ASSOCIATION OF EPSTEIN-BARR VIRUS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: RELATION TO DISEASE ACTIVITY AND FLARE-UPS

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KEY WORDS: EPSTEIN BARR VIRUS, SLE, RELATED ACTIVITY & FLARE-UPS.

ABSTRACT

Objectives: *To determine the prevalence of IgM, IgG and IgA antibodies against Epstein Barr, virus capsid antigens (EBV-VCA) in systemic lupus erythematosus (SLE) patients and to clarify their relation to disease activity and flare.*

Methodology: *The study comprised forty adults SLE patients; they were 35 females and 5 males, 'their ages ranged from 21-35 years (mean ± SD 29.3 ± 4.2) and forty normal subjects; 36 females and 4 males with a mean age value of 29.2 ± 3.9 as a control group. Patients were subjected to thorough medical history taking, clinical examination, laboratory investigations, disease activity assessment and disease flare assessment within one year and detection of EBV IgG, IgM and IgA antibodies in the serum against EBV –VCA for patients and control groups.*

Results: *There was non significant difference as regards the prevalence of anti EBV IgG and IgM in both SLE patients and control groups. A significant difference of serum IgA antibody against EBV-VCA between SLE patients and control groups was found; 15/40 (37.5%) vs. 2/40 (5%); $p<0.001$. The systemic lupus erythematosus disease activity index (SLEDAI) score was significantly higher in the SLE patients with IgA antibody against EBV-VCA than in the SLE patients without IgA antibody (29 ± 7.7 VS 23.4 ± 3.2; $p<0.001$). As regard the disease flare we found that the SLE patients with IgA antibody against EBV-VCA had higher prevalence of disease flare compared to those without IgA antibody 10 (66.7%) vs. 2 (8%), $p<0.001$.*

Conclusion: *The close clinical data association between EBV infection and SLE suggests a possible role of the EBV as a trigger in the Pathogenesis, disease activity and flare of SLE patients. Further, studies should be done to elucidate the complex relationship between EBV infection and SLE patients.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with no known cure. Lupus patients suffer from a myriad of clinical symptoms which variably include arthritis, pleuritis, pericarditis, vasculitis and nephritis. The underlying mechanisms behind these clinical findings and the etiologic events preceding and causing disease onset, however, remain largely unknown *(Mc Clain et al., 2001).*

The role of infectious agents in the pathogenesis of autoimmune disease has long been a matter of debate *(Tsai et al.,*

1995). Viruses have long been postulated to play a role in autoimmune disease.

Epstein-Barr virus (EBV) is suspected to play a role in predisposing to SLE for several reasons. First, EBV promotes proliferation of B-cells after infection and thus it poses a prolonged antigenic challenge. This prolonged EBV antigen expression may trigger SLE in genetically prone individuals. Second, EBV-infected B-cells can become a continuous source of autoantibodies. Third, sequence homologies exist between SLE autoantigens and some EBV proteins, such as EBV nuclear antigen EBNA-1 and EBNA-2. The antibodies elicited by these viral antigens may cross-react with autoantigens and trigger SLE *(Moon et al., 2004)*.

James et al. (2001) examined more than 100 SLE patients and found that the EBV sero conversion rate was significantly greater in SLE patients than in normal control individuals. *Gross et al. (2005)* suggested that EBV infection may be perturbed in a broad range of autoimmune disease but the most profound effect occurs with SLE.

Aim Of The Work:

To determine the prevalence of IgM, IgG and IgA antibodies against Epstein-Barr virus capsid antigens in SLE patients and to clarify their relation to disease activity and flare.

PATIENTS AND METHODS

Forty adult SLE patients (35 females and 5 males), with a mean age value of 29.3±4.2 years, recruited from Rheumatology and Rehabilitation and internal medicine departments, Zagazig University Hospitals.

All patients fulfilling the revised criteria for classification of SLE of the American College of Rheumatology *(Tan et al., 1982)*. All patients were followed at Zagazig University Hospitals for one year (mean disease duration 5.5±2.1 years). Forty healthy subjects; 36 females and 4 males with a mean age value of 29.2 ±3.9 years were taken as a control group.

Patients were subjected to the following:

(1) Full history taking with special attention to articular and extra-articular symptoms.

(2) Thorough general and local clinical examination.

(3) Laboratory investigations including:

- Complete blood picture.

- Erythrocyte sedimentation rate (ESR).

- Complete urine analysis (pus cells/HPF, RBCs/HPF and abnormal casts) and protein in urine collected for 24 hours (gm/24 h).

- Antinuclear antibodies (ANA) using Kallestad kits and Anti-ds DNA antibodies were detected by ELISA.

- Complement 3, 4 by use of immunodiffusion plate.

- Chest X-ray and electrocardiography (ECG).

(4) Disease activity assessment was done according to systemic lupus erythematosus disease activity index (SLEDAI) score *(Bombardier et al., 1992)*. Changes in SLEDAI scores from baseline to each follow-up visit through one year were assessed.

SLEDAI score >10 were considered in a state of active disease *(Gilkeson et al., 1999)*. Mild lupus flares and severe flares were defined as increases in the SLEDAI of 3 or more; 12 or more points, respectively from the previous visit *(Guerrero et al., 2005)*.

(5) Detection of EBV IgG, IgM and IgA antibodies in the serum for patients and controls against EBV-VCA using an indirect immunofluorescence technique

(Lynn et al., 1985). The titer of IgG antibody against EBV-VCA at a 1:160 or greater dilution was regarded as positive which indicated exposure to EBV infection. The titer of serum IgM antibodies against EBV-VCA at a 1:10 or greater dilution was regarded as positive indicating acute phase of EBV infection. The titer of IgA antibody against EBV-VCA at a 1:40 or greater dilution was regarded as positive and indicated frequent reactivation of latent EBV in B cells, repeated viral infection or both.

Statistical analysis:

The results of the study were statistically analyzed on a standard computer program using the student's "t" test for paired and unpaired data

RESULTS

Table (1): Clinical and laboratory findings of SLE patients.

Item	Number of patients (No.)	Percent (%)
CNS manifestations	18	45
Vasculitis	12	30
Arthritis	21	52.5
Muscle Weakness, myalgia	17	42.5
Myositis	5	12.5
Urinary casts	24	60
> 5 RBCs/HPF	15	37.5
> 5 WBCs/HPF	15	37.5
Proteinuria > 0.5 mg/24 hrs	24	60
Skin rash	28	70
Hair loss	28	70
Oral ulcers	15	35.5
Pleurisy	6	15
Pericarditis	5	12.5
Anti-dsDNA	30	75
Low complement	11	27.5
Pyrexia	31	77.5
< 100,000 PLT/mm^3	10	25
< 3,000 WBCs/mm^3	2	5

From table (1), CNS manifestations in form of seizures, psychosis, visual disturbance, organic brain syndrome or cranial nerve disorders were found in 18 patients (45%), vasculitis were found in 12 patients (30%), arthritis were found in 21 patients (52.5%), muscles disorder in form of muscle weakness, myalgia or myositis were found in 22 patient (55%), urinary casts were found in 24 patients (60%), red blood cells or pus cells > 5 were found in 30 patients (75%), proteinuria > 0.5 mg/24hrs were found in 24 patients (60%), skin rash was found in 28 patients (70%), alopecia was found in 28 patients (70%), oral ulcers were found in 15 patients (35.5%), pulmonary or cardiac affection was found in 11 patients (27.5 %), anti-dsDNA was found in 30 patients (75%), low complement was found in 11 patients (27.5%), pyrexia was found in 31 patients (77.5 %), thrombocytopenia was found in

10 patients (25 %) and leucopenia was found only in 2 patients (5 %).

Table (2): Comparison between SLE patients and control groups regarding clinical and serological findings.

	SLE patients No= 40	Controls No=40	p
Age (mean ± SD) years	29.3 ± 4.2	29.2 ± 3.9	> 0.05 NS
Positive IgG anti-EBV/VCA (%)	34/40(85%)	33/40 (82.5 %)	> 0.05 NS
Mean titer ± SD	720 ± 40.4	380 ± 20.5	< 0.001
Positive IgM anti EBV/VCA (%).	6/40 (15%)	5/40 (12.5%)	> 0.05 NS
Mean titer ± SD	90 ± 2.9	75 ± 2.7	< 0.05
Positive IgA anti EBV- VCA (%).	15/40 (37.5%)	2/40 (5%)	<0.001 HS
Mean titer ± SD	120 ± 7.5	60 ± 5.2	< 0.001

NS = Non Significant HS = Highly Significant

Regarding serological findings in SLE Patients and control groups we found that the prevalence of positive IgG antibody against EBV-VCA was 34/40 (85%) in the SLE patients versus 33/40 (82.5%); in the control group ($p>0.05$). The prevalence of positive IgM antibody against EBV-VCA was 6/40 (15%) in SLE patients versus 5/40 (12.5%) in the control group ($p>0.05$). Regarding IgA antibody against EBV-VCA we found that IgA antibody against EBV-VCA is significant elevated in SLE patients versus controls 15/40 (37.5) versus 2/40 (5%); ($p<0.001$).

SLEDAI score was significantly higher in the SLE patients with positive IgA antibody against EBV-VCA than in the SLE patients with negative IgA antibody (29 ± 7.7 versus 24.4 ± 3.2 respectively) $p<0.001$.

Regarding disease flare 10 (66.7%) SLE patients with positive IgA antibody against EBV-VCA had exposed to disease flare; mild flare and severe flare were 7 (46.7%) and 3 (20%) respectively. While, 2 (8%) of negative IgA antibody against EBV-VCA, SLE patients had exposed to disease flare (mild flare).

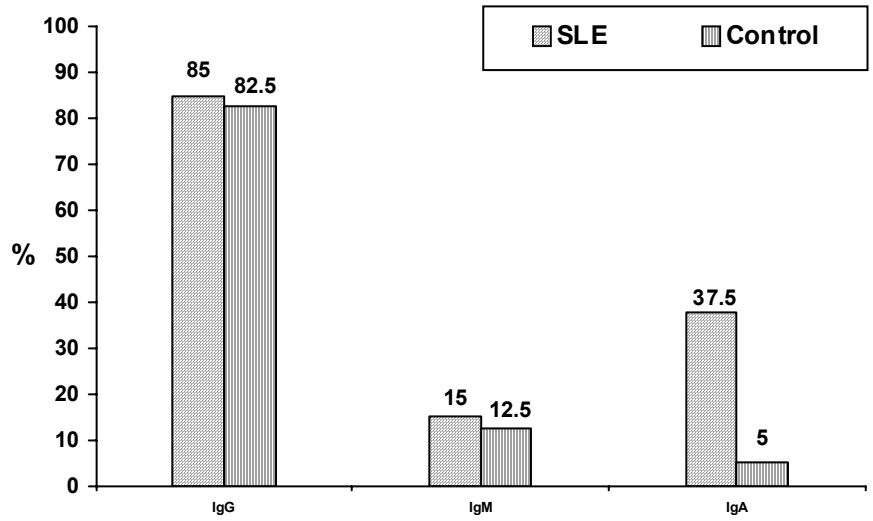

Fig. (1): Sero-Prevalence of EBV-VCA in SLE patient and control groups.

Table (3): Clinical activity and disease flare association in SLE patients.

	With IgA antibody	without IgA antibody	P
Mean SLEDAI ± SD	29 ± 7.7	23.4 ± 2.3	< 0.001
Flare (No., %)			
No flare	5 (33.3)	23 (92)	< 0.001
Mild flare	7 (46.7)	2 (8)	< 0.001
Severe flare	3 (20)	-	< 0.001

Insignificant difference were found between SLE patients and control group regarding seroprevalence of IgG and IgM antibodies against EBV-VCA. Regarding IgA antibody against EBV-VCA we found that IgA antibody against EBV-VCA is significantly elevated in SLE patients versus controls; 15/40 (37.5%) versus 2/40 (5%); respectively, p< 0.001 (Fig. 1).

SLEDAI score was significantly higher in SLE patients with IgA than SLE patients without IgA. (29± 7.7 versus 23 ± 2.3 respectively) p < 0.001.

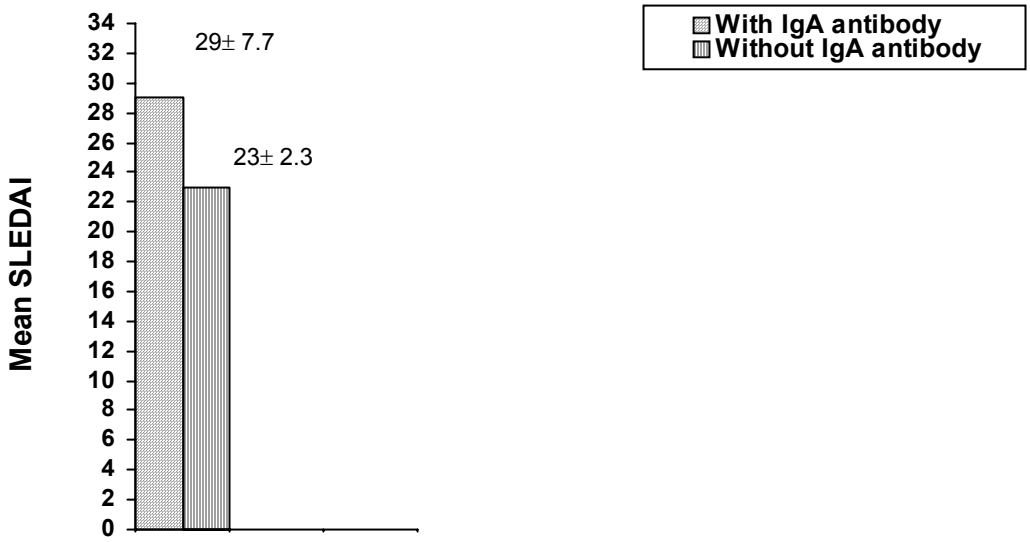

Fig. (2): Association between disease activity in SLE patients with and without IgA.

DISCUSSION

Various genetic and environmental factors appear to be involved in systemic lupus erythematosus (SLE). Epstein-Barr virus (EBV) is among the environmental factors that are suspected of predisposing to SLE, based on the characteristics of EBV itself and on sequence homologies between autoantigens and EBV antigens. In addition, higher titers of anti-EBV antibodies and increased EBV sero conversion rates have been observed in SLE patients as compared with healthy individuals *(Moon et al., 2004)*.

The goal of our study was to determine the sero prevalence of several antibodies (IgM, IgG and IgA) against Epstein Barr virus capsid antigens in SLE patients and to clarify their relation to disease activity and flare. Our study demonstrated non significant difference between SLE patients and controls who had been exposed to EBV-VCA infection (Positive IgG antibody against EBV-

VCA), 34 (85%) and 33 (82.5%), P > 0.05 respectively. While SLE patients and controls who had acute EBV infection (defined by positive IgM antibody against EBV-VCA) were 6 (15%), 5 (12.5%), p>0.05 respectively. The increase percentage of Epstein-Barr virus antibodies in the control group was explained by *Macsween & Crawford (2003)* who attributed this to that EBV infecting over 90% of human and persisting for the lifetime of the person and by *Linde (2003)* who concluded that EBV, the causative agent of infectious mononucleosis is extremely prevalent worldwide, infecting more than 98% of the human population by the age of 40 years.

Huggins et al. (2005) tested sera from SLE patients for antibodies to several EBV antigens and found a significantly higher prevalence of immunoglobulin G antibodies against EBV antigens than in controls and suggested that recent EBV infection or virus reactivation was occurring in SLE patients. Our study demonstrated a significant difference of serum IgA antibody against EBV-VCA between SLE patients and controls "15 (37.5%) versus 2 (5%) p<0.001. *Parkis et al. (2005)* agreed with our results who found that EBV –IgA sero prevalence was strongly associated with SLE and the sero prevalence of EBV-IgM and that of EBV-IgG were not associated with SLE.

The mean SLEDAI score was significantly higher in the SLE patients with IgA antibody against EBV-VCA than in the SLE patients without IgA antibody (29± 7.7 versus 23 ± 2.3 respectively) p<0.001. These findings goes hand by hand with that of *Gross et al. (2005).*

Infectious agents like EBV, Cytomegalovirus and Parovirus B19 may have a role in the occurrence or the exacerbation of SLE *(Kasapcopur et al., 2006).* SLE is a disease of flares and remissions. Although the cause of this is unknown, it is thought that disease flares represent times of greatest immune dysfunction *(Bermas et al., 1994).* We found that the SLE patients with IgA antibody against EBV-VCA had higher prevalence of disease flare compared to those without IgA antibody 10 (66.7%) versus 2 (8 %); p<0.001.

Our results are in agreement with *Chen et al. (2005)* who confirmed that the prevalence of IgA antibody against EBV-VCA was indeed higher in adults with SLE (38.9% Vs 2.8%, p<0.001) and adult SLE patients with IgA antibody against EBV-VCA had higher disease activity compared to SLE patients without IgA antibody against EBV-VCA and also SLE patients with flare showed much higher prevalence of IgA antibody against EBV-VCA compared to those without flare; 13 (81.3%) versus 3 (18.7 %); p<0.001.

Most SLE patients exposed to recurrent flares even under regular medication. This may be due to reactivation of EBV, repeated infection or both (indicated by a positive serum IgA antibody against EBV-VCA). EBV reactivation may cause increased production of autoantibodies with subsequent disease flare; because EBV reactivation in EBV-infected B-cell lines has been found to induce the production of interleukin 10 which is known to elicit B-cell proliferation and anti-double-stranded DNA production *(Liorente et al., 1995 and Sairenji et al., 1998).*

Kang, et al. (2004) found that defective control of latent EBV infection may result in frequent reactivation of EBV, Which in turn may result in disease flare.

Gross et al. (2005) found that patients with SLE have abnormally high frequencies of EBV-infected cells in their blood and this is associated with the occurrence of SLE disease flares.

Parks et al. (2005) suggested that repeated or reactivated EBV infection,

which results in increased EBV-IgA sero prevalence and higher IgG antibody titers, may be associated with SLE.

Moon et al. (2004) concluded that the abnormally increased proportion of EBV-infected B-cells in SLE patients may contribute to enhanced auto antibody production in this disease. *Verdolini et al. (2002)* in their study provided further evidence supporting the hypothesis that EBV infection could work as a trigger in some cases of SLE.

In contrast to this study *Katz et al. (2001)* who found that active EBV infection was not seen in most SLE patients, despite serologic data that could be interpreted as a primary or reactivated infection.

Also, in contrast to this study *Rothfield et al. (1973),* in their study about clinical and laboratory aspects of raised virus antibody titers in systemic lupus erythematosus, they found that the highest mean of EBV antibody titers occurred when clinical activity was absent and declined as signs and symptoms of severity increased.

Conclusion:

The close clinical data association between EBV infection and SLE suggests a possible role of this virus as a trigger in the pathogenesis, disease activity and flare of SLE patients. Further, studies should be done to elucidate the complex relationship between EBV infection and SLE patients.

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مصاحبة فيروس ابشتين بار وعلاقته بمرض الذئبة الحمراء وشدته ونشاطه

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الهدف من البحث: تحديد مدى وجود الأجسام المضادة لفيروس ابشتين بار فى المصل فى مرضى الذئبة الحمراء وعلاقته بنشاط وشدة المرض.

الطريقة: هذه الدراسة أجريت على 40 مريض بالغ بالذئبة الحمراء و 40 من الأصحاء وتتوافق المجموعتان فى الجنس والسن، وتم أخذ تاريخ المرض وإجراء فحص إكلينيكى لتقييم نشاط وشدة المرض والفحوصات المعملية بما فيها اكتشاف الأجسام المضادة أم، جى ، أيه لفيروس ابشتين بار فى المصل بطريقة الفلوروسين الغير مباشر.

النتائج: وقد أظهرت النتائج إرتفاع نسبة الأجسام المضادة وخاصة ايه لفيروس ابشتين بار فى المرضى عنهم فى الأصحاء.

كما وجدت علاقة ذات دلالة إحصائية بين درجة نشاط وشدة المرض فى المرضى الذين يحملون الأجسام المضادة أيه لفيروس ابشتين بارعن الذين لا يحملون الأجسام المضادة ايه لفيروس ابشتين بار .

الإستنتاج: نستنتج من هذا البحث أن الأجسام المضادة وخاصة نوع ايه لفيروس ابشتين بار يزيدوا فى مرضى الذئبة الحمراء ولذا فإنه من الممكن أن يكون لهم دور فى حدوث مرض الذئبة الحمراء.