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Detection of Epstein - Barr virus Capsid antigen (EBV CA) in Sera of Rheumatoid Arthritis, Reactive Arthritis and Ankylosing Spondylitis Patients

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Abstract

To determine the role of Epstien barr virus (EBV) in the pathogenesis of Rheumatoid arthritis (RA) , Reactive arthritis (ReA) and Ankylosing spondylitis (AS) patients, sixty-two patients (52 patients with RA ,five patients with ReA and five patients with AS) have been investigated. The mean age (47.3, 47.7, 34.5) of RA, ReA , AS respectively and compared to 24 apparently healthy individuals with the mean age 28.3. All the study groups were carried out to measure antibodies of EBV (VCA) IgG and IgM by enzyme-linked immunosorbent assay (ELISA) technique. The result showed that there was a highly significant elevation ($p< 0.01$) in the concentration of EBV (VCA) IgG Ab compared to control group, while there was no significant different ($p> 0.05$) in EBV (VCA) IgM Ab compared to control group. when the study groups were compared between each other in EBV (VCA) IgG Ab, the AS group had the higher mean of concentration 51.24 ± 2.19 U/ml while the RA mean concentration 40.82 ± 2.36 U/ml and finally ReA mean concentration was 38.24 ± 8.79 U/ml. compared to control group2.55 ± 0.03 U/ml . while comparison in mean level of EBV CA IgM Ab, the concentration was(3.32 ± 0.40 , 5.24 ± 2.30 and 10.70 ± 6.89) U/ml for RA, ReA and AS reapectively compared to control group 0.122 ± 0.01 U/ml.The results of the present study indicate that infection with EBV play a role as a triggering factor in pathogensis of RA,Rea,AS.

Keywords: EBV CA, Rheumatoid arthritis, Reactive arthritis, Anklosing spondylitis.

التحري عن مستضدات الغلاف الفيروسي لفيروس ابشتاين بار في مصول المرضى المصابين بالتهاب المفاصل الرثوي ، التهاب المفاصل التفاعلي وتشمع العمود الفقري

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الخلاصة

لغرض تعيين دور فيروس ابشتاين بار في امراضية التهاب المفاصل الرثوي ، التهاب المفاصل التفاعلي وتشمع العمود الفقري .تم التحري عن 62 مريضا (52 مريض مصاب بالتهاب المفاصل الرثوي ،خمسة مرضى مصابين بالتهاب المفاصل التفاعلي وخمسة مرضى مصابين بتشمع العمود الفقري.وكان متوسط

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العمر (47.3 ،47.7 ،34.5) لمرضى التهاب المفاصل الرثوي ، التهاب المفاصل التفاعلي وتشمع العمود الفقري على التوالي وبالمقارنة مع 24 فردا من الاشخاص الاصحاء وكان متوسط اعمارهم 28.3. خضعت جميع عينات ا لدراسة لقياس مستوى اضداد الغلاف الفيروسي لفيروس ابشتاين بار الصنف(IgM , IgG) باستخدام تقنية الامتزاز المناعي المرتبط بالانزيم. اضهرت نتائج الدراسة ارتفاعا معنويا عاليا (p< 0.01) في تركيز اضداد EBV (VCA) IgG مقارنة بمجاميع السيطرة .بينما لا يوجد ارتفاع معنوي في اضداد الصنف EBV (VCA) IgM (p> 0.05). وعند المقارنة بين مجاميع الدراسة في تركيز EBV (VCA) IgG اعلى تركيز لوحظ عند مرضى تشمع العمود الفقري وكان 51.24 ± 2.19 U/ml بينما كان التركيز في مرضى التهاب المفاصل الرثوي40.82 ± 2.36U/ml واقل تركيز كان في مرضى التهاب المفاصل التفاعلي 38.24 ± 8.79U/ml مقارنة بمجاميع السيطرة 38.24 ± 8.79 U/ml. اما عند مقارنة تركيز EBV (VCA) IgM كانت التاكيز(3.32 ± 0.40 ، 5.24 ± 2.30 ، 10.70 ± 6.89) U/ml لمرضى التهاب المفاصل الرثوي ، التهاب المفاصل التفاعلي وتشمع العمود الفقري على التوالي مقارنة بمجاميع السيطرة0.122 ± 0.01 U/ml. تشير نتائج الدراسة الحالية بان الاصابة بفيروس ابشتاين بار تلعب دورا كعامل تحفيز في امراضة التهاب المفاصل الرثوي ، التهاب المفاصل التفاعلي وتشمع العمود الفقري .

Introduction:

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease. Inflammatory polyarthritis is the primary clinical manifestation, mainly affecting small joints of the hands and the feet [1]. RA is the most common systemic autoimmune disease in the world [2]. Reactive arthritis (ReA) is an acute inflammatory joint disease belonging to the group of spondyloarthritides (SpA)[3]. Reactive arthritis (ReA) is an autoimmune condition that develops in response to an infection in other part of the body [4]. ReA usually affects young people aged 20–40 years. Men are 15 times more likely to develop symptoms than women[5]. Ankylosing spondylitis is the prototype, a subtype, and an outcome of spondyloarthritis, particularly of the axial form of spondyloarthritis. AS is a chronic inflammatory joint disease that predominantly affects the sacroiliac joints and spine [6], it is a disease that affects young people, who generally present at around 26 years of age. Men are more often affected than women [7]. Several microorganisms have been implicated in the development of RA based on higher titres of the relevant antibodies in patients with RA. Infectious agents reported to trigger RA (Human parvovirus B19, Rubella virus, Human retrovirus 5, Alphaviruses , Hepatitis B virus and Epstein–Barr virus)[8]. Epstein-BarrVirus (EBV) is a gammaherpesvirus, infecting over 95% of the adult population worldwide [9].

EBV replicates primarily in B lymphocytes but also may replicate in the epithelial cells of the pharynx and parotid duct.The infection is spread primarily by saliva, and the incubation period is four to eight weeks [10].An association between EBV and rheumatoid arthritis (RA) was first proposed on the basis of high titres of EBVspecific antibodies found in some patients with RA [11].

They have also high numbers of EBV-specific T cells in their affected joints [12]. The number of EBV-specific CD8+ T-cells correlates positively with the viral load, whereas CD4+ T-cell responses against EBV and CD8+ T-cell responses to CMV antigens do not[13]. Some research shown that a virus could act as an adjuvant in the development of autoimmunity, non-specifically stimulating innate immune responses, including mast cells, dendritic cells, Toll-like receptors and complement receptors [14]. The aim of present study was to estimate the seroprevalence of EBV CA in sera of patients with RA, ReA and AS.

Material and method

Patients included in this study were 62 patients, 52 with rheumatoid arthritis (RA ; 46f/ 6m) , 5 with Reactive arthritis (ReA ; 2 f/3m) ,5 with anklosing spondylitis (AS ; 0 m/ 5f).The age of the total patients range from (28-70) years.24 samples of apparently healthy individuals (HC ; 15f /9m) were studied as a control groups of same age and sex. The study population attending at AL-Yarmouk teaching hospital/Rheumatology clinic . The samples were collected from the first of November 2014 until February 2015. Blood samples (3ml) were collected by disposable syringe which stand at room temperature until blood was clotted. Then the samples were centrifuged at 3000 rpm for 5 minutes.

Serum samples were dispended separated on Eppendroff tubes. All samples stored at (-20) until carried out to immunological examination.

Serological Examination

ELISA test was used to measure EBV (VCA) IgG Ab and IgM Ab (NovaTecImmundiagnostica/ Germany) according to instruction of the kit in the sera of all the study groups.

Statistical Analysis

The Statistical Analysis System- SAS [15] was used to study the effect of different factors in study parameters. Least significant difference –LSD test was used to compare the significance between means and Chi-square test was used to compare between percentages in this study.

Results and Discussion

A total of sixty two patients 52 with RA, 5 patients with ReA and 5 patients of AS . The range of RA patients age were (30-50) years, the range of ReA patients age were more than 50 years while in AS patients the range of age less than 30 years. The results show that highly significant different ($P<0.01$) between patients and control in EBV (VCA) IgG Ab while no significant different ($P>0.05$) in EBV (VCA) IgM Ab as shown in Table-1.

When comparing between patient groups and control in the EBV (V CA) IgG Ab, the AS patients have the highest concentration of Ab which was 51.24 ± 2.19 U/ml , then RA patients 40.82 ± 2.36 U/ml and the less concentration of EBV CA IgG Ab appear in ReA patients which was 38.24 ± 8.79 U/ml compared to control group 2.55 ± 0.03 U/ml as shown in Figure-1 , But comparison in mean level of EBV CA IgM Ab, the concentration was (3.32 ± 0.40 , 5.24 ± 2.30 and 10.70 ± 6.89) U/ml for RA, ReA and AS reapectively compared to control group 0.122 ± 0.01 as shown in Figure-2.

Table 1- Shows the result between patients and control in EBV (VCA) IgG Ab and EBV (VCA) IgM Ab

Group	Means ± SD	
	IgG Ab	IgM Ab
Patients	41.00 ± 2.14	4.25 ± 0.69
Control	19.36 ± 0.79	3.70 ± 0.34
LSD value	7.099 **	2.304 NS
P-value	0.0001	0.6352
** ($P<0.01$), NS: Non-significant.		

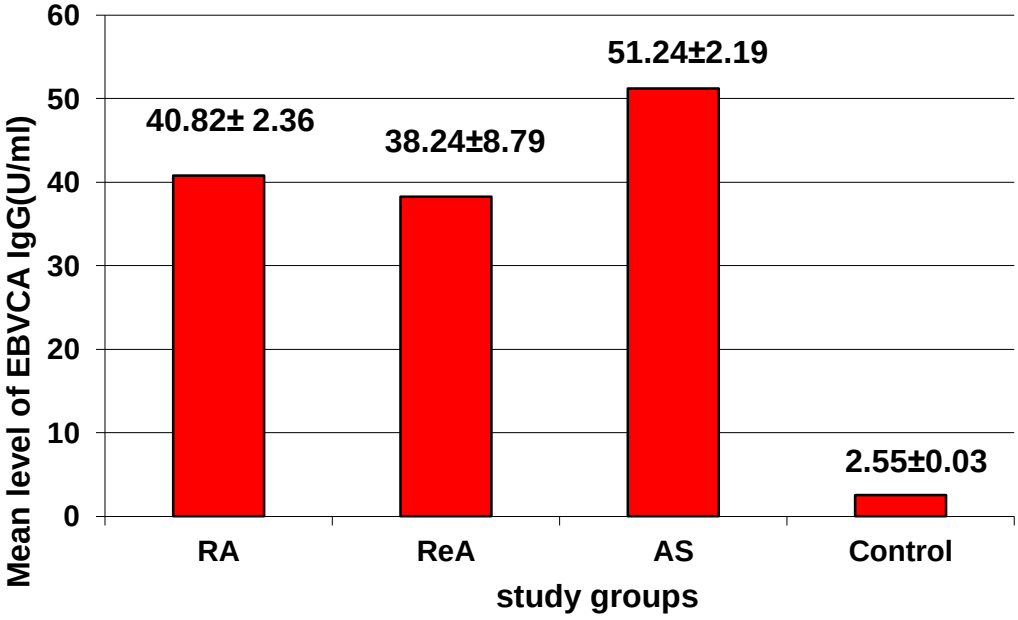

Figure 1- Compare between RA , ReA, AS & Control in EBVCA IgG

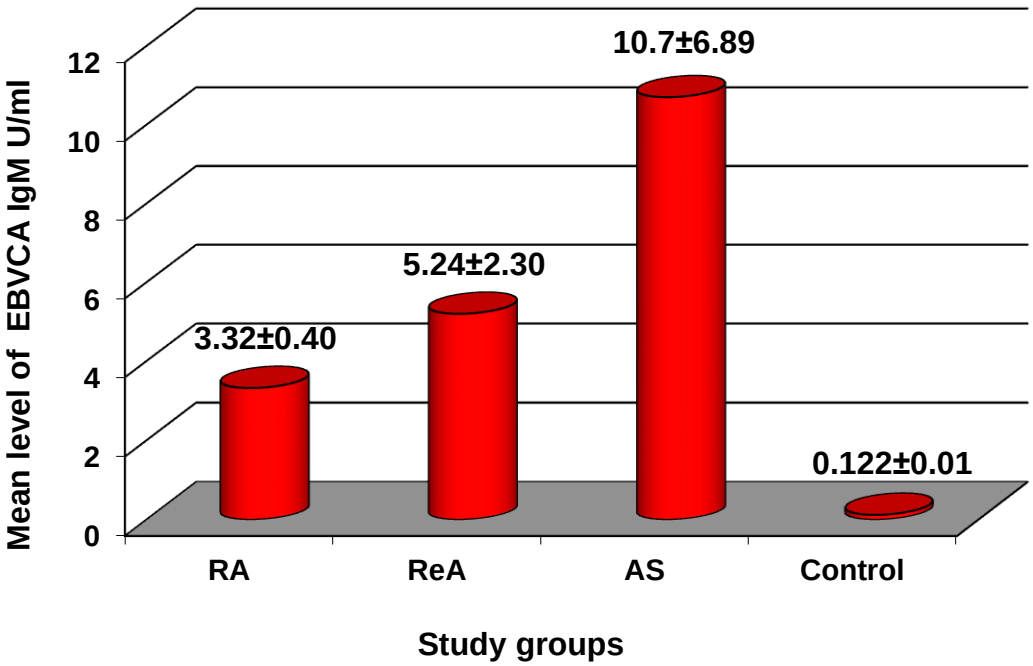

Figure 2- Compare between RA , ReA, AS & Control in EBVCA IgM

The results of present study were agreement with several other studies. By using another technique in [16] the study show that Elevated anti-VCA titers (>1: 160) were more common in RA patients than controls, 31% compared with 15%. The geometric mean titer of anti-VCA was significantly higher in the RA group.in another study it was demonstrated that elevated titers (≥320) of antibodies of Epstein-Barr viral capsid antigen (VCA) in 64% of 28 seropositive RA patients [17] Another study showed that patients with RA show higher titers of IgG Abs specific for both latent and lytic EBV encoded Ags compared with healthy EBV carriers [18].

Patients with RA have increased anti-EBV antibody levels in their sera compared to healthy subjects.[19] .Humoral response to both latent and lytic EBV antigens with elevated titers of antibodies against EBNA1, VCA, and EA/R in both sera and synovial fluids from RA patients compared to healthy controls [20].Patients with RA have an abnormally increased frequency of circulating EBV-infected B cells [21], they have very high EBV load in peripheral blood lymphocytes than healthy controls.[22] .EBV infection may contribute indirectly to the pathophysiology of RA by impairing immune control of EBV replication, causing increased exposure to EBV antigens and, thereby, chronic inflammation [23]. Mechanisms by which infectious agents may initiate RA and/or autoimmunity include molecular mimicry and epitope spreading. Molecular mimicry can be initiated when lingering autoreactive T cells become activated by peptides from infecting organisms that bear similar structure and/or amino acid sequence to that of same host peptide, i.e proteins/peptides that share a similar molecular structure to that of host tissue peptides, and which therefore can perpetuate inappropriate immune reactions to self.[24].Acute primary infection is marked by a rise in immunoglobulin M against VCA, which usually disappears after 2 months of infection. This is followed by the appearance of immunoglobulin G against VCA and nuclear antigen (EBNA) at least 1 month after primary infection and persists for many years or throughout life [25].

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