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Immunological Prevalence of EBV in Rheumatoid Arthritis Iraqi Patients

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Abstract

It has been hypothesized that infection with the Epstein-Barr virus (EBV) may play a role in the development of autoimmune illnesses, including rheumatoid arthritis (RA). This study aimed to investigate whether or not a history of infection with the virus is more common in RA patients compared to controls. Ninety samples ranging from 20 to 70 years old were obtained (45 patients plus 45 controls) between October 2022 and March 2023; each group included 37 females and 8 males. Rheumatoid arthritis (RA) patients who were chosen for this study were sent with a physician report for RA regular tests at Baghdad Teaching Hospital in Baghdad Province. The apparently healthy individuals were obtained (from family, friends, and other acquaintances after routine examinations) with age and sex matched with patients. We immunologically examined all 90 samples to detect Epstein-Barr virus (EBV) infection using both Epstein-Barr viral capsid Ag (VCA) IgG and Epstein Barr nuclear Ag (EBNA) IgG enzyme-linked immunosorbent assay (ELISA). The results showed that (66.7%) of RA patients and (60%) of controls were positive for VCA, while (57.8%) and (51.1%) were positive for EBNA in patients and controls, respectively. According to disease activity, there were (33.33%) and (34.62%) at high disease activity, while (66.67%) and (65.38%) at moderate disease activity were positive for VCA and EBNA, respectively. In conclusion, these findings do not reveal a relationship between EBV seroprevalence and RA, and as a result, they do not support the concept that a previous infection with EBV predisposes individuals to the development of RA.

Keywords: Epstein-Barr virus, viral capsid Ag , Epstein Barr nuclear Ag , Rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a persistent inflammatory disease that predominantly impacts the synovial membrane lining of the joints that ultimately leads to joint destruction (1). There is a correlation between rheumatoid arthritis and worsening disability, as well as premature mortality and socioeconomic problems (2). In rheumatoid arthritis, your immune system attacks the tissue lining the joints on both sides of your body; other parts of the body may also be affected (3). The cause is unknown, but in up to 20% of cases, infections have been thought to be environmental

triggers. Researchers are especially interested in viral causes; viral infection and immunity had been suggested to play a role in the pathogenesis of autoimmune diseases (4,5). So, multiple viral diseases, like rubella, parvovirus B19, human T-lymphotropic virus type 1 (HTLV-1), hepatitis B and C viruses (HBV and HCV), can cause polyarthritis that looks like (6); CMV might be one of the triggering factors for RA (7). The Epstein-Barr virus (EBV) has been suspected as a contributor to rheumatoid arthritis (RA). This is due to the fact that Epstein-Barr virus (EBV) has been related to several other autoimmune illnesses, including multiple sclerosis (MS) and lupus, while there was a non-significant association between EBV infection and reactive arthritis (ReA) disease development (8). EBV is a DNA virus belonging to the gamma herpesviruses subfamily of the Herpesviridae family that infects more than 98 percent of the global population by the age of forty. It is the infectious agent that causes infectious mononucleosis (IM), which is a contagious disease. It is highly associated with a number of cancers, such as lymphoproliferative diseases in those with immunodeficiency, including Hodgkin's disease, Burkitt's lymphoma, T/NK cell lymphomas, and nasopharyngeal carcinoma. Also, EBV induces polyclonal lymphocyte proliferation and resides in B lymphocytes for the life of the host, despite the immune response preventing it from reactivating. According to (6), it is possible that the immunomodulating effects that it possesses in its dormant and replicating forms could contribute to the progression of this autoimmune disease over time (6). Antibodies against EBV antigens are commonly detected in a laboratory setting for the purpose of identifying EBV infection. Antigens like early antigen complex diffuse (EA-D), early antigen complex restricted (EA-R), Epstein-Barr virus nuclear antigen (EBNA) -1, and viral capsid antigen (VCA) and. A patient's stage of EBV infection can be identified using antibodies against these antigens. Antibodies to EA, on the other hand, are believed to point to ongoing replication of EBV, whilst antibodies against VCA and EBNA-1 are expected to remain in the host during their whole lifetime (9). This study intends to determine whether prior infection with the EBV virus occurs more frequently in patients with RA compared to controls.

2. Materials and Methods

In total, 90 samples were taken between October 2022 and March 2023. This study included 45 RA patients with high and moderate disease activity, while the RA patients with low disease activity were excluded, from the Baghdad Teaching Hospital in Baghdad Province, who were referred for standard RA testing by their treating physician (10). The remaining 45 were apparently healthy individuals who were obtained from family, friends, and other acquaintances after routine examinations and had age and sex matched with the patient group. After letting 3 ml of blood clot for 30 minutes at room temperature (25 °C) (10), it was centrifuged at 3000 RPM/min for 15 minutes, and the serum was separated out and stored at -20°C until it was needed for the ELISA procedure, according to (11). Serological detection of EBV was applied using both EBV-VCA IgG and EBNA IgG ELISA kits (Monocent/USA). Statistical analysis was done using the program IBM SPSS version 27.0, and the test used for parametric data was the chi-square test.

3. Results

The results showed that (66.7%) of RA patients and (60%) of controls were positive for VCA. While for EBNA there were (57.8%) and (51.1%) positive for patients and controls, respectively.

The **Tables (1)** and **(2)** showed that there were no significant differences between patients and controls in infecting with EBV-VCA and EBNA.

Table 1. The percentage of infection with EBV- viral capsid antigen

Item	VCA results No. (%)		Probability
	Patients group	Control group	
Positive	30 (66.7)	27 (60.0)	0.512
Negative	15 (33.3)	18 (40.0)	
Total	45 (100.0)	45 (100.0)	

Using $\chi 2$ test

Table 2. The percentage of infection with EBV- nuclear antigen

Item	EBNA results No. (%)		Probability
	Patients group	Control group	
Positive	26 (57.8)	23 (51.1)	0.525
Negative	19 (42.2)	22 (48.9)	
Total	45 (100.0)	45 (100.0)	

Using $\chi 2$ test

To measure the activity of the disease, the Clinical Disease Assessment Index (CDAI) were used. There was significant differences in **Tables (3)** and **(4)** in high and moderate RA disease activity infected with the virus.

Table 3. : RA disease activity and EBV- viral capsid antigen

Disease activity	VCA results No. (%)	Probability
High	10 (33.33)	
Moderate	20 (66.67)	0.010*
Total	30 (100.0)	

$\chi 2$ test, * is significant at $P \leq 0.05$.

Table 4. RA disease activity and EBV- nuclear antigen

Disease activity	EBNA results N. (%) of patients group	Probability
High	9 (34.62)	
Moderate	17 (65.38)	0.027*
Total	26 (100.0)	

$\chi 2$ test * is significant at $P \leq 0.05$.

4. Discussion

Tables (1) and **(2)** showed that there were no significant differences between patients and controls in infecting with EBV-VCA and EBNA. These results agree with (12); the number of patients with a positive EBV viral load did not vary between groups: 80% of people in the control group, 65% of people with pondylarthropathy (SpA), 79% of people with RA who were taking methotrexate (MTX), and 85% of people with RA who were not taking disease-modifying anti-rheumatic drugs (DMARDs) (p = 0.42). Ball et al. showed that there is no link between EBV seroprevalence and RA. This means that the hypothesis that a previous infection with EBV makes you more likely to get RA is not true. In contrast, a meta-analysis showed that EBV infection is linked to multiple sclerosis and SLE (8). The study disagreed with some studies (13-

15) that indicated that there are significant differences in RA patients; EBV was higher than that in the control group. The explanation of the current results may be due to the seroprevalence of EBV rising with age, going from 69.9% in the age category of 1–4.9 years old to 96.0% in the age range of 20–25 years old (16), especially since all the samples in this study were adults. Most people are susceptible to EBV at some point in their life, and the virus is widespread. Saliva is the primary vehicle for the transmission of EBV (17). Most adults and adolescents who contract EBV and experience symptoms recover completely within a month. But for some, exhaustion might last for months at a time (18). The EBV virus, once infected, goes dormant (inactive) in the host's body (19). The virus can reactivate itself in some people. Reactivation of EBV may not necessarily result in symptoms, although those with compromised immune systems are likely to experience them (20).

The phenomenon of molecular mimicry is the mechanism that is cited the most frequently in order to explain autoimmune diseases that occur after infections (21). This study is in agreement with this mechanism. On the other hand, both the hypothesis that EBV immortalizes self-reactive B cells that would otherwise be deleted and the idea that EBV resets the global immune system to a more active state imply that EBV infection should cause a genetic predisposition to autoimmune disease, which is difficult to reconcile with the findings of the present study (22). Two further mechanisms suggested explaining the associations are mistaken self and bystander damage surrounding EBV reactivation, both of which are consistent with the disease associations being confined to specific diseases (9, 23, 24).

To measure the activity of the disease, a variety of composite scales are available. Some examples of these scales include the Disease Activity Score utilizing 28 joints (DAS-28), the Simplified Disease Activity Assessment Index (SDAI), and the Clinical Disease Assessment Index (CDAI) (2). The significant differences in **Tables (3)** and **(4)** in high and moderate RA disease activity infected with the virus return to the fact that most of the collected RA patients were in the moderate stage of the disease. It's important to note that the lack of a link between EBV antibodies and RA doesn't mean that EBV doesn't play a role in the disease, as there is other evidence of this (25, 26).

5. Conclusion

These findings do not reveal a significant relationship between EBV seroprevalence and RA, and as a result, they do not support the concept that a previous infection with EBV predisposes individuals to the development of RA.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

This study was approved by Baghdad University/College of Science Ethics committee (Ref: CSEC/0922/0079). Everyone signed a study-related informed consent. In compliance with the Helsinki Declaration, all human rights have been observed.

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