IAR Journal of Clinical & Medical Biochemistry
ISSN Print : 2789-5998 | **ISSN Online :** 2789-6005
Frequency : Bi- Monthly
Language : English
Origin : Kenya
Website : https://www.iarconsortium.org/iarjcmb/

OPEN ACCESS JOURNALS

Research Article

Detection of Epstein-Barr Virus (EBV) among Chronic Autoimmune Diseases Patients in Mosul City/Iraq

Article History

Received: 10.01.2024
Revision: 19.01.2024
Accepted: 05.03.2024
Published: 30.04.2024

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How to Cite the Article:

Duha Adil Jasim & Anmar A. AlTaie (2024). Detection of Epstein-Barr Virus (EBV) among Chronic Autoimmune Diseases Patients in Mosul City/Iraq. *IAR Jr. Clnc. Med Biochem. 4*(2), 1-5

Abstract: ***Aim:*** This study was designed to detect EBV among (40) Autoimmune Diseases patients in Mosul city/Iraq over 4 months since the 1st of October 2023. ***Methodology and results***: Serum samples were examined for EBV infection using ELISA and IFA. The results revealed that all of the cases were Negative for EBV and interestingly the IFA showed that the majority of cases had past EBV infection. However, the results did not show a significant association between the distribution of EBV among Autoimmune Diseases patients and their age. ***Conclusion:*** Significance and impact of study: Epstein–Barr virus (EBV) is one of the most prevalent viruses in the world. It has been linked directly or indirectly with many diseases like cancers and autoimmune diseases, however, the link between EBV and Autoimmune Diseases are still controversial. Our results indicate that there is a possible role for EBV in the development of Autoimmune diseases, however, more studies of larger scale are required to confirm the results.

Keywords: Epstein-Barr virus, Autoimmune diseases (SLE, RA, CD), Indirect immunofluorescence.

INTRODUCTION

Autoimmune Diseases (AIDs) are a group of complicated diseases with unknown etiologies, different AIDs may share certain similarities in their clinical presentations and yet preserve unique characteristics in each individual of autoimmune disease [1]. Autoimmune Diseases generally result from the loss of self-tolerance i.e., failure of the immune system to distinguish self from non-self, and are characterized by autoantibody production and hyper activation of T cells, which leads to damage of specific or multiple organs. Thus, AIDs can be classified as organ-specific or systemic [2]. Autoimmune Diseases are categorized into organ-specific autoimmune diseases, such as Systemic Lupus Erythematosus SLE, Rheumatoid Arthritis RA and Celiac Diseases CD [3].

The role of Epstein-Barr Virus in Autoimmune diseases has the ability to cause chronic relapsing/reactivating infections, Chronic or recurrent EBV infection of epithelial cells has been linked to SLE, whereas chronic/recurrent infection of B cells has been associated with RA and other diseases [4]. Viruses are obligate parasites that rely on host cellular factors to replicate and spread [5]. EBV is defined by a discrete viral life cycle with primary infection, latency, and lytic reactivation phases [6], EBV establishes lifelong latent infection in B lymphocytes Rarely, EBV latent infection results in malignancy, while In oral epithelial tissues, EBV establishes a lytic infection of differentiated epithelial cells to facilitate the spread of the virus to new hosts [7].

EBV is a ubiquitous double-stranded DNA virus that belongs to the family Herpesviridae and subfamily Gammaherpesvirinae. Gammaherpesvirinae includes two important human gammaherpesviruses, EBV also known as human Herpesvirus 4 and Kaposi's sarcoma-associated herpesvirus also known as human herpesvirus 8 HHV8 [8] . EBV is a lymphotropic Herpesvirus and the causative agent of Infectious Mononucleosis IM [9]. It was originally discovered in cells isolated from African Burkitt's lymphoma and first later on, was it recognized that EBV is highly prevalent worldwide [4].

Serologic tests are usually performed using ELIZA or Immune fluorescent assays IFA [10]. IFA is considered the gold standard for EBV serology Nevertheless, because performing and interpreting IFA is labor-intensive and sometimes subjective [11]. Indirect Immunofluorescence Assay was accepted as the reference method and the concordance of IFA and ELISA methods was investigated. VCA (IgG , IgM) and EBNA IgG antibodies were evaluated according to both tests and their sensitivity and specificity rates were determined for ELISA method [12].

MATERIALS AND METHODS:

Studying Groups:

Samples of serum were collected from 40 Autoimmune Diseases groups of patients (females and males) collected from private laboratories and Ibn- Sina, Al-salam hospitals in Mosul city over 4 months from the 1st of October 2023 to 29 of February 2024. They involved 20 cases of Rheumatoid Arthritis (RA), 10 cases of Systemic Lupus Erythematosus (SLE) , and 10 cases of Celiac Disease (CD) of ages ranging from (10-65) years. All the cases were firmly diagnosed based on clinical signs, symptoms and Laboratory Diagnosis by using immune technique. The patients were tested for EBV infections using an ELISA-EBV specific antigen kit and indirect immunofluorescence test (IFA) and the results were interpreted according to the manufacturers' criteria.

Blood sample collection:

A sample of 2.5 ml of fresh blood was drawn from each patient and collected in a gel tube. Then the sample was centrifuged at (3000) rpm for 3 minutes and the obtained serum was divided into 2-3 aliquots in sterile Eppendorf and stored at -20 ◦C.

EBV antigen detection by ELISA:

Human Epstein-Barr Virus ELISA kits from SUNLONG (C.N. SL2574Hu) was used, according to the manufacturer's manual. Then the results were recorded as absorbance A0 at 450 nm using a Microtiter Plate Reader.

Indirect immuno-Fluorescence Assay (IFA):

Different anti-EBV antigens were evaluated simultaneously using BIOCHIPs of IFA from Euro immune AG (Lüebeck, Germany). Briefly, the fields of BIOCHIP (A, B, C, D, and E), coated with different EBV antigens, were treated with diluted serum samples and incubated for 60 min at ambient temperature. Then, the chips were washed, mounted and evaluated using a fluorescence microscope under magnification power of 10X and 40X.

RESULTS

Serologically, EBV infection was evaluated in Autoimmune Diseases (n = 40) groups of patients SLE, RA and CD by ELISA and IFA. As reported in **Table 1**, it was clear that the results of ELISA revealed that all patients were seronegative to EBV (antigen test). Indirect immunofluorescence assay was used to detect EBV by investigating Antibodies against different Antigens. Therefore, 40 samples were selected and investigated by Euro immune BIOCHIP. The test determines antibodies that are selectively directed towards different EBV antigens, including IgG CAV, IgM CAV, IgG EA, and EBNA. It is well known that the presence and level of these antibodies reflect the different stages of EBV infection, so using Euro immune BIOCHIP would also indicate whether the virus is latent or reactivated. In this term, the presence of IgM VCA and IgG VCA with a lack of anti-EBNA IgG indicates an acute primary infection. While the positive IgG VCA with high avidity and anti-EBNA with an absence of anti-VCA IgM indicates past infection. A positive IgG EA can be considered a marker of reactivation . The results of IFA, as reported in **Table 2, and Figure 1,** indicated that all the samples were positive, IFA suggested that the majority of samples were past infection, as they were positive for IgG CAV with moderate to high avidity and also positive for anti- EBNA. However, a large number of these samples were also positive IgG EA, and some of them for IgM CAV also, which is evidence of the virus reactivation. This may be due to impaired immunity of some patients as a result of the disease or the received treatment.

Table 1: Results the ELISA Technique among Autoimmune Diseases patients

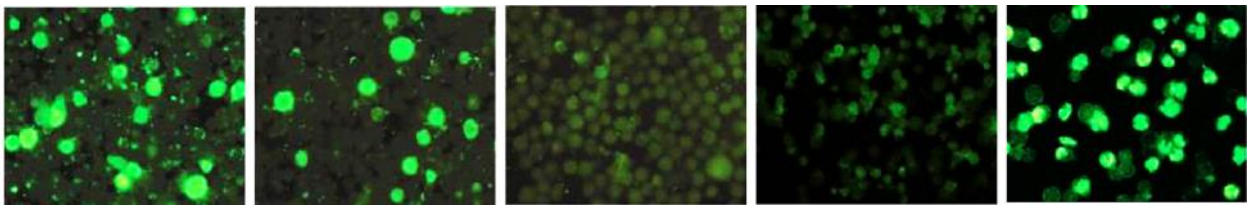
NO.	Autoimmune Diseases	Sex and Number of the patients	Age	Results of ELISA Technique
P(1-20)	RA	M (1-5) F (1-15)	(20-35) (40-65)	Negative
P(20-30)	SLE	M (1-3) F (1-7)	(10-25) (35-60)	Negative
P(30-40)	CD	M (1-4) F(1-6)	(20-37) (45-60)	Negative

Table (2): describe the result of IFA test to the EBV

IFA test slids	IgG CAV	IgG CA with urea	IgM CAV	IgG EA	IgG NA	Phase infection
1	+	+	+	+	+	Past infection
2	+	+	+/-	+	+	Late primary infection or reactivation
3	+	+	+	+	+	Late primary infection or reactivation
4	+	+	+/-	+	+	Late primary infection or reactivation
5	+	+	+/-	-	+	past infection

6	+	+/-	+	+/-	+	Past infection or reactivation
7	+	+	-	+	+	past infection or reactivation
8	+	+	+	+	+	Past infection or reactivation
9	+	+/-	-	-	+	past infection
10	+	+	-	+	+	Past infection or reactivation
11	+	+/-	-	-	+	past infection
12	+	+/-	+	+	+	Past infection or reactivation
13	+	-	-	-	-	past infection
14	+	+/-	-	+	-	Past infection or reactivation
15	+	+	-	+	+	Past infection or reactivation
16	+	+	-	+	+	Past infection or reactivation
17	+	+	-	+	+	Past infection or reactivation
18	+	+/-	-	-	+	past infection
19	+	+	+	+	+	Late primary infection or reactivation
20	+	+/-	+	+/-	+	Past infection or reactivation
21	+	+	-	+	+	Past infection or reactivation
22	+	+	+/-	-	+	past infection
23	+	+	+	+	+	Past infection
24	+	+/-	+	+/-	+	Past infection or reactivation
25	+	+/-	-	-	+	past infection
26	+	+	+	+	+	Past infection or reactivation
27	+	+	-	+	+	Past infection or reactivation
28	+	+	+/-	-	+	past infection
29	-	+/-	-	-	+	past infection
30	+	+/-	+	+	+	Past infection or reactivation
31	+	-	-	-	-	past infection
32	+	+/-	-	+	-	Past infection or reactivation
33	+	+	-	+	+	Past infection or reactivation
34	+	+/-	+	+	+	Past infection or reactivation
35	+	+/-	-	-	+	past infection
36	+	+	-	+	+	Past infection or reactivation
37	+	+	+/-	-	+	past infection
38	+	+	+/-	-	+	past infection
39	+	+	-	+	+	Past infection or reactivation
40	+	+	+	+	+	past infection

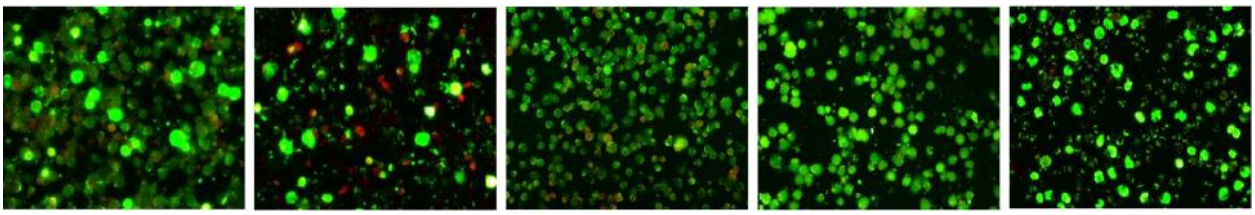
1- Positive control



EBV-CA EBV-CA(IgG) EBV-CA(IgM) EBV-EA(IgG) EBNA(IgG)

Urea treated

2-Test sample



EBV-CA EBV-CA(IgG) EBV-CA(IgM) EBV-EA(IgG) EBNA(IgG)

Urea treated

Figure 1: EBV-Profile Instructions for the indirect immunofluorescent test (BIOCHIP Test). 1: Positive Control 2: EBV Infection.

DISCUSSION

This study focused on Autoimmune Diseases patients in Mosul city and the prevalence of EBV among them. Autoimmune Diseases are usually presented in three categories of Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) and Celiac Diseases (CD) cases as used in this study. The present study demonstrated that EBV was not detected by ELISA technique (determine Antigen by use Sandwich technique) in patients, this result mean the EBV is latent [6] and the patients in chronic phase of the Autoimmune Diseases However, no significant association between the EBV infection among Autoimmune Diseases patients and their sex, age, but the risk of autoimmune diseases is significantly higher in females between (40-65)years more likely than the mal, Kronzer and others in 2021 reported that the risk of autoimmune diseases is significantly higher in females relative to males, a point that is attributed to hormonal, genetic, and environmental factors [13]. Additionally, IFA revealed that the majority of Autoimmune Diseases patients were in past infections, this is a point that supports the possible connection between EBV and Autoimmune diseases. In a meta-analysis study, it was found that EBV infection increased the risk of multiple sclerosis by 2.2 folds [14]. In another study, it was found that the high titers of anti-EBNA complex IgG antibodies were associated with a 36-fold increase in the risk of multiple sclerosis [15]. Also, some EBV-specific antibodies have shown cross-reactivity with SLE autoantigens [16].

Autoimmune diseases such as scleroderma, rheumatoid arthritis, SLE, and multiple sclerosis have been reported in about 23%, 21%, 17.9%, and 9.1% of thyroid cases respectively [17]. It is well known that EBV immobilizes memory B-cells and has several mechanisms to modulate and escape from immune responses of the body, which could be the rationale behind the link between EBV and the initiation and exacerbation of autoimmune diseases [18]. Finally, it is clear from the studies reported above that the association between EBV and Autoimmune diseases is still an active subject and more studies on large scale should be conducted to establish this association or link and reveal the possible mechanism by which EBV could affect Autoimmune patients.

CONCLUSION

This study aimed to investigate the distribution of EBV among 40 Autoimmune Diseases patients (20 cases of Rheumatoid Arthritis (RA), 10 cases of Systemic Lupus Erythematosus (SLE) , and 10 cases of Celiac Disease (CD) of ages ranging from (10-65) years at the hospitals teaching in Mosul city/Iraq. The results indicated that all the patients were sero-Negative to EBV of the cases using ELISA. Then , 40 samples of EBV-sero-Negative cases were tested by IFA using Euro immune BIOCHIPs. So the majority of tested cases were in the past stage of EBV infection. These results may suggest that EBV has a potential role in the development of Autoimmune diseases, however more studies on a larger scale were needed.

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