Original Article

# Epstein-Barr Virus Antibodies in Rheumatoid Arthritis Iraqi patients

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***Summary:***

**Background:** Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. Many cases are believed to result from an interaction between genetic factors and environmental exposures. Epstein-Barr virus (EBV) is associated with several autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis.

**Objective:** To study the association between EBV antibodies and RA.

**Patients and methods:** This prospective study was carried out in the period between March 2013 and May 2013. The study involved 30 patients diagnosed with RA attending rheumatology outpatient's clinic Baghdad medical city teaching hospital and 30 apparently healthy individuals as a control group. Serum samples were collected to investigate the level of EBV-IgG and IgM by using ELISA technique.

**Results:** This study showed that 70% of control group had positive EBV-IgG while only 6 out of 30 had positive EBV-IgM. The mean values of EBV-IgG were significantly higher among RA patients (0.542 IU/ml) in comparison with that in control group(0.269 IU/ml). Also mean value of serum EBV-IgM (0.723 IU/ml) were significantly higher than that in healthy individuals(0.354 IU/ml)

**Conclusions:** The serum levels of EBV-IgG and IgM were significantly higher in RA patients in contrast with those in healthy individuals.

**Keywords:** Rheumatoid arthritis, Epstein-Barr virus, EBV-IgG, EBV-IgM, ELISA.

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## Introduction:

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. It can be a disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated (1&2). RA is a chronic inflammatory polyarthritis (arthritis that affects 5 or more joints)(3). The etiology of RA is unknown (4). Many cases are believed to result from an interaction between genetic factors and environmental exposures (5). There is no evidence that physical and emotional effects nor stress could be a trigger for the disease (2). The many negative findings suggest that either the trigger varies, or that it might in fact be a chance event inherent with the immune response (6).

All of the risk for RA is believed to be genetic, it is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4 (most specifically DR0401-4)(7). Although the cause of RA is unknown, autoimmunity plays a big part and autoimmune diseases are believed to result from interactions between genetic, environmental and psychiatric factors. Systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and multiple sclerosis (MS) are associated with certain HLA genes and more weakly to several other immune related genes(8). RA is diagnosed clinically on the basis of symptoms, physical exam, radiographs (X-rays) and labs, but classified according to the American College of Rheumatology (ACR),2010 and European League Against Rheumatism (EULAR) classification criteria for rheumatoid arthritis (9).

Epstein-Barr virus (EBV) is associated with several autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. However, it is not clear whether EBV plays a role in the pathogenesis of these diseases, and if so, by which mechanisms the virus may contribute(10). The EBV also called human herpesvirus 4 (HHV-4), is a virus of the herpes family, and is one of the most common viruses in humans. It is best known as the cause of infectious mononucleosis (glandular fever). It is also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV) such as hairy leukoplakia and central nervous system lymphomas(11). Evidence of an aberrant T-cell response against EBV has been reported in SLE, RA and MS, and also T-cells were unable to control the production of immunoglobulins (Ig) from EBV-infected B-cells(12). Since EBV infection is very common in normal individuals it is also uncertain whether

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the higher levels EBV antibodies in rheumatoids are due to some particular defect in the handling of the EBV, or whether the high titers are consequent on the generalized increase in antibody titers seen in established rheumatoid disease(13). This study estimate the association between EBV antibodies and RA.

**Materials and method:**

This prospective study was carried out in the period between March 2013 and May 2013. The study involved 30 patients diagnosed with RA attending rheumatology outpatient's clinic Baghdad medical city teaching hospital and 30 apparently healthy individuals as a control group. The study was approved by the ethical committee of the microbiology department in Baghdad university. The age was matched between the patients and controls groups, ranging from (19-60) years.

Blood samples were obtained from each individual by venous puncture, then was left to clot at room temperature, centrifuged and serum was collected and stored in aliquots at -20 □ C. Repeated freezing and thawing was avoided. EBV IgG and IgM antibodies were detected with commercially available enzyme immunosorbent assay kits as (Human Gesellschaft fur Biochemica and Diagnostica mbH ELISA Test for the Detection of IgG Antibodies to Epstein-Barr Virus in Human Serum) and (Human Gesellschaft fur Biochemica and Diagnostica mbH ELISA Test for the Detection of IgM Antibodies to Epstein-Barr Virus in Human Serum) respectively. We have considered 0.12 HU/ml and 0.45 IU/mi as the upper limits of normality for EBV-IgG and EBV-IgM respectively.

The results were expressed as mean and SD by using Statistical package for Social Sciences (SPSS) version 19.0. The value of ($p<0.001$) was considered statistically highly significant.

**Results:-**

Results of tests applied for the included individuals were distributed in Table (1), which shows that 70% of control group have positive EBV-IgG while only 6 out of 30 have positive EBV-IgM.

**Table (1): Distribution of study samples according to their applied tests results**

| | EBV-IgG | | | | EBV-IgM | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive | | negative | | Positive | | Negative | |
| | N | (%) | N | (%) | N | (%) | N | (%) |
| RA patients | 28 | (93.3) | 2 | (6.7) | 16 | (53.3) | 14 | (46.7) |
| Healthy Control | 21 | (70) | 9 | (30) | 6 | (20) | 24 | (80) |

The differences in mean of serum EBV-IgG level (IU/ml) among included study groups were shown in table (2).The mean values of EBV-IgG were significantly higher among RA patients in comparison with that in control group(P value <0.001).

**Table (2): The differences in mean of serum EBV-IgG level (IU/ml) among included study groups**

| Values | Patient | Control | P value |
|---|---|---|---|
| Number | 30 | 30 | |
| EBV-IgG(IU**/ml) Mean | 0.542 | 0.269 | p<0.001 |
| EBV-IgG(IU/ml) SD* | 0.255 | 0.236 | |
| P value | highly significant | | |

*SD=standard deviation **IU=International Unit

Table (3) shows the differences in the mean of serum EBV-IgM level between RA patients in comparison to healthy individuals were it seems to be highly significant also (P value <0.001).

**Table (3): The differences in mean of serum EBV-IgM level (IU/ml) among included study groups**

| Values | Patient | Control | P value |
|---|---|---|---|
| Number | 30 | 30 | |
| EBV-IgM(IU/ml) Mean | 0.723 | 0.354 | p<0.001 |
| EBV-IgM(IU/ml) SD | 0.586 | 0.143 | |
| P value | highly significant | | |

**Discussion:-**

EBV was suggested early as an environmental trigger of autoimmune disease and remains a main candidate. Several studies have suggested that EBV is associated with autoimmune diseases, such as SLE, RA, MS, autoimmune thyroiditis, inflammatory bowel diseases, insulin-dependent diabetes mellitus, Sjögren's syndrome, systemic sclerosis, myasthenia gravis and autoimmune liver diseases(1,2.4,14). However it is possible that recrudescence of EBV would also lead to a transient rise in antibodies to EBV, which would suggest that early rheumatoid patients control their EBV infections less well than normal individuals. It would be necessary to titre the IgM antibodies to the EBV to differentiate between these two hypotheses. Data on antibodies to the EBV early antigen would be of interest, to see whether the onset of recent infection can be associated with the onset of RA in some patients(15). Further evidence for the abnormal handling of herpes viruses comes from reports that the T cells' control of EBV-dependent spontaneous B cell outgrowth in culture is defective in rheumatoids(16). It could be that these phenomena are merely a reflection of general immunoregulatory defects which independently produce abnormal virus handling and the development of IgG autoimmunity which is also a feature of the RA patients(17). In this study, EBV-IgG were positive in 70% of healthy people while EBV-IgM were positive only in 6% of them. Thus we depend on the mean of serum level to compare between the two groups. The serum levels of both EBV-IgG and EBV-IgM were significantly higher in RA patients than in healthy control individuals. These results agree with many previous studies which give the evidences for the B-cell and T-cell response against EBV in RA patients(12,13,14&16).

An early study in RA demonstrated that T-cells were unable to control the production of immunoglobulins (Ig) from EBV-infected B-cells .Later studies have reported a functionally impaired EBV specific CD8+ T-cell response characterized by the decreased production of cytokines (interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-2 and macrophage inflammatory protein-1β) and decreased cytotoxicity in RA patients(18). In RA, it is also possible to measure the concentration of EBV-specific antibodies in body fluids that are contiguous with the diseased organs and, thus, reflect local synthesis of antibodies. Early studies on synovial fluid from RA joints did not show any evidence of the local production of antibodies against EBV. As for antibodies, it is also possible to study T-cells from body fluids contiguous with the diseased organs in RA. EBV specific CD8+ T-cells were shown early to be enriched in the synovial fluid compared to blood in patients with RA(19).

**Conclusions:-**

The serum levels of EBV-IgG and IgM were significantly higher in RA patients in contrast with those in healthy individuals.

Author Contributions:-

Aida R. Al-Derzi / study conception, design, interpretation of data and critical revision

Muhammad M. Alani / acquisition of data analysis and interpretation of data

Hayfaa S. AL-Hadithi / drafting of manuscript, design and interpretation of data.

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