ORIGINAL ARTICLE

Cytomegalovirus and Epstein–Barr Virus Infection: Two Triggers flaring up Systemic Lupus Erythematosus Patients

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ABSTRACT

Background: *Systemic lupus erythematosus (SLE) is an autoimmune, multi-system, chronic inflammatory disease The effect of Cytomegalovirus(CMV) and Epstein-Barr virus(EBV) in triggering SLE has been investigated for many years.* ***Objectives:*** *To study the association of viral load of CMV-EBV in the serum of SLE patients' with SLE disease parameters.* ***Methodology:*** *48SLE patients and 40controls were enrolled. Disease activity was assessed using SLE disease activity index (SLEDAI). Quantitation of CMV and EBV-DNA in serum were detected by real-time polymerase chain reaction.* ***Results:*** *Patients were 91.8% females and 8.2%males. Mean age was 26.6±8.0 years, mean disease duration 4.5±3.1 years, and age at onset 22.1±7.8 years. 41.7% of SLE patients had CMV-DNA, 54.2% patients had EBV-DNA. Neither EBV-DNA nor CMV-DNA were found in the healthy controls. Copy numbers of CMV and EBV found in the serum of SLE patients were 26827±25879copies/µl, and 25309±22852copies/µl, respectively. Regarding SLEDAI; 83.3% showed high disease activity. Renal biopsy revealed that, 66.7% of the patients had lupus nephritis; 50% with CMV-DNA, and 56.25% with EBV-DNA. Regarding the association between CMV and EBV with different disease parameters in SLE patients; we found significant associations with: photosensitivity, Raynaud's and thrombocytopenia (pvalue<0.05). EBV-DNA were significantly associated with: pyuria, oral ulcers, photosensitivity, vasculitis and involvement of nervous system (r=0.43, p=0.002), (r=0.36, p=0.01), (r=0.42, p=0.003), (r=-0.33, p=0.023), (r=0.32, p=0.029) respectively.* ***Conclusions:*** *A high incidence of CMV and EBV was detected in SLE patients with increased viral load. Disease activity of SLE patients is significantly higher in patients infected with CMV and EBV compared to non-infected.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune, multi-system, chronic inflammatory disease that is characterized by bouts of remission and exacerbation. Several endogenous and exogenous factors are contributed in the pathogenesis of SLE[1].The condition involves the generation of self-reactive antibodies, increased antigenic load, regulatory T-cell dysfunction and polyclonal B-cell activation[2].The genetic, environmental and infectious factors, especially viral infections, may represent a potent role in the immune dys-regulation. The effect of viruses in triggering SLE has been investigated for many years. Incriminated viruses include: Epstein–Barr virus (EBV)[3], cytomegalovirus(CMV)[4], parvovirus B19[5] and human endogenous retroviruses[6]. Both, CMV and EBV belong to the human herpes virus family. They are characterized by persistence and latency, with acute replicative reactivation of infections[7].

Infectious mononucleosis is caused by Epstein–Barr virus (EBV), is the most commonly stressed viral agent that eases autoimmunity in SLE[3]. EBV infects B cells, resulting in polyclonal B-cell activation, which plays a major role on the immune system. Laboratory and clinical investigations have exposed that EBV triggers the development of SLE. EBV activates CD4þT lymphocytes, which secrete huge amounts of cytokines, leading to a chronic inflammatory response in SLE. In addition, several studies have shown that SLE patients had higher sero-prevalence rates of anti-EBV antibodies with an increase in the viral load compared to healthy controls[8-11]. Moreover, immunological similarity has been detected between EBV nuclear antigen (EBNA) and lupus specific Smith (Sm) nuclear antigens[12]. CMV is a common pathogen that infects 60–90% of the

world's population. Next a primary infection, it exists in latently infected premonocytic or monocytes cells, and reactivation often motivated by inflammation may periodically occur. An association between CMV infection and SLE have been speculated[10,11,13-15].

The co-existence of CMV and EBV had been studied in a different geographical regions; however, there is still limited data about EBV and CMV in Egyptian patients with SLE [7].

So, the purpose of the current study was to assess the association of the viral load of EBV and CMV in Egyptian SLE patients in correlation with other disease parameters in the study population.

METHODOLOGY

This cross-sectional study included forty eight SLE patients fulfilling the European League Against Rheumatism and the American College of Rheumatology (EULAR/ACR) 2019 classification criteria[16], recruited from Internal Medicine and Rheumatology and Rehabilitation Departments, Fayoum University Hospitals within one year. We exclude patients if: patients had other autoimmune diseases like rheumatoid arthritis, scleroderma, demato/polymyositis and mixed connective tissue disease. Patients excluded also were, those complaining from other viral or bacterial infections or suffering from malignancies, lymphoproliferative disorders, hematologic diseases, hepatosplenic diseases, gastrointestinal diseases, endocrinological disorders or diabetes. Forty age and sex matched healthy adults were included as control. The patients' consents were attained and the study was approved by the local Ethics Committee of Fayoum University, reseaech number (R175) in its session (83) on 13/6/2021, and in accordance to the 1964 Helsinki declaration.

All patients included in our study were subjected to full history taking, and thorough clinical examination. Their age ranged from 18 to 60 years old. Disease activity was evaluated using Systemic lupus erythematosus disease Activity Index (SLEDAI) score[17].

Laboratory, immunological and radiological investigations

Laboratory investigations were done including routine laboratory tests: complete blood picture, erythrocyte sedimentation rate (ESR), creatinine, aspartate transaminase (AST) and alanine transaminase (ALT), random blood sugar, and complete urine analysis. Other specific laboratory for SLE: 24hours protein in urine, aiding in assessment of lupus nephritis.

The immune profile included: antinuclear antibody by indirect immunofluorescence (ANA) on Hep-2 cells, antibody against double stranded DNA (anti-dsDNA) by modified Farr assay and complement components (C3-C4) using immunodiffusion plates.

Quantitation of Cytomegalovirus (CMV) and Ebestein Barr virus (EBV-DNA) in patient's serum:

Quantitation of CMV and EBV- DNA was performed by real-time polymerase chain reaction (PCR). Serum was prepared from blood drawn by centrifugation. Each sample was separated into two aliquots; stored at -70ºC until being tested.

DNA was isolated using the QIAamp DNA mini kit (QIAGEN,Valencia, CA) as described by the manufacturer.

Quantitation of the EBV and CMV - DNA was conducted using CMV- and EBV specific primers sequences aiming, the conserved 105-bp region of the major immediate-early antigen in the CMV genome and 97 bp region of the conserved Epstein-Barr nuclear antigen 1 (EBNA-1) region in the EBV genome as designated by the Artus® CMV TM PCR Kit and the Artus® EBV TM PCR Kit (QIAGEN,Valencia, CA), respectively[18].

Quantitative real-time PCR assay amplification, data acquisition, and data analysis were carried out on ABI 7000 real-time PCR system (Applied Biosystems, Life technologies).The Artus EBV & CMV TM PCR kit primer and probes are proprietary and not made publicly available. Four quantitation standards were involved in each run to generate a standard curve involving 5-10^4 copies/ µl, 5 - 10^3 copies/ µl, 5-10^2 copies/ µl and, 5-10^1 copies/ µl for EBV and 1-10^4 copies/ µl, 1 -10^3 copies/ µl, 1-10^2 copies/ µl and 1-10^1 copies/ µl for CMV. To modify this copy number to copies/mL for patient samples, a conversion factor was used. The detection limit of the Artus CMV TM PCR Kit and the Artus EBV TM PCR Kit is 0.20 copies/ µl and 5.3 copies/ µl respectively. For either reactions, a negative control was added in the form of PCR grade water, in addition to the internal control which was used to validate DNA isolation procedure and to check for probable PCR inhibition.

Imaging techniques: Plain X-ray of the affected joint was performed and ultrasound guided renal biopsy was obtained to those with suspected lupus nephritis.

Statistical analysis

We analyzed data using SPSS (Statistical package for the social sciences) version 20. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test. Spearman-rho method was used to test correlation between numerical variables. p value < 0.05 was considered significant.

RESULTS

Forty eight SLE patients; 44 (91.8 %) females and 4 (8.2%) males with mean age 26.6 ±8.0 years (16–43 years) and 40 age and sex matched controls were involved in the current study. The mean disease duration was 4.5 ±3.1 years (0.25-12 years), and age of disease onset was 22.1±7.8 years (9-37years). Regarding viral DNA detection about 20 (41.7%) of SLE patients has a CMV-DNA versus 26 (54.2%) for EBV-DNA, from them, 12 (25%) SLE patients had both EBV and CMV- DNA. Demographic, clinical characteristics and laboratory features of the patients are presented in table 1a, table 1b and table 1c.

Table 1a: Demographic, characteristics of systemic lupus erythematosus (SLE) patients

Parameter mean±*SD* (range) or n (%)	SLE patients (n=48)
Demographic data	
Age/ years	26.6 ±8.0(16–43)
Age of onset/years	22.1±7.8 (9-37)
Disease duration/years	4.5 ±3.1 (0.25-12)
Female % / Male%	91.7/ 8.3
Female: Male	11: 1

SLE systemic lupus erythematosus, n number,
SD standard deviation,

Table 1b: Clinical characteristics of systemic lupus erythematosus (SLE) patients

Parameter n (%)	SLE patients (n=48)
Clinical features	
Arthritis/arthralgia	32 (66.7)
Oral ulcer	24 (50)
Malar rash	30 (62.5)
Photosensitivity	18 (37.5)
Alopecia	22 (45.8)
Raynaud's phenomenon	12 (25)
Vasculitis	10(20.8)
Serositis	18 (37.5)
Renal involvement	32 (66.7)
Nervous system	6 (12.5)

SLE systemic lupus erythematosus, n number,
SD standard deviation,

Table 1c: Laboratory characteristics of systemic lupus erythematosus (SLE) patients

Parameter mean±*SD* (range) or n (%)	SLE patients (n=48)
Laboratory investigations.	
Hemoglobin (g/dl)	9.8±1.6(6.2-12.1)
TLC (x10³/mm³)	5.9±3.1(1.8-13.8)
Platelets (x10³/mm³)	261.7±70.99 (146-475)
ESR (mm/1st hr)	75.5±5.3 (15-140)
Creatinine (mg/dl)	1.4±1.3 (0.45-6.37)
AST(U/L)	23.6± 12.98 (10-59)
ALT (U/L)	21± 12.6 (7-60)
Serum C3(mg/dl)low (< 76 mg/dl)	38(79.2)
Serum C4 (mg/dl) low (< 9 mg/dl)	32 (66.7)
CMV- DNA (copies/ µl) 20	26827±25879 (1200-70410) (range69210)
EBV- DNA (copies/ µl) 26	25309±22852 (2130-90940) (range 88810)
Urine analysis with casts	22(45.8)
Pyuria	6(12.5)
ANA (positive)	44 (91.7)
Anti-dsDNA antibody(>100 IU/ml)	28 (58.3)
Pturia 24 hrs (> 0.5 g/24hrs)	24(50)
CMV-DNA	20(41.7)
EBV-DNA	26(54.2)
CMV-EBV DNA	12(25)

SLE systemic lupus erythematosus, n number, SD standard deviation, ANA anti nuclear antibody, C complement, % percentage, Inv. investigations, ds DNA double stranded deoxynucleic acid antibody, Pturia 24 hrs proteinuria in twenty four hours, CMV-DNA cytomegalovirus deoxybonucleic acid, EBV-DNA Epestein- barr virus deoxybonucleic acid.

Regarding viral DNA no viral detection in healthy control for both CMV-DNA and EBV-DNA, with viral load below the detection limit for both kits. The mean ± *SD*, copy numbers of CMV and EBV found in the serum of SLE patients were 26827±25879 (1200-70410) copies/µl and 25309±22852 (2130-90940) copies/µl, respectively.

Disease activity in SLE was scored by SLEDAI, with correlation to the presence of either CMV-DNA and\or EBV-DNA. Results are shown in table 2.

Table 2: Frequency of Cytomegalovirus and Epstein-Barr virus infection with disease activity in systemic lupus erythematosus patients

n (%)	SLE patients (n=48)		
SLEADI	Not active	Mild- moderate	Severe
	0	8 (16.7)	40/48(83.3)
CMV-DNA	0	0	20/40(50)
EBV-DNA	0	0	26/40(65)
CMV-EBV	0	0	12/40(30)

SLEDAI Systemic lupus erythematous disease activity Index, CMV-DNA Cytomegalo virus deoxynucleic acid, EBV-DNA Epstein-barr virus deoxynucleic acid.
N.B.SLEDAI (9 organs, scoring : 0-3= not active, 4-12 mild or moderate, >12=severe)

Comparison between Epstein-Barr viruses and Cytomegalovirus viral DNA load in different in SLE clinical and laboratory parameters are shown in table 3a and table 3b

Renal biopsy revealed that, 32 (66.7%) patients had lupus nephritis, out of which CMV-DNA was detected in16 (50%) patients, eighteen (56.25%) lupus nephritis patients had EBV-DNA, while 10 (31.25%) patients had both EBV- DNA and CMV- DNA. Table 3d

In this work we revealed the frequency between CMV and EBV virus in blood with different disease parameters in SLE patients; significant associations were found with photosensitivity, Raynaud's and thrombocytopenia (p= 0.045), (p= 0.013), (p = 0.046) respectively are shown in table 3c and table 3d.

Table 3a: Comparison between Cytomegalovirus and Epstein-Barr viruses viral load in different in systemic lupus erythematosus clinical parameters

SLE disease parameters (no. of Patients)	CMV-DNA mean± SD	EBV-DNA mean ±SD	p
Arthritis (32)	25143±27421	23299±14112	0.815
Alopecia (22)	30818±26711	30655±26701	0.988
Oral ulcers (24)	29275±25445	36309±26156	0.486
Malar rash (30)	29028±24546	29533±27989	0.960
Photosensitivity (18)	33983±20904	37343±28219	0.80
Raynaud's (12)	20500±0	25568±16125	0.682
Serositis (18)	38560±10599	24524±5032	0.219
Vasculitis (10)	5660±5150	2130±0	0.412
Renal involvement(32)	29706±28230	28551± 26900	0.90
Nervous system(6)	7627±11131	46535±51274	0.262

CMV-DNA cytomegalovirus deoxybonucleic acid, EBV-DNA Epstein-barr virus deoxybonucleic acid

Table 3b: Comparison between Cytomegalovirus and Epstein-Barr viruses viral load in different in systemic lupus erythematosus laboratory parameters

SLE disease parameters (no. of Patients)	CMV-DNA mean± SD	EBV-DNA mean ±SD	p
Laboratory			
Anemia(hemoglobin<11g/dl)(32)	26061±26581	17306±9472	0.207
Leucopenia (<4000/µl) (16)	23505±24119	23878±5019	0.959
Thrombocytopenia(<200000/µl)	10370±0	0±0	-
ANA (44)	29674±25742	27241±22737	0.75
anti-dsDNA (28)	36360± 25405	26816±25971	0.319
C3 (low) (38)	24551±25564	24848±23764	0.970
C4 (low) (32)	25130±27407	25717±26054	0.950
Pturia 24 hrs(>0.5 g/24hrs) (24)	26145±26527	27900±28659	0.873
Pyuria (6)	50597±23713	37287±41561	0.511

CMV-DNA cytomegalovirus deoxybonucleic acid, EBV-DNA Epstein-barr virus deoxybonucleic acid, ANA anti nuclear antibody, C complement, % percentage, ds DNA double stranded deoxynucleic acid antibody, Pturia 24 hrs protein uria in twenty four hours, Pyuria pus in urine

Table 3c: Comparison between Cytomegalovirus and Epstein-Barr viruses blood frequency in different in systemic lupus erythematosus clinical parameters.

SLE disease parameters (no. of Patients	CMV-DNA no. (%)	EBV-DNA no. (%)	p
Arthritis (32)	14/32(43.8)	16 /32(50)	0.619
Alopecia (22)	12/22(54.5)	14/22(63.6)	0.539
Oral ulcers (24)	12/24 (50)	14/24 (58.3)	0.564
Malar rash (30)	12/30 (40)	16/30 (53.3)	0.302
Photosensitivity (18)	6/18(33.3)	12/18(66.7)	**0.045**
Raynaud's (12)	2/12 (16.7)	8/12(66.7)	**0.013**
Serositis (18)	8/18 (44.4)	10/18 (55.6)	0.502
Vasculitis (10)	4/10 (40)	2/10(20)	0.329
Renal involvement(32)	16/32 (50)	18/32 (56.2)	0.619
Nervous system(6)	3/6(50)	4 /6(66.7)	0.557

CMV-DNA cytomegalovirus deoxybonucleic acid, EBV-DNA Epstein-barr virus deoxybonucleic acid,

Table 3d: Comparison between Cytomegalovirus and Epstein-Barr viruses blood frequency in different in systemic lupus erythematosus laboratory parameters.

SLE disease parameters (no. of Patients	CMV-DNA no. (%)	EBV-DNA no. (%)	p
Laboratory			
Anemia(hemoglobin<11g/dl)(32)	12/32(37.5)	18/32 (56.3)	0.132
Leucopenia (<4000/µl) (16)	8/16 (50)	12/16 (75)	0.144
Thrombocytopenia(<200000/µl)	2/2 (100)	0/2 (0)	**0.046**
ANA (44)	18/44(40.9)	24/44 (54.5)	0.202
anti-dsDNA (28)	14/28(50)	16/28 (57.1)	0.594
C3 (low) (38)	16/38 (42.1)	24/38 (63.2)	0.065
C4 (low) (32)	14/32 (43.7)	20/32 (62.5)	0.132
Pturia 24 hrs(>0.5 g/24hrs) (24)	12/24 (50)	18/24 (75)	0.074
Pyuria (6)	6/6(100%)	6/6(100%)	1

CMV-DNA cytomegalovirus deoxybonucleic acid, EBV-DNA Epstein-barr virus deoxybonucleic acid, ANA anti nuclear antibody, C complement, % percentage, ds DNA double stranded deoxynucleic acid antibody, Pturia 24 hrs protein uria in twenty four hours, Pyuria pus in urine

To reveal whether the increased EBV and CMV load in SLE patients resulted from immune suppressive drug treatment. We found four (8.3%) patients were on current methotrexate dosage; out of them, two (50%) patients had CMV- DNA while four (100%) patients had EBV- DNA.

Forty (83.3%) SLE patients take corticosteroids; out of them, eighteen (45.0%) patients had CMV- DNA while twenty two (55.0 %) patients had EBV- DNA.

We found significant correlation between Epstein-Barr virus viral load with: pyuria, oral ulcers, photosensitivity, vasculitis and nervous system (r= 0.43, p= 0.002) (r= 0.36, p= 0.01) ,(r=0.42, p = 0.003) ,(r=-0.33 ,p=0.023) ,(r=0.32 ,p = 0.029) respectively, and Cytomegalovirus viral load with: pyuria, and anti-dsDNA (r=0.71, p=0.000), (r=0.40, p=0.005) respectively. Table 4a and table 4b.

Table 4a: Correlation between viral load of Cytomegalovirus with systemic lupus erythematosus clinical and laboratory parameters

Parameter mean± SD or n(%)	CMV r	CMV p
Disease duration/ years	-0.020	0.894
Arthritis/arthralgia 32	-0.012	0.936
Alopecia 22	0.247	0.090
Oral Ulcers 24	0.165	0.263
Malar rash 30	0.027	0.85
Photosensitivity 18	0.006	0.970
Raynaud's 12	-0.214	0.145
Vasculitis 10	0.218	0.317
Serositis 18	0.220	0.133
Renal involvement 32	0.248	0.090
Nervous system 6	-0.71	0.632
Laboratory		
Anemia 32	-0.095	0.522
Leucopenia 16	0.019	0.869
Thrombocytopenia 2	-0.009	0.954
ANA 44	0.152	0.303
anti-dsDNA 28	**0.395**	**0.005**
C3 (low) 38	-0.078	0.598
C4 (low) 32	-0.012	0.933
Pturia 24 hrs 24 (> 0.5 g/24hrs)	0.172	0.347
Pyuria	**0.710**	**0.000**

CMV-DNA cytomegalovirus deoxybonucleic acid, ANA anti nuclear antibody, C complement, % percentage, ds DNA double stranded deoxynucleic acid antibody, Pturia 24 hrs protein uria in twenty four hours, Pyuria pus in urine

Table 4b: Correlation between viral load of Epestein-Barr virus with systemic lupus erythematosus clinical and laboratory parameters

Parameter mean± SD or n(%)	EBV r	EBV p
Disease duration/ years	0.180	0.220
Arthritis/arthralgia 32	-0.139	0.345
Alopecia 22	0.258	0.077
Oral Ulcers 24	**0.360**	**0.012**
Malar rash 30	0.128	0.386
Photosensetivity 18	**0.418**	**0.003**
Raynaud's 12	0.092	0.533
Vasculitis 10	**-0.328**	**0.023**
Serositis 18	-0.004	0.979
Renal involvement 32	0.161	0.275
Nervous system 6	**0.315**	**0.029**
Laboratory		
Anemia 32	-0.270	0.064
Leucopenia 16	0.144	0.329
Thrombocytopenia 2	-0.138	0.350
ANA 44	0.183	0.212
anti- dsDNA 28	0.092	0.532
C3 (low) 38	0.184	0.210
C4 (low) 32	0.160	0.278
Pturia 24 hrs 24 (> 0.5 g/24hrs)	0.066	0.721

EBV-DNA Epstein-barr virus deoxybonucleic acid, ANA anti nuclear antibody, C complement, % percentage, ds DNA double stranded deoxynucleic acid antibody, Pturia 24 hrs protein uria in twenty four hours, Pyuria pus in urine

DISCUSSION

Systemic lupus erythematosus (SLE) is an autoimmune disease with bouts of exacerbation and remission, accompanying infections are of major concern causing life-threatening complications[1]. It has been noticed that dissemination of viruses in blood as EBV and CMV are a growing recognized risk factor for development of simultaneous clinical abnormalities in their patients[7,8].CMV primarily infects monocytes and macrophages, but also infects fibroblasts, epithelial cells, endothelial and dendritic cells cells, while EBV primarily infects B cells but can also exists in latent phase within nasopharyngeal epithelial cells[19].

Therefore, it was assumed that EBV gene expression might also be sensitive to the disruptions of normal B cell function seen in SLE patients. These comprise the existence of unusual B cell subsets, expression of activation markers, and alarm of intracellular signaling[20].Detection of cell-free DNA in serum samples is addressed to be an indicator of active infection[21].

Our study had explored active infection with CMV and EBV in 48 SLE patients and 40 healthy controls by detecting viral load of both viruses in serum. Our results revealed that 41.7% of patients had CMV DNA *versus* 54.2% for EBV, serum of healthy controls had not EBV DNA or CMV DNA. A long same line, Mohamed and colleagues found that both EBV and CMV IgG 100% and 96.6%, respectively) were significantly higher in Egyptian SLE patients compared to healthy controls. In addition, one-third of SLE patients had PCR positive for CMV, whereas half of SLE patients had EBV DNA[22].

These present results are consistent with other reported studies from different studies; Kang *et al.,* [23] found that, by the usage of real-time quantitative PCR, a 40-fold increase of EBV load in SLE patients compared to healthy controls**.** Moon and colleagues, [24] assessed the level of EBV-DNA in the peripheral blood of patients with SLE and healthy controls. They reported more than 15-fold increase of EBV load in SLE patients. Lu *and co-workers* [25] found a significantly elevated level of EBV DNA in serum of SLE patients compared the healthy controls, in addition to positive PCR results for CMV were detected in 30.3% of their SLE patients. Furthermore, Hrycek *et al*[26]**.** reported in a qualitative analysis of the CMV genome that 100% of the studied SLE patients were infected with CMV versus 73% in controls**.** On the contrary, Barzilai and others[27]**,** found non -significant results for IgM anti-CMV and anti-EBV in both SLE patients and the control group. Determination of the existence of anti-EBV IgM specifies recent infection and/or reactivation**.**

In our study both viruses revealed a relatively high viral load specially for CMV, one of the possible clarifications is the deficiencies in cellular immunity in SLE which may results in increased viral load, alternative clarification is the reactivation of these viruses as a consequence of immunosuppressive drug treatment[7,8,15]**.** EBV affects the majority of the world's population, and is considered to be included in the commencement or promotion of several autoimmune diseases, including SLE. It acts mainly by triggering induction and self-reactivation of pro-inflammatory cytokines[28]**.** The autoreactive T- and B-cell activation and prolonged antigenic expression are considered to be common topographies of both EBV infection and SLE[29]**.** The linkage between EBV and SLE was first thought- out in 1971[30]**]**, and since then, numerous studies have advocated the role of EBV in the beginning and exacerbation of SLE[3,7,9,23,24,25,29,31]. Increased EBV sero-conversion and high titers of anti-EBV antibodies have been frequently, reported in SLE patients[9-14,23-25]. The first systematic review on the association between SLE and anti-EBV antibodies was performed by Hanlon and colleagues, who reported a higher sero-prevalence of EBV EA-D IgG and EBV VCA IgG (although not EBNA-1 IgG) in SLE patients compared to healthy controls, which corresponds to our results[12]. According to a review by Li *et al.* the sero-positivity of EBV EA-D IgG and EBV VCA IgG was also more frequent in SLE patients[32]**.** These findings support that EBV is an imperative factor in the development of SLE due to either immune dysregulation or molecular similarities.

The present study was attempting to compare the frequencies of SLE disease activities in CMV infected patients as opposed to those infected with EBV; disease activity in SLE was assessed by SLEADI score and comparison was done in relation to the presence of either CMV-DNA or EBV-DNA. Eight patients (16.7%) with mild to moderate disease activity had not EBV DNA or CMV DNA, but in the 40 patients (83.3%) with high disease activity had detected. In 65% of patients with high disease activity had EBV DNA and in 50% of patients with high disease activity had CMV DNA. The increased SLE disease activity in our study may be due to up-regulation of leukocyte immunoglobulin-like receptor1 (LIR-1) by CMV[19,33,34]**.** In contrast, *Mohamed* and coworkers[22]', assessment showed a statistically significant lower SLEDAI score in patients with EBV viremia (positive PCR) compared to those with negative results. Also, they found a lower percentage of SLE patients with major organ involvement in the EBV positive PCR group compared to those in the negative group. However, this difference did not reach statistical significance. Furthermore, in their study they reported EBV PCR positive patients compared to negative patients were a statistically significant with SLEDAI**.**

To assess whether the increased EBV and CMV load in SLE patients was the effect of an immune suppressive drug treatment. Four SLE patients were on current methotrexate dosage; ٢(50%) patients had CMV-DNA while 4 (100%) patients had EBV- DNA. Forty SLE patients took corticosteroids; in 18 (45%) patients had CMV- DNA while in 22 (55%) patients had EBV- DNA. Various studies had reported the effect of immunosuppressive drug in reactivation of latent viral infections[15,27,28]. *Lossius* and others[35] conveyed that EBV load was nearly the same in patients receiving or not receiving immunosuppressive drugs**.** Even after long-term treatment with methotrexate there was no increase in EBV load[36]**.**

Renal biopsies that show renal involvement revealed that, 66.7% of patients had lupus nephritis; The mean ± *SD*, copy numbers of CMV and EBV found in the serum of SLE patients with renal involvement were

29706±28230 copies/ µl and 28551± 26900 copies/ µl, respectively; of them, 50% of patients with CMV-DNA, 56.25%patients with EBV -DNA, while 31.25% patients had both EBV- DNA and CMV- DNA. To our knowledge this is the first time to target this issue in spite of the implication of both viruses in SLE etio-pathogenesis.

SLE patients have dysfunctional control of EBV infection resulting in frequent reactivations and disease progression. These involve compromised functions of EBV-specific T-cells with a reverse correlation to disease activity and raised serum levels of antibodies against lytic cycle EBV antigens. The presence of EBV proteins in renal tissue from SLE patients with nephritis suggests direct involvement of EBV in lupus nephritis development. As predictable for patients with immuno-deficiencies, similar study, revealed that SLE patients show dysfunctional responses to other viruses as well [23].

We detected negative significant correlation in this work between EBV-DNA and ANA. A positive significant correlation was found between EBV-DNA and pyuria, oral ulcers, photosensitivity, vasculitis and involvement of nervous system. Other clinical or laboratory variables had not significant values. Though, there was no supporting evidence from other studies.

Buonavoglia and colleagues[37], comprehensive the knowledge about a possible insinuation of EBV in SLE etio-pathogenesis. Unlike other studies, since finding of EBV in saliva is constant with active viral replication, they used saliva as a favored sample. By screening oral swabs, they identified EBV DNA in 50% SLE patients and only in 6.6% healthy subjects. Remarkably, they observed that the majority of the SLE patients with EBV DNA positivity had oral lesions. A higher frequency (37.5%) of ulcerative oral lesions in SLE patients rather than in healthy subjects (6.66%) was found, and a statistically significant difference was found between the two groups. In another study, it was detected that herpes virus DNA in patients with oral lesions[38], and persistent EBV infection can consequence in oral manifestations, oral hairy leukoplakia and EBV-positive muco-cutaneous ulcers that are suggestive of SLE-related ulcerative lesions, mainly in immunocompromised patients[39].

In this work we found that, the frequency and association between EBV and CMV with different disease parameters in SLE patients; significant associations were found with: photosensitivity, Raynaud's and thrombocytopenia; $p < 0.05$. *Sekigawa and colleagues*, [10] found in SLE patients with CMV infection, the presence of skin ulceration and subcutaneous nodules mimicking the cutaneous manifestations of SLE developed, but a skin biopsy revealed a diagnosis of CMV-mediated vasculitis. They also found that thrombocytopenia was induced by CMV infection during maintenance therapy (prednisolone (PSL) at 5 mg/day) for symptoms of SLE (a malar rash and proteinuria).

Several reports have revealed an association between EBV EBNA IgG EBV EA-D IgG and EBV EBNA IgG positivity and the existence of lupus autoantigens and Raynaud's phenomenon[15,16,20]. ***Aygunetal***, [34] found that EBV VCA IgG positivity was related with malar rash and immunological disorder. However, there was no significant relationship between EBV sero-positivity and other clinical symptoms, haematological findings, autoantibody positivity and disease duration.

Despite the significant various studies investigating the seropositivity of EBV in SLE, there are limited reports on EBV DNA burden in SLE[35,36] reported a higher EBV DNA load in adult SLE. EBV DNA viral load is also reported to be associated with disease activity. Another review found that 55.1% of adult SLE patients and 20.7% of healthy controls were reported to be EBV DNA positive [18]. Additionally in two SLE patients had CMV DNA, whereas in 90% of patients had CMV IgG. However, the result was not statistically significant compared to the healthy controls[34].

The reduced clinical input is among the study limitations, in the era of global COVID -19 pandemic and further analyses in view of the patients' disease characteristics, activity and medications received are recommended.

Conclusion, SLE patients have increased sero-prevalence of EBV-DNA and CMV-DNA signifying more frequent viral reactivation and increased viral loads. Frequencies of SLE disease activities are significantly higher in CMV and EBV infected patients. Additional efforts are essential to entirely translate how these viruses may modify the immune system in SLE patients. An interdisciplinary approach will be necessary to better understand the pathways by which both viruses might impact the pathogenesis and progress of SLE.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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