

## RESEARCH ARTICLE

# Correlation between Epstein-Barr Virus Infection and Disease Activity of Systemic Lupus Erythematosus: a Cross-Sectional Study

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### Abstract

**Background:** Systemic lupus erythematosus (SLE) is an autoimmune disease for whose pathogenesis viral infections are important. The Epstein-Barr virus (EBV) is the main infectious etiological agent. This study aimed to quantitative evaluation of EBV in SLE patients. **Materials and Methods:** In this cross-sectional study, 40 patients with SLE diagnosed based on American College of Rheumatology criteria were selected using purposive sampling. All were included in the study after obtaining informed consent for participation. Whole blood samples were taken and buffy coat preparations were isolated to determine viral load using the real-time polymerase chain reaction method and assessment with the SLE disease activity index (SLE-DAI). **Results:** From a total of 40 patients, 37 cases (92.5%) were women. The EBV test was positive in 67.5% and mean viral load was  $5396 \pm 1891.9$  copy/ml. Twenty of forty patients had active and 50% inactive disease, mean EBV viral loads being 6798 and 28.25 copy/ml, respectively (P-value = 0.003). In terms of the severity of disease activity, 17.5 % of female patients had mild to moderate activity, whilst 32.5% of them had severe activity, with respective viral loads of 5,803.3 and 29.73 copy/ml (P-value = 0.003). **Conclusion:** The Epstein-Barr viral load in SLE patients with active disease was found to be markedly higher than in inactive cases. Thus, EBV may have an important role in the pathogenesis and activity of SLE.

**Keywords:** Epstein-Barr virus- viral load- systemic lupus erythematosus- real-time PCR- human- infection

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### Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease with incidence of 6-35 cases per 100,000 people in year and appears mainly in fertility age of women (Draborg et al., 2012). Disease signs are butterfly rash in face, discoid rash, sensitivity to light, mouth ulcer, arthritis, serositis, renal involvement, blood disorder (anemia, leukopenia, lymphocytopenia, thrombocytopenia) and neuropathy and positive tests anti-nuclear antibody(ANA) and anti dsDNA antibody (Fauci & Morens, 2012). Ethology of lupus is multifactorial and influenced by genetic and peripheral factors including sex, age and social-economic situation.

Typical revolution of disease is seen as alternative periods of exacerbation and remission. Environmental risk factors for SLE are including UV lights, drugs, special chemistry substances and infections specially EBV (Draborg et al., 2012; Fauci et al., 2012). EBV is known as human herpes type 4 (HHV-4) that is linear double stranded DNA virus with capsid and envelope. EBV virus is an infectious agent over the world and is approximately latent in 95% of population. Virus transmits from saliva and become transcribed in mucosal surfaces

of oropharynx and nasopharynx specially in tonsils region and then enters to underlying tissue and make infection by binding of its envelope glycoprotein to special receptor on B lymphocyte. This virus has ability to shifting from lytic cycle to latency (Draborg et al., 2012).

Infection with this virus is asymptomatic in childhood but in adolescence causes infectious mononucleosis with symptoms of skin rash, palatal exanthema, arthralgia, renal disorder and anemia (Straus et al., 1993). During the lytic cycle, DNA of virus is transcribed and strengthen of expressed genes reached to 100-1000 times. This events causes the pouring virus to saliva which can infect other B lymphocytes and epithelial cells. Virus enters become latent in response to host immunity. Progress of infection in B lymphocytes during lytic cycle can be controlled specifically by T cytotoxic cells which declines infected B lymphocytes and induces the latent phase (Draborg et al., 2012). EBV stimulates the proliferation of B lymphocytes after infection and EBV become long standing in SLE patients with genetically susceptibility. On the other hand B lymphocytes infected with virus are a continual source for producing auto antigen and auto antibody; also some of protein sequences of virus have homology with human auto antigens. The reaction of antinuclear 1, 2

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autoantibody created against virus interacts with human auto antigen and produces SLE disease (Amaral et al., 2014; Namjou et al., 2007).

High titer of anti EBV antibody and serum appearance of virus in persons with SLE in compare with control people was seen. Antibody response in serum lonely cannot show the presence of virus directly, but other methods like as PCR on saliva or peripheral blood samples will be helping to show the presence and activity of virus (Draborg et al., 2012).

PCR product was quantified through real-time PCR using a fluorogenic probe. Nowadays this method is become a suitable alternative for other quantitative PCR methods and it is used extensively in the world for measuring load of EBV virus. Lupus patients have the infected B lymphocytes at least 10 times more than normal people and this increase can be correlated to increase in activity of disease (Gross et al., 2005).

In one study by using this method, 40 times increasing of EBV virus load in SLE patients compared to normal people was reported (Kang et al., 2004). In one another study it was shown a significant increase in serum level of EBV DNA in 42% of SLE patients compared with 3% of controls (Lu et al., 2007). Moon and Colleagues in 2004 showed the prevalence and type of infection with EBV was equal in people with SLE and controls (94-98.5%), but viral load in peripheral blood mononuclear cells of persons with SLE was 15 times of control people (Moon et al., 2004).

Recently there are reports that some people after affecting of infectious mononucleosis had acquired lupus erythematous which is documentation for the theory of lupus caused by EBV. It was seen in normal people that out of 100,000 to 1000,000 B lymphocytes, one cell is latently infected by EBV, but in people with SLE the number of infected cell is higher and every cell contains 30 viral episome (Ito et al., 2016; Macsween et al., 2014; Rocchi et al., 1977).

Katz and Colleagues studied on 13 SLE patients using PCR method and they did not find any viral genome in peripheral blood mononuclear cells; that is maybe because of problem(s) in type of used PCR method (Katz et al., 2001).

With respect to slight studies in the case of viral serum load in SLE patients, we tried to study about load of this virus in SLE patients and inactive status of disease using real-time PCR method in order to understanding the role of EBV virus in create and activity enhancement of disease, through the understanding of this role we can prevent SLE disease and it's activity in future.

## Materials and methods

In this Cross-sectional study 40 SLE patients referred to rheumatology clinic of Beheshti Hospital and diagnosed as based on American rheumatology Association (ACR) criteria and included in study according to the purpose and as a census were selected during the year of 2014.

Activity of disease was measured by SLEADI standard questionnaire (Lotfi et al., 2016; Saba et al., 2017; Sharif et al., 2016). This questionnaire Contains some clinical

and laboratory findings related to SLE disease (Fauci and Morens, 2012). In the base of questionnaire, score less than 6, disease is inactive form and score equal or more than 6 until 12 reveals the mild to moderate active form and score more than 12 consider as severe active form.

After obtaining the informed consent from all patients and giving sufficient explanation for them regarding to security of information, 10 mL vein blood sample was taken and was sent to PCR center of Kashan Beheshti Hospital (Ferdosian et al., 2015; Jalali et al., 2016; Kashani et al., 2015). In order to assess the serum viral load of EBV, first buffy coat DNA was extracted by High Pure Viral Nucleic Acid Kit (Roche, kit Mannheim, Germany) and was kept in -20°C freezer. For measuring viral load , extracted DNA along with other substances included in Master mix and primer /probe from BKRF1 gen region (table1) and applied Bio-system real time PCR (Taq-Man PCR method) was used.

In summary 250 ng of DNA extracted from 5 ml buffy coat added to PCR master mix containing; 10mM Tris (PH:8.3), KCl(50mM), EDTA(10mM), MgCl<sub>2</sub>(5mM), 100mM of dATP, dCTP, dGTP and dTTP, primers(0.2mM each one), 0.1 mM Taq Man, Enzyme (Applied Biosystems). Following activation of Ampli-Taq gold Enzyme for 10 min in 95°C, 45-50 cycles including 15 sec in 95°C, 1 min in 62°C for PCR protocol was considered. Mean of EBV virus load is obtained in 2 groups with active and inactive disease and was recorded as copy/ml.

## Statistical method

In this study we applied one way, but 2-tailed independent samples Student's t tests. These analyses were done using analysis of variance (ANOVA) (Hosseini et al., 2016; Kashani et al., 2012; Nikzad et al., 2013). P-values <0.05 were considered statistically significant. The test compared the average of two independent groups with each other. In this test, the average obtained from a random sample, are judged. This means that the samples from two different groups, whether equal or unequal number of samples are randomly selected and the average of the two groups were compared. The Kolmogorov-Smirnov test was applied to determine the normal distribution of variables (Dehghani et al., 2016a; Dehghani et al., 2016b; Kashani et al., 2013). All statistical analyses were done using the Statistical Package for Social Science version 19 (SPSS Inc., Chicago, Illinois, USA) (Dehghani et al., 2016c; Kashani et al., 2013).

## Results

In the present study, that is done on 40 SLE patients, there was 37(92.5%) women and 3 (7.5%) men that abundance ratio of women to men was 9 to 1. The duration of the disease was in 27 person (67.5%) less than 5 years, in 12 person (30%) between 5-10 years and in 1 person (2.5%) higher than 10 years. EBV DNA was negative in 13 person (32.5%) and positive in 27 person (67.5%). In active form of lupus, the amount of negative and positive DNA test was 15% and 85% respectively, but in inactive form was 50% in each group (Table 1). On the other hand most people with positive DNA test (63%) had active form

Table 1. Primers and Probes Used for EBV Viral Load Assessment

Viral gene	Sequence (5'-3')	Primer/probe
BKRF1	CGG TGT GTT CGT ATA TGG AGG TAG TA	Forward
BKRF1	AGA CCA TGA AAT AAC AGA CAA TGG AC	Reverse
BKRF1*	AGT CGT CTC CCC TTT GGA ATG GC	3'-Fluorescein probe

Star arrow\*, 3'-Fluorescein probe

Table 2. The Average and Standard Error of EBV Viral Load with SLE Disease/ EBV Positive in Form of Active or Inactive of Disease and Patients with Active Lupus in Term of Severity of Disease

	SLE disease	N	Mean	SD	P-value*
	Active	18	5803.3	7178.0	0.003
	Inactive	19	29.7	43.3	
EBV Positive	Active	17	7997.6	9003.5	0.002
	Inactive	10	56.5	45.5	
Severity	Mild to moderate	7	6608.6	7769.1	0.946
	Severe	13	6900.0	9563.7	

\*Statistical significance was attained when (P-Value < 0.05).

& most people with negative DNA test (76.9) had inactive form of SLE disease (P-value < 0.01).

In terms of the severity of disease activity, 17.5 % of patients had mild and moderate activity, whilst 32.5% of them had severe activity. The average EBV load in active and inactive was 6,798 copy/ml and 28.2 copy/ml respectively; this difference was statistically significant (P-value =0.003). The average load of virus in women with active form was more than inactive form, 5803.3copy/ml and 29.73copy/ml respectively; that was statistically significant (P-value =0.003) (Table 2).

## Discussion

The results of this study showed that the 7.5% of SLE patients were men and 92.5% were women. In different forms of lupus, the proportion of women is more than men which in text books, this ratio of male to female involvement is 9-10 to 1 respectively. The majority of women (51 %) had an inactive form and the majority of men (66.7%) had the active form of Lupus, but these differences, weren't statistically significant. In a study on 76 patients with inactive lupus and 42 patients with active lupus it was showed that in both patients with active and inactive lupus, number of women was more than men (Larsen et al., 2011).

In another study 24 out of 27 SLE patients with positive EBV were women and 3 out of 27 were men. Among patients with Lupus and negative EBV test , 68 and 14 patients were women and men respectively (Ulff-Møller et al., 2010). Also other study on 120 lupus patients it showed 129 out of them were women and 85% of women had active lupus (Shoenfeld et al., 2009). In a study that conducted by Chen et al., (2005) in Taiwan on 36 patients, there was 34 woman and only 2 man, and most of active lupus patients were women. Moreover other research on 82 out of 96 SLE patients were woman (94% of them with active SLE) and the rest were men; most of them had inactive Lupus. These findings are contrary to the findings of our study (D.Y. Chen et al., 2010). Study of Tazi et al., (2009) in 44 SLE patients, revealed that

39 woman and 5 man had SLE, most of men and women had active lupus.

The results of present study showed 67.5% of SLE patients had positive EBV and 32.5% were negative. In active form the percentage of negative and positive EBV test was 15% and 85% respectively. In inactive form was 50% and 50%. The most of patients with positive EBV test (63%) had active form of disease, whereas the most of people with negative EBV test (76.9%) had inactive form, these differences were statistically significant.

Lu et al., (2007) in study on 93 SLE patients showed 32.1% of them were positive EBV test and in control group had 4.1% positive test and also in this study it was found that prevalence of EBV in patients with active form is more than inactive form , similar to the results in our study. In another study on 33 Egyptian patients with Lupus and 30 healthy person, 32 (96.9%) were positive EBV test and in control group 20 people (66.6%) had positive test (Mohamed, Hasen, Mohammed and Elmaraghy, 2015).

The results of the studies carried out by others is different from the present study, which can be the cause of this difference in laboratory methods to be used, so that in some of these studies only of serum antibodies against the virus is used. That is not an accurate test and it shows a higher rate of being positive, while in present study we used Real-Time PCR which is more accurate. Average of EBV viral load in patients with active and inactive lupus was 6,798 copy/ml and 28.25copy/ml respectively, that this difference is statistically considerable. on the other hand, the average of EBV viral load in women with active form was 5803.3 copy/mol and in women with inactive form was 29.73copy/ml, that showed the average virus load in women in active form is more than inactive that these differences was statistically significant. In a study by Gross in order to check EBV viral load in patients with systemic lupus, it was shown that viral load of EBV in patients compared to the healthy group has been significantly higher, so that comparison of average viral load in two group has showed viral load in lupus patients is about 40 times more than control group. In this study it was found that average viral load in patients with active

lupus is more than inactive ones and there was statistically significant difference. And also it was shown that the viral load has a direct relationship with the severity of disease activity (Gross et al., 2005). Kang in 2004 by studying on 32 lupus patients in New York USA, showed that increase in viral load in patients with Lupus is 40 times more than healthy group and this factor in patients with active lupus EBV virus load is more than patients with inactive lupus (Kang et al., 2004).

In a study Larsen on 76 patients affected by inactive lupus and 42 patients with active lupus showed that average of viral load in patients with active and inactive lupus is 19,300copy/ml (35,400-2,800) and 17300copy/ml (28,900-2,900) respectively. This difference is statistically significant and its results is similar to our results, but in this study amount of viral load in patients with inactive lupus is higher than that in our study (Larsen et al., 2011). Present study showed that the amount of EBV viral load in patients with mild to moderate active lupus and severe was 6608 copy/mol and 7769 copy/ml respectively that this difference was not statistically significant. In a research on 33 Egyptian lupus patients and 30 healthy controls, the frequency of virus in patients with higher severity was more (Mohamed et al., 2015). The results of this study was different from our study results.

In a study by Moon on 24 lupus patients and 29 healthy controls showed EBV viral load in patients with active lupus was higher than inactive lupus and also the amount of viral load in patients with mild active lupus compared to patient with severe active lupus is significantly different. The difference is approximately 5 times the estimated and this is different from the results of the present study (Moon et al., 2004).

In another study on 24 lupus patients and 44 healthy controls showed that average viral load and Antibody against EBV virus in lupus patients was higher than control group and in patients with more severe lupus, average viral load is 3 times more than patients with mild lupus that this difference was statistically significant. These results are different from the results of our study. The differences in the studies could be due to the use of different methods and devices with different precision or perhaps due to the conditions of the society and the society of our sample (Tazi et al., 2009).

The results of present study showed the average viral load in patients with diploma or lower education level in active and inactive form was 10,138copy/ml and 18.1 copy/ml respectively and in patients with persons with education level higher than diploma was 4,517.7copy/ml and 36.5 copy/mol respectively, that this difference was statistically meaningful. It also was shown in the present study that the viral load in patients with intermediate economic situation in active and inactive form was 7,113copy/ml and 28.2 copy/ml respectively. This result was statistically significant; Of course these variables in other studies have not yet been reviewed. At the current study due to the low number of men in we did statistical analysis on women only.

According to this study the amount of infection with EBV virus in SLE patients is high and in viral load of virus in patients with active lupus is higher than patients

with inactive lupus; this is evidence for the role of virus on activity and pathogenesis of disease.

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### Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments.

### Conflict of interest

The authors declared that they have no competing interests.

## References

- Amaral B, Murphy G, Ioannou Y, et al (2014). A comparison of the outcome of adolescent and adult-onset systemic lupus erythematosus. *Rheumatology*, **53**, 1130-35.
- Chen C-J, Lin K-H, Lin S-C, et al (2005). High prevalence of immunoglobulin a antibody against Epstein-Barr virus capsid antigen in adult patients with lupus with disease flare: case control studies. *J Rheumatol*, **32**, 44-7.
- Chen D-Y, Chen Y-M, Lan J-L, et al (2010). Polymyositis/dermatomyositis and nasopharyngeal carcinoma: The Epstein-Barr virus connection?. *J Clin Virol*, **49**, 290-5.
- Dehghani R, Sharif A, Assadi MA, et al (2016a). Fungal flora in the mouth of venomous and non-venomous snakes. *Comp Clin Path*, **25**, 1207-11.
- Dehghani R, Sharif A, Madani M, et al (2016b). Factors influencing animal bites in Iran: a descriptive study. *Osong Public Health Res Perspect*, **7**, 273-7.
- Dehghani R, Sharif M R, Moniri R, et al (2016c). The identification of bacterial flora in oral cavity of snakes. *Comp Clin Path*, **25**, 279-83.
- Draborg A H, Duus K, Houen G (2012). Epstein-Barr virus and systemic lupus erythematosus. *Clin Dev Immunol*, **2012**, 370516.
- Fauci AS, Morens DM (2012). The perpetual challenge of infectious diseases. *N Engl J Med*, **366**, 454-61.
- Ferdosian M, Khatami MR, Malekshahi ZV, et al (2015). Identification of immunotopes against Mycobacterium leprae as immune targets using PhDTm-12mer phage display peptide library. *Trop J Pharm Res*, **14**, 1153-9.
- Gross AJ, Hochberg D, Rand WM, et al (2005). EBV and systemic lupus erythematosus: a new perspective. *J Immunol*, **174**, 6599-6607.
- Hosseini ES, Moniri R, Goli YD, et al (2016). Purification of antibacterial CHAPK protein using a self-cleaving fusion tag and its activity against methicillin-resistant staphylococcus aureus. *Probiotics Antimicrob Proteins*, **8**, 202-10.
- Ito Y, Suzuki M, Kawada J-I, et al (2016). Diagnostic values for the viral load in peripheral blood mononuclear cells of patients with chronic active Epstein-Barr virus disease. *J Infect Public Health*, **22**, 268-71.
- Jalali HK, Salamatzadeh A, Jalali AK, et al (2016). Antagonistic activity of nocardia brasiliensis PTCC 1422 against isolated enterobacteriaceae from urinary tract infections. *Probiotics Antimicrob Proteins*, **8**, 41-5.

- Kang I, Quan T, Nolasco H, et al (2004). Defective control of latent Epstein-Barr virus infection in systemic lupus erythematosus. *J Immunol*, **172**, 1287-94.
- Kashani HH, Nikzad H, Mobaseri S, et al (2012). Synergism effect of nisin peptide in reducing chemical preservatives in food industry. *Life Sci*, **9**, 496-501.
- Kashani HH, Moniri R (2015). Expression of recombinant pET22b-LysK-cysteine/histidine dependent amidohydrolase/peptidase bacteriophage therapeutic protein in Escherichia coli BL21 (DE3). *Osong Public Health Res Perspect*, **6**, 256-60.
- Kashani HH, Moshkdanian G, Atlasi MA, et al (2013). Expression of galectin-3 as a testis inflammatory marker in vasectomised mice. *Cell (Yakhteh)*, **15**, 11.
- Katz BZ, Salimi B, Kim S, et al (2001). Epstein-Barr virus burden in adolescents with systemic lupus erythematosus. *Pediatr Infect Dis J*, **20**, 148-53.
- Larsen M, Sauce D, Deback C, et al (2011). Exhausted cytotoxic control of Epstein-Barr virus in human lupus. *PLoS Pathog*, **7**, e1002328.
- Lotfi A, Shiasi K, Amini R, et al (2016). Comparing the effects of two feeding methods on metabolic bone disease in newborns with very low birth weights. *Glob J Health Sci*, **8**, 249.
- Lu JJ, Chen D-Y, Hsieh C, et al (2007). Association of Epstein-Barr virus infection with systemic lupus erythematosus in Taiwan. *Lupus*, **16**, 168-75.
- Macswen KF, Johannessen I (2014). Epstein-Barr virus (EBV): infectious mononucleosis and other non-malignant EBV-associated diseases *Viral Infections of Humans*, Springer, pp 867-96.
- Mohamed AE, Hasen AM, Mohammed GF, et al (2015). Real-Time PCR of cytomegalovirus and Epstein-Barr virus in adult Egyptian patients with systemic lupus erythematosus. *Int J Rheum Dis*, **18**, 452-8.
- Moon UY, Park SJ, Oh ST, et al (2004). Patients with systemic lupus erythematosus have abnormally elevated Epstein-Barr virus load in blood. *Arthritis Res Ther*, **6**, R295.
- Namjou B, Kilpatrick J, Harley JB (2007). Genetics of clinical expression in SLE. *Autoimmunity*, **40**, 602-12.
- Nikzad H, Kashani HH, Kabir-Salmani M, et al (2013). Expression of galectin-8 on human endometrium: Molecular and cellular aspects. *Iran J Reprod Med*, **11**, 65.
- Rocchi G, de Felici A, Ragona G, et al (1977). Quantitative evaluation of Epstein-Barr-virus-infected mononuclear peripheral blood leukocytes in infectious mononucleosis. *N Engl J Med*, **296**, 132-4.
- Saba M, Valeh T, Ehteram H, et al (2017). Diagnostic value of neuron-specific enolase (NSE) and cancer antigen 15-3 (CA 15-3) in the diagnosis of pleural effusions. *Asian Pac J Cancer Prev*, **18**, 257.
- Sharif MR, Kashani HH, Ardakani AT, et al (2016). The Effect of a yeast probiotic on acute diarrhea in children. *Probiotics Antimicrob Proteins*, **8**, 211-14.
- Shoenfeld Y, Berkun Y, Zandman-Goddard G, et al (2009). Infectious antibodies in systemic lupus erythematosus patients. *Lupus*, **18**, 1129-35.
- Straus SE, Cohen JI, Tosato G, et al (1993). Epstein-Barr virus infections: biology, pathogenesis, and management. *Ann Intern Med*, **118**, 45-58.
- Tazi I, Fehri S, Elghrari K, et al (2009). Systemic lupus erythematosus and Epstein-Barr virus. *Viruses*, **15**, 701-8.
- Ulf-Møller CJ, Nielsen NM, Rostgaard K, et al (2010). Epstein-Barr virus-associated infectious mononucleosis and risk of systemic lupus erythematosus. *Rheumatology*, **49**, 1706-12.