than primary

*Corresponding author: kasya.othman@hs.hmu.edu.krd

infection [5]. Some researchers have tracked these antibody levels over time and found something remarkable: fluctuations in VCA-IgM levels sometimes happen right before or during RA flares, this timing is too coincidental to ignore it suggests that viral reactivation might trigger periods of worsening arthritis, creating a vicious cycle where inflammation allows the virus to reactivate, which then fuels more inflammation [6]. Recent research has revealed that the EBV-RA connection may begin years before people develop any symptoms of arthritis. When scientists examined stored blood samples from people who later developed RA, they found that specific EBV antibodies, particularly those against early antigens, were already elevated up to a decade before the arthritis appeared [7], suggesting that viral-related immune dysregulation might be an early event in RA development rather than a consequence of existing disease. The presence of viral genetic material in both joint fluid and blood cells of RA patients suggests that ongoing viral activity, rather than just past infection, might be relevant to disease processes [8]. However, the findings of the relation between EBV and RA from various studies were examined. The serological evidence for contribution of EBV infection in the pathophysiology of RA has not been studied in our region. The present study aims to investigate the relationship between RA and IgM and IgG antibody responses to EBV infection (VCA and EBNA) markers, with particular attention to EBNA-IgG and VCA-IgM antibody responses.

2 MATERIALS AND METHODS

2.1 STUDY DESIGN

A case-control study was performed on 91 study objectives to investigate patients with Rheumatoid arthritis and healthy controls, aged 25-65 years, including 79 females and 12 males, referring to the Rizgari Teaching Hospital Department of Rheumatology in Erbil City, Kurdistan region, Iraq, from September 2024 to January 2025. The control group was selected from healthy volunteers with the age, sex, and body mass index (BMI) matched with the RA cases. The rheumatoid arthritis patients were diagnosed according to the ACR-EULAR (European Alliance of Associations for Rheumatology) 2010 criteria. A Questionnaire Form was designed for data collection of demographic information and history of both patients and control groups. The exclusion criteria included: patients with diabetes, asthma, cancer, pregnancy, and women on lactation.

2.2 SAMPLE COLLECTION

Blood samples were collected from the studied groups (RA patients, and control groups). The blood samples were allowed to clot at room temperature and centrifuged at 3000 rpm for ten minutes. Serum was separated from each sample into Eppendorf tubes, which were labeled with the patient's full information. The tubes were then stored at -20°C for various immunological analyses. Severity was assessed by the disease activity score 28 (DAS28), which depends on assessment of 28 joints, CRP, or ESR tests. A mathematical formula calculated the overall score, and scores greater than 5.1 suggested highly active disease, between 3.2 and 5.1 indicated moderate disease activity, less than 3.2 indicated low disease activity, and less than 2.6 indicated a state of remission [9].

2.3 ETHICAL APPROVAL

A scientific ethics committee reviewed and approved the study proposal at the College of Health Sciences, Hawler Medical University, with reference number Sc.E.C.10A3 dated September 9, 2024.

2.4 SAMPLE ANALYSIS

Quantitative detection of serum EBNA-IgG and VCA-IgM using Indirect Enzyme-Linked Immunosorbent Assay (ELISA) kit, according to the manufacturer's instructions (Sunlong, China). Standard calibrators were used to calculate index values/optical density (OD) ratios, which served as a quantitative measure of IgG antibody levels and IgM antibody levels. The positivity of IgG antibody presence was defined by a cut-off value of (0.198) relative units (RU/mL). The positivity of IgM antibody presence was defined by a cut-off value of (0.2025) relative units (RU/mL). Absorbance was read at 450 nm using a microplate reader (BioTek 800TS, BioTek Instruments Inc., USA).

2.5 STATISTICAL ANALYSIS

The statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA). Continuous variables were summarized using median (min-max), and categorical variables were presented as frequencies and percentages. The distribution of continuous variables was assessed using the Shapiro-Wilk test for normality. The Mann-Whitney test was employed for abnormally distributed continuous variables to compare medians between groups; results are represented as median (minimum-maximum). Chi-square tests were applied to examine associations between categorical variables and group membership (case vs. control status). A correlation matrix was constructed to evaluate the relationships between the studied variables. Statistical significance was set at $p < 0.05$ for all analyses.

3 RESULTS

The demographic characteristics of 66 RA patients and 25 healthy controls were presented in (Table 1). There were no significant differences in age (P<0.7937) between RA patients and healthy controls. The RA patients were mainly

observed among the age group (46-55), representing 23(34.85%). The study also showed no significant difference between patients and healthy controls in terms of body mass index (BMI) (P<0.4221). Also, due to the result of this study, the prevalence of RA in women was higher than in men (87.88% and 12.12%) respectively (p<0.7302).

Table 1. Baseline characteristics of Rheumatoid arthritis patients and the control group.

Characters		Cases (%)	Controls (%)	P-value
Age	25-35	7 (10.61)	2 (8)	0.7937
	36-45	19 (28.79)	7 (28)	
	46-55	23 (34.85)	9 (36)	
	56-65	17 (25.76)	7 (28)	
BMI	<25	12 (18.18)	1 (4)	0.4221
	25-29	22 (33.33)	14 (56)	
	≥30	32 (48.48)	10 (40)	
Sex	Female	58 (87.88)	21 (84)	0.7302
	Male	8 (12.12)	4 (16)	

According to seropositivity of RA patients to EBV antigens, the comparison between the RA patients and control groups showed a significant difference in VCA-IgM serum concentration of cases 50(75.76%) and controls 5(20%) with p value (P < 0.0436). At the same time, no significant difference was found between serum concentration of EBNA-IgG of cases 46(69.7%) and controls 14(56%) with (P < 0.6096), as mentioned in (Table 2) and (Figure 1).

Table 2. EBV serological markers in rheumatoid arthritis patients versus healthy controls.

EBV antibodies	RA cases (n=66)	Healthy controls (n=25)	P-value
EBNA-IgG			
Concentrations	0.229(0.1590-3.359)	0.214(0.1290-2.179)	0.6096
Positivity (n, %)	46(69.7%)	14(56%)	
VCA-IgM			
Concentrations	0.223(0.1280-2.868)	0.193(0.1030-2.303)	0.0436
Positivity (n, %)	50(75.76%)	5(20%)	

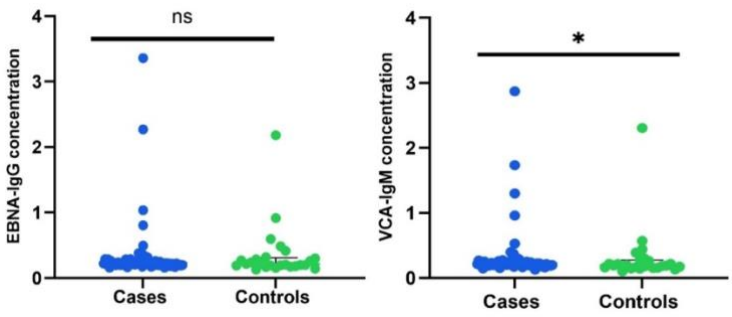

FIGURE 1. Serum EBNA-IgG and VCA-IgM levels in study participants.

Regarding the association between immunological parameters and disease activity (DAS28), no significant differences were found in the median levels of EBNA-IgG and VCA-IgM among 27 cases with moderate activity, 22 in remission, 13 with severe activity, and 4 with low activity, as shown in (Table 3).

Table 3. EBV antibody titers in different DAS28 disease activity groups.

Variable	DAS 28				P-value
	Remission (n=22)	Low (n=4)	Moderate (n=27)	Severe (n=13)	
EBNA-IgG	0.229 (0.189-3.359)	0.179 (0.159-0.224)	0.239 (0.164-1.034)	0.209(0.169- 0.379)	0.0838
VCA-IgM	0.215 (0.148- 2.868)	0.213 (0.193- 0.233)	0.223 (0.128- 1.298)	0.253 (0.198- 0.398)	0.2947

The EBV status between cases and controls shows an apparent significance (p<0.0001). The non-infected (VCA-IgM-/EBNA-IgG-) status in cases 5 (7.58) and controls 11 (44%) shows the highest significance, then reactivated (VCA-IgM+/EBNA-IgG+) in cases 35 (53%) and controls 5 (20%), and recent primary (VCA-IgM+/EBNA-IgG-) in cases 15 (22.73) and controls 0 (0%), with a P-value of (<0.0049, <0.0089) respectively. In comparison, the past infection (VCA-IgM-/EBNA-IgG+) in cases 11 (16.67) and controls 9 (36%) shows no significancy (<0.0859), as shown in (Table 4) and (Figure 2).

Table 4. Distribution of EBV infection phases in RA cases versus controls.

EBV status	Recent Primary (VCA-IgM+/EBNA-IgG-)	Reactivated (VCA-IgM+/EBNA-IgG+)	Past Infection (VCA-IgM-/EBNA-IgG+)	Non-Infected (VCA-IgM-/EBNA-IgG-)
Cases (n, %)	15 (22.73)	35 (53%)	11 (16.67)	5 (7.58)
Controls (n, %)	0 (0%)	5 (20%)	9 (36%)	11 (44%)
P-value	0.0089	0.0049	0.0859	0.0002
P-value (overall)	<0.0001			

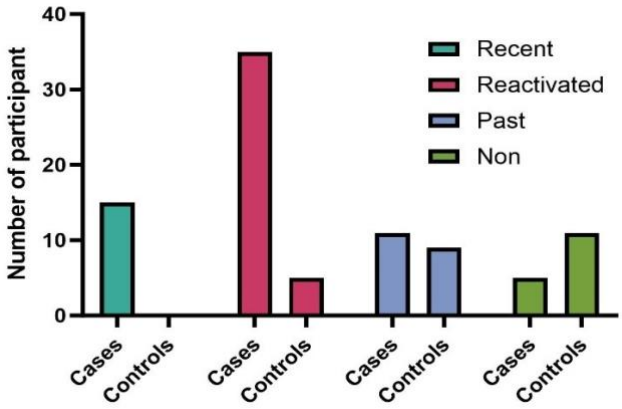

FIGURE 2. Number of participants in each EBV infection status between RA cases and controls.

Our study also showed no significant correlation between DAS28 with EBV-IgG and EBV-IgM (r = -0.2, p = 0.4 and r = 0.2, p = 0.11), respectively, as seen in (Table 5).

Table 5. Pearson correlation coefficients among EBV markers and DAS28.

Variables	IgG		IgM		DAS28	
	r value	P-value	r value	P-value	r value	P-value
IgG	1	-	0.329424948	0.006913823	-0.108283491	0.386797462
IgM	0.329424948	0.006913823	1	-	0.195086408	0.116477593
DAS28	-0.108283491	0.386797462	0.195086408	0.116477593	1	-

4 DISSCUSSION

The association between Epstein-Barr virus (EBV) and rheumatoid arthritis (RA) has gained considerable attention in recent years, with accumulating evidence suggesting a potential role for EBV in RA pathogenesis. EBV may contribute

to autoimmune processes through molecular mimicry, where viral proteins cross-react with self-antigens, potentially triggering the characteristic inflammatory responses observed in RA [10]. Additionally, studies have demonstrated increased EBV reactivation and altered immune responses to viral antigens in RA patients compared to healthy controls, indicating a dysregulated virus-host interaction that may perpetuate joint inflammation [4].

In this study, the demographic characteristics of our patients showed peak prevalence in the 46-55 age group (34.85%), which matches well with other studies in Iraq. In corresponding with the age factor, [11] was found a mean age of 49.9 years in RA patients from Basrah. The successful age matching between cases and controls (p=0.7937) strengthens the study's validity, and the middle-age predominance reflects typical RA onset patterns seen in Kurdish populations [12, 13].

The striking female predominance in our study (87.88% females vs 12.12% males) creates a 7.3:1 ratio that's higher than the global 2-3:1 average but consistent with regional patterns. [12] reported 86.2% female patients in Duhok, and [11] found 81.9% in Basrah. This strong female bias appears characteristic of RA, with women being 2-3 times more likely to develop the disease than men, and is primarily attributed to the complex interplay of hormonal and immunological factors. Estrogen enhances autoimmune processes by promoting pro-inflammatory cytokine production and increasing the survival of autoreactive immune cells. At the same time, the hormonal fluctuations during pregnancy and postpartum periods further demonstrate the significant influence of sex hormones on RA pathogenesis and disease activity [14-16].

Additionally, nearly half of the study RA patients (48.48%) were obese (BMI ≥30), with another third overweight (33.33%). This high obesity prevalence exceeds many international studies but matches regional trends. An study in Iraq reported a mean BMI of 27.1, indicating widespread weight issues among RA populations [11]. This pattern is concerning because obesity worsens RA outcomes, reduces treatment response, and makes remission harder to achieve [17, 18].

Our study uncovered some remarkably striking differences in how rheumatoid arthritis (RA) patients respond to the Epstein-Barr virus compared to healthy people. The numbers reveal a compelling narrative; we saw much higher levels of both EBNA-IgG and VCA-IgM antibodies in RA patients, which backs up what researchers have been suspecting for years about EBV playing a role in RA [3].

By looking at EBNA-IgG results, the difference was notable: nearly 70% of our RA patients (46 out of 66) tested positive compared to just 56% of healthy controls (14 out of 25). This is not just a random finding; this result matches with what other researchers have found. [5] where they showed similar patterns.

In addition, it has been demonstrated that RA patients consistently have higher antibody levels against various EBV proteins, including EBNA-1. What makes this interesting from a clinical standpoint is that these EBNA-IgG levels might tell us something useful about how patients are doing. Recent research by [4] found that lower anti-EBNA1-IgG levels could predict when RA patients are going into remission, regardless of their age or gender. The biological explanation for why EBNA-1 antibodies are elevated in RA gets into some complex immunological mechanisms. Various epitopes of EBNA-1 are suggested to mimic several autoantigens in RA, such as cytokeratin, type II collagen, actin, keratin, and citrullinated fibrin, where molecular mimicry between viral and self-antigens can lead to cross-reactive antibodies and T-cell receptors, resulting in an immune response against autoantigens [19]. This molecular mimicry mechanism can initiate autoimmune diseases through EBV's antigen EBNA1, which resembles host proteins, triggering autoimmune reactions in genetically predisposed individuals [20].

Moreover, the VCA-IgM results were even more dramatic. Our results showed that 75.76% of RA patients (50 out of 66) tested positive for these antibodies, compared to only 20% of controls (5 out of 25). That's more than a threefold difference, and its highly compelling evidence that something active is happening with EBV in these patients. The VCA-IgM antibodies are significant because they typically show up early in EBV infections and usually disappear within a month or two [21]. When someone tests positive for VCA-IgM, it usually means they either have a new EBV infection or the virus has reactivated [6].

Furthermore, this timeline suggests that EBV changes aren't just a consequence of having RA; they might be part of what causes it. Moreover, [4] also found that successful RA treatment led to decreases in EBV antibody levels, particularly VCA-IgM and early antigen antibodies, suggesting that controlling RA inflammation also helps normalize the EBV immune response.

The relationship between EBV antibody levels and RA activity (DAS28) remains controversial, with studies showing conflicting results. In a large Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) cohort study involving 990 RA patients and 700 controls, researchers found that both anti-EBV levels were significantly lower in ACPA-positive RA compared to controls [22]. This finding is consistent with our observation of lower EBV IgG levels in active disease states compared to remission.

Notably, the Swedish study found no correlation between anti-viral antibody levels and baseline clinical characteristics, specifically inflammation measured as CRP and disease activity measured as DAS28 [22]. This supports our finding of non-significant associations (p=0.0838 for EBV IgG) despite apparent clinical patterns.

A prospective study investigating EBV infection markers for remission in RA patients found that lower anti-EBNA1 IgG antibody titer levels could be considered as a significant marker of RA remission regardless of age and gender (OR = 0.99, 95% CI OR = 0.98–0.99, p = 0.038) [4]. This finding supports the potential clinical relevance of monitoring EBV antibody responses in RA management.

The borderline statistical significance in our study (p=0.0838) may reflect insufficient sample size, particularly in the low activity group (n=4). The Swedish study's authors noted that longitudinal studies measuring viral DNA and anti-viral IgM in addition to anti-viral IgG would be needed to dissect the role of common viruses in RA etiology in detail, particularly the relationship between viral load and anti-viral immune response in the context of shared epitope and ACPA [22].

The overall p-value of <0.0001 in Epstein-Barr virus (EBV) infection patterns between cases and controls indicates a highly significant association between EBV status and the condition under investigation, supporting the hypothesis that EBV infection plays a crucial role in disease development. The most striking finding is the absence of recent primary EBV infection (VCA-IgM+/EBNA-IgG-) in the control group (0%) compared to 22.73% in cases (p<0.0089). This pattern suggests that recent EBV infection may serve as a triggering factor for disease onset. These findings are consistent with the landmark study by [23], who demonstrated that the risk of multiple sclerosis increased 32-fold after infection with EBV but was not increased after infection with other viruses, including the similarly transmitted cytomegalovirus. The temporal relationship between recent infection and disease development has been documented in autoimmune conditions, where EBV's sophisticated strategies to evade immune responses facilitate lifelong persistence and contribute to the pathogenic roles in autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus [24]. Recent studies have identified EBV as playing a significant role in exacerbating and potentially triggering autoimmune and autoinflammatory diseases, with research demonstrating that EBV employs several mechanisms of evading the host immune system and has been implicated in inducing host immune dysfunction, potentially resulting in exacerbation or triggering of inflammatory processes [25].

The significantly higher proportion of reactivated EBV infection (VCA-IgM+/EBNA-IgG+) in cases (53%) compared to controls (20%) (p<0.0049) indicates that viral reactivation is strongly associated with disease status. This finding aligns with recent literature showing that EBV reactivation can occur during periods of immunosuppression or stress, where the virus may cause harm in immunocompromised conditions [26]. The high frequency of reactivated infection in RA cases suggests that the underlying disease process may create conditions favorable for viral reactivation, possibly through immune dysregulation or chronic inflammation. In genetically predisposed individuals, EBV can cause immune dysregulation and the induction of autoimmunity, with evidence showing impaired control of EBV infection leading to more frequent viral reactivations [27]. This bidirectional relationship between viral reactivation and disease progression has been documented in autoimmune conditions. EBV reactivation is associated with a substantial risk of autoimmune disease development or exacerbation, with dysregulated infection in the host potentially triggering or promoting autoimmune responses [28]. Furthermore, studies demonstrate that EBV reactivation and progression to chronic active EBV weaken the immune response, creating a cycle where immunosuppression facilitates further viral activation [26].

Additionally, the distribution of past infections (VCA-IgM-/EBNA-IgG+) shows a lower proportion in cases (16.67%) compared to controls (36%), though this difference approaches but does not reach statistical significance (p<0.0859). This pattern suggests that individuals with past, resolved EBV infections may have some degree of protection against disease development, possibly through the development of effective immune memory responses [22].

The significantly lower proportion of non-infected (VCA-IgM-/EBNA-IgG-) in cases (7.58%) compared to controls (44%) (p<0.0002) supports the hypothesis that EBV infection, in some form, is closely associated with disease development. An extensive prospective cohort study by [1] involving 133 RA patients and 50 healthy controls demonstrated that patients with active/recent EBV infection had a five times higher chance of having RA. Additionally, [29] conducted a comprehensive systematic review and meta-analysis of 23 studies examining the Sero-epidemiological association between EBV and rheumatoid arthritis. However, they found mixed results when controlling for study quality.

However, our findings contrast with some meta-analyses that reported weaker associations. [29] found that when studies were restricted to those reporting more plausible levels of EBV exposure in control groups, no significant association was apparent (OR 1.47, 95% CI 0.88–2.46, p = 0.14). These discrepancies may be attributed to differences in study populations, diagnostic criteria, or EBV detection methods.

Our finding of a weak negative correlation between EBV-IgG and DAS28 (r = -0.108, p = 0.387) aligns with recent paradigm shifts in EBV-RA research. This pattern contrasts with earlier assumptions but matches contemporary findings from sophisticated studies. [10] A comprehensive review published in Nature Reviews Rheumatology discussed how EBV acts as a potentiator of autoimmune diseases, including rheumatoid arthritis, through complex mechanisms, including molecular mimicry and B cell reprogramming, supporting our observation that chronic EBV markers don't correlate linearly with current disease activity.

Interestingly, recent studies on twins provide context for our negative trend. [30] found that IgG-EBNA1 antibodies were elevated in healthy co-twins from RA-discordant pairs but not in affected twins, suggesting these antibodies may reflect genetic predisposition rather than active disease processes. This mirrors historical findings by [31], who showed that while EBV-infected B cells increased in RA synovial tissue, systemic antibody levels didn't correlate straightforwardly with disease severity.

However, the negative correlation we observed may reflect immune tolerance mechanisms. This is in agreement with the study by [32], which proposed that chronic EBV infection in RA patients might lead to altered antibody responses over time, a concept now supported by recent mechanistic studies.

Therefore, our positive correlation trend between EBV-IgM and DAS28 ($r = 0.195$, $p = 0.116$) gains support from both historical and recent research. This pattern aligns with [8], who reported similar associations between elevated EBV-IgM and inflammatory markers in German RA patients, and is now mechanistically supported by recent experimental work. [33] provided crucial experimental evidence showing that EBV DNA increases arthritis incidence and severity in murine models, with enhanced IL-17A and IFN-γ responses. This supports our clinical observation that viral reactivation markers (IgM) may influence disease activity even when correlations don't reach statistical significance.

Importantly, regional studies support these patterns. Recent research from neighboring Pakistan [34] found EBV Type-1 in 45% of RA patients versus 9% of controls, with significant correlations between EBV presence and family history ($p = 0.0001$). While focused on viral DNA rather than antibodies, this South Asian study supports the biological relevance of EBV in regional populations, similar to the recent study

Finally, the significant positive correlation between EBV-IgG and IgM in our study ($r = 0.329$, $p = 0.007$) mirrors both classical and recent findings. This pattern has been consistently observed across populations. Recent work by [35, 36] suggests this reflects ongoing viral activity rather than cross-reactivity, supporting the concept of chronic viral reactivation in autoimmune disease.

CONCLUSION

In conclusion, the dramatically higher VCA-IgM antibody levels in RA patients (75.76% positive) compared to controls (20% positive) indicate potential viral reactivation patterns that warrant clinical attention. The overall antibody response patterns strengthen the growing evidence connecting EBV to RA development. The study also confirmed expected demographic patterns in Kurdish RA patients, particularly the strong female predominance and middle-age onset we observe clinically. Although we found no direct correlations between EBV markers and current disease activity (DAS28), the substantial differences between patients and controls suggest these viral responses may be more relevant to disease initiation than ongoing severity. These findings add valuable Middle Eastern perspectives to global RA-EBV research and indicate that EBV testing could become a valuable diagnostic tool in our clinical practice, and also targeting it in management of RA.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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