Original Research Article

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Does herpes simplex virus have a role in the pathogenesis of systemic lupus erythematosus in Egyptians?

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Abstract

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by immune mediated tissue damage affecting a wide range of organs. The pathogenesis of SLE is complex. Infectious agents, including viruses, can act as environmental triggers, inducing or promoting onset and exacerbations of autoimmune disease in genetically predisposed individuals. Viral infections may be involved in the pathogenesis of SLE. To date, there is no published data about role of herpes simplex virus (HSV) in pathogenesis of SLE in Egyptian population. This study was designed to investigate a possible role of HSV in pathogenesis of SLE and its relation to disease activity. This study included 90 SLE female patients and 83 apparently healthy age-matched female subjects. SLE disease activity was assessed using SLEDAI-2K score. Qualitative assessment of anti-HSV antibodies (HSV1/2 IgM and IgG) was performed using ELISA kits. There was no statistically significant difference in frequency of HSV1/2 IgG positive test between SLE patients (97.6%) and control subjects (94.4%). There was a statistically significant increase in frequency of HSV1/2 IgM positive test in SLE patients compared to control subjects (P<0.001). There was no difference in the frequency of HSV1/2 IgM and HSV1/2 IgG positive test results between SLE patients with higher disease activity score (60% and 95.6%, respectively) and those with lower disease activity score (60% and 93.3%, respectively). High prevalence of HSV1/2 IgG antibodies was observed among Egyptians. The lack of significant difference in frequency of HSV1/2 IgG between SLE patients and control subjects may indicate that HSV is not involved in SLE pathogenesis. Also, HSV infection may have no role in SLE disease exacerbation due to the absence of significant difference in the frequency of HSV1/2 IgM and HSV1/2 IgG antibodies in SLE patients with higher disease activity compared to those with lower disease activity.

Keywords: Autoimmunity, Herpes Simplex Virus, Systemic Lupus Erythematosus, Pathogenesis.

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Introduction

Autoimmunity is the result of self-tolerance breakdown, so, the immune system attacks own tissues and organs with subsequent development of an overt autoimmune disorder. This requires the combination of immunologic,

genetic and environmental factors.[1] Systemic lupus erythematosus (SLE) is an autoimmune disorders, caused by the effect of multiple polyclonal autoantibodies, which usually appear years before clinical manifestations are evident.[2] The onset might occur at whatever age with periods of flare and remission, resulting in irreversible tissue damage and even premature death.[3] SLE is characterized by a persistent inflammatory state affecting different organs, such as skin, joints, kidney, serous membranes, central nervous system and blood. This chronic inflammation results from both adaptive immunity dysregulation and hyper-production of different autoantibodies.[4]

Several studies have suggested that the interaction of genetic and infectious factors may have a role in determining the outcome of an autoimmune process.[5-8] The role of environmental factors in the etiology of autoimmune disorders is suggested by the disease discordance rate between monozygotic twins.[9] Infectious agents, including viruses, may initiate and be involved in the pathogenesis of an autoimmune disease by several mechanisms such as mimicry, epitope spreading, polyclonal stimulation, super antigens and others.[10] Infections can act as environmental primers, inducing or promoting onset and exacerbations of SLE in genetically-predisposed individuals. [11]

Viruses which have been linked to the pathogenesis of SLE include Epstein-Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, transfusion-transmitted virus, human herpes virus, human papilloma virus, dengue virus, human T cell lymphotropic virus and HIV.[12]

Antibodies to these viruses, including herpes virus, were detected at higher levels in SLE patients as compared to controls together with enhanced viral loads which suggest viral triggering or exacerbation of SLE.[12] In Egypt, some studies investigated the role of EBV as a pathogenic factor for SLE,[13-15] two studies on parvovirus B19 role in SLE[16,17] with controversy results, and one study on CMV role in SLE [14] without published data on other viruses yet. This study was designed to detect the frequency of antibodies against herpes simplex virus 1 and 2 aiming to determine if they have a role in the pathogenesis of SLE or a role in the flaring of the disease.

Subjects and Methods

The study was conducted on 90 female SLE patients who fulfilled the 1997 American College of Rheumatology (ACR) revised criteria for SLE diagnosis.[18] Patients were recruited from the Rheumatology Clinic, Department of Internal Medicine, Assiut University hospital, Assiut, Egypt from January 2018 to May 2019. Patients were subjected to complete history taking including medication and clinical examination to assess clinical signs and symptoms of systemic lupus disease. SLE disease activity was assessed according to Systemic Lupus Disease Activity Index (SLEDAI-2K) score, the range of possible SLEDAI-2K scores is 0–105.[19] Also, 83 apparently healthy females, age matched to SLE patients, were included in the study as a control group.

The study protocol was reviewed and approved by Ethical Committee, Faculty of Medicine, Assiut University (approval date: 8/11/2016). Each subject or his parent / guardian gave an informed consent before being included in the study.

Specimen collection and handling

Blood specimens, 2 ml were collected on EDTA for blood count and direct Coombs' test, and sera were isolated from 4 ml for serology and blood chemistry tests. Random urine samples were collected for urine analysis and 24 hours urine sample was collected for measuring 24-hour urine protein.

Laboratory investigations

Complete blood count was done using Celldyn Ruby (Abbott, USA) and reticulocytic count was performed by Brilliant cresyl blue staining.[20] In SLE patients with anemia (hemoglobin <10g/dl) and/or reticulocytosis, direct Coombs' test was done using LISS/Coombs ID cards (REF 004015, DiaMed Gmbh, Switzerland).

Serum urea (BUN), creatinine and albumin (Alb) were done on the automated chemistry analyzer Dimension RxL Max (Siemens, USA), according to the manufacturer’s instructions,

using Siemens flex reagent cartridge BUN (REF DF 2, Siemens, USA), Siemens flex reagent cartridge CRE2 (REF DF33B, Siemens, USA) and Siemens flex reagent cartridge Alb (REF DF13, Siemens, USA), respectively.

Antinuclear antibody (ANA) was performed by indirect immunofluorescence technique on HEP-2 cells using ANA Fluoro kits (cat.no. 1660, DiaSorin Inc, USA).

Qualitative assessment of antibodies to HSV was done by using NovaLisa HSV 1+2 IgM and IgG kits (REF HSVM0250 and HSVG0250, NovaTec Immunodiagnostics GMBH, Germany) according to manufacturer's instructions.

For assessment of disease activity in SLE patients, the following laboratory investigations were done

Anti-double strand DNA (anti-dsDNA) was performed by indirect immunofluorescence technique on Crithidia luciliae using nDNA Fluoro Kit (cat.no.1790, DiaSorin Inc, USA). C3 and C4 was done by nephelometric technique on BN ProSpec analyzer (Siemens, USA) according to the manufacture instructions using Siemens N Antiserum to Human C3c (Ref OSAP09, Siemens, USA) and Siemens N Antiserum to Human C4 (Ref OSAO09, Siemens, USA), respectively. Erythrocyte sedimentation rate (ESR) was performed by Westergren method.[20] Urine analysis was performed by urine strips comboStik 10 (DFI Co. Ltd, Korea) and microscopic examination.[21] The protein in urine was measured by automated chemistry analyzer Erba XL 300 (Mian Scientific, Pakistan) then 24 hours protein was calculated by the equation (protein in urine (mg) × volume of urine (ml) /100).[22]

Statistical analysis

Data were verified, coded, and analyzed using IBM-SPSS 21.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive statistics: Means, standard deviations, medians and ranges were calculated. Chi-square (Fisher's Exact test) was calculated to compare frequencies between groups. For continuous variables, independent t-test analysis was carried out to compare the means of normally distributed data, while Mann-Whitney U test was calculated to test the median differences of the non-parametric data. A *P*-value of ≤ 0.05 was considered significant.

Results

The study was conducted on 90 SLE female patients, their age ranged from 18-54 years. In addition, 83 apparently healthy age-matched females were included in the study as a control group, their age ranged from 15-45 years. There was no significant difference in age distribution between SLE patients and control subjects (Table 1).

Table 1. Age distribution in SLE patients and control subjects.

	Control subjects (n=83)	SLE patients (n=90)	*P*-value
Age (years)			
Mean ± SD	32.06 ± 7.3	30.73 ± 8.5	NS
Median (Range)	33 (18 - 45)	30 (14 - 54)	

n=number, SLE=Systemic Lupus Erythematosus, SD=Standard Deveiation. *P* value>0.05 is not significant (NS).

Disease duration in SLE patients ranged from 1-14 years (Mean ± SE= 4.5 ± 3.3 years). Thirteen SLE patients were receiving steroids only (14.4%), one patient was receiving non-steroidal anti-inflammatory drugs (NSAID) only (1.2%) and 76 patients were receiving both steroids and NSAID (84.4%); 6 of them (6/76) were receiving cyclophosphamide.

All control subjects had negative ANA test. ANA test was positive in 88/90 SLE patients (97.8%) and anti-dsDNA test was positive in

34/90 SLE patients (37.8%). Two patients showed negative ANA test while their anti-dsDNA test was positive. Direct Coombs' test was done for 23 SLE patients and 4 out of them (17.4%) showed a positive test result.

Table 2 shows the results of different laboratory tests in SLE patients compared to control subjects. There was a statistically significant increase in frequency of HSV1/2 IgM positive test in SLE patients (60%) in comparison to control subjects (25.3%), *P*<0.001, while no significant difference was found in frequency of HSV1/2 IgG positive test.

Table 2. Laboratory tests findings in SLE patients and control subjects

	Control subjects (n=83)	SLE patients (n=90)	*P*-value
Haemoglobin (g/dl)			
Mean ± SD	11.84 ± 1.2	10.77 ± 1.5	< 0.001
Median (Range)	12 (9 - 14.5)	11 (5.5 - 14)	
WBCs (10^3 /µl)			
Mean ± SD	6.99 ± 2.5	8.12 ± 5.5	NS
Median (Range)	6.7 (3 - 15)	6.3 (1.5 - 33)	
Platelet (10^3 /µl)			
Mean ± SD	325.16 ± 86.8	226.58 ± 91.9	< 0.001
Median (Range)	328 (177 - 593)	212 (26 - 717)	
Urea (mmol/l)			
Mean ± SD	4.64 ± 1.2	7.96 ± 6.9	< 0.001
Median (Range)	4.5 (2.4 - 8)	6.1 (2 - 71)	
Creatinine (µmol/l)			
Mean ± SD	76.56 ± 15.4	136.70 ± 126.1	< 0.001
Median (Range)	76.5 (52 - 111)	94 (57 - 900)	
Albumin (g/l)			
Mean ± SD	40.58 ± 4.1	32.56 ± 6.7	< 0.001
Median (Range)	41 (34 - 50)	31.5 (16 - 46)	
HSV1/2 IgM			
Negative	62 (74.7%)	36 (40%)	< 0.001
Positive	21 (25.3%)	54 (60%)	
HSV1/2 IgG			
Negative	2 (2.4%)	5 (5.6%)	NS
Positive	81 (97.6%)	85 (94.4%)	

n=number, SLE=Systemic Lupus Erythematosus, SD=Standard Deveiation, WBCs= White blood cells, HSV= Herpes simplex virus, IgM= Immunoglobulin M, IgG= Immunoglobulin G. *P* value>0.05 is not significant (NS).

SLE disease activity was assessed according to SLEDAI-2K score. SLE patient's disease activity score ranged from 4 to 30 (mean ± SD = 15.8 ± 7.9, median = 17). SLE Patients were classified into two groups; patients with SLEDAI-2K score < 17 (median) were considered in a relatively low disease activity state and patients with SLEDAI-2K score ≥17, considered in a high disease activity state. There was no significant difference in age at diagnosis and disease duration between SLE patients with SLEDAI-2K score ≥17 and those with SLEDAI-2K score < 17 (Table 3).

Table 3. Age distribution and disease duration in SLE patients with higher and lower disease activities.

	SLE patients with SLEDAI-2K ≥ 17 (n=45)	SLE patients with SLEDAI-2K < 17 (n=45)	*P*-value
Age (years)			
Mean ± SD	30.91 ± 8.3	30.56 ± 8.7	NS
Median (Range)	30 (14 - 54)	29 (15 – 51)	
Age at Diagnosis (years)			
Mean ± SD	25.69 ± 6.9	26.42 ± 7.9	NS
Median (Range)	26 (10 - 42)	26 (13 - 42)	
Disease Duration (years)			
Mean ± SD	4.98 ± 3.7	4.18 ± 2.9	NS
Median (Range)	4 (1 - 14)	3 (1 - 12)	

SLE=Systemic Lupus Erythematosus, n=number, SD=Standard Deveiation. *P* value>0.05 is not significant (NS).
SLEDAI-2K ≥ 17=higher disease activity. SLEDAI-2K< 17=lower disease activity.

Table 4 shows results of different laboratory tests in SLE patients with SLEDAI-2K score ≥17 compared to those with SLEDAI-2K score < 17. There was no difference in the frequency of HSV1/2 IgM and HSV1/2 IgG positive test results between SLE patients with SLEDAI-2K score ≥17 compared to those with SLEDAI-2K score < 17.

Table 4. Laboratory tests findings in SLE patients with higher disease activity and patients with lower disease activity.

	SLE patients with SLEDAI-2K ≥ 17 (n=45)	SLE patients with SLEDAI-2K < 17 (n=45)	*P*-value
Anemia (Hb < 10 g/dl)	17 (37.8%)	6 (13.3%)	0.008
Direct Coombs' test (Positive)	3/17 (17.7%)	1/6 (16.7%)	NS
Leukopenia (WBCs < 4 ×10^3/μl)	10 (22.2%)	8 (17.8%)	NS
Thrombocytopenia (Plt < 100 ×10^3/ul)	3 (6.7%)	2 (4.4%)	NS
ESR- first hour (mm)			
Mean ± SD	44.53 ± 24.9	35.00 ± 24.0	0.039
Median (Range)	35 (8 - 102)	28 (6 - 124)	
Anti-dsDNA (Positive)	19 (42.2%)	15 (33.3%)	NS
C3 (g/l)			
Mean ± SD	0.72 ± 0.4	0.99 ± 0.3	< 0.001
Median (Range)	0.7 (0.2 – 1.8)	0.9 (0.3 – 1.8)	
C4 (g/l)			
Mean ± SD	0.19 ± 0.1	0.28 ± 0.1	< 0.001
Median (Range)	0.2 (0.01 – 0.4)	0.3 (0.01 – 0.4)	
Albumin (g/l)			
Mean ± SD	30.92 ± 6.8	34.17 ± 6.3	0.020
Median (Range)	31 (16 – 44)	35 (19 – 46)	

Table 4. (Continued).

	SLE patients with SLEDAI-2K ≥ 17 (n=45)	SLE patients with SLEDAI-2K < 17 (n=45)	*P*-value
Urea (mmol/l)			
Mean ± SD	8.81 ± 6.1	7.11 ± 5.4	NS
Median (Range)	6 (2 - 71)	6 (2 - 30)	
Creatinine (μmol/l)			
Mean ± SD	153.24 ± 120.2	120.16 ± 104.3	NS
Median (Range)	109 (57 - 900)	89 (32 - 670)	
24-h Urine Protein			
Mean ± SD	1.28 ± 1.2	0.81 ± 0.8	< 0.001
Median (Range)	0.82 (0.2 – 7.6)	0.38 (0.1 – 7.7)	
Urine RBCs (/HPF)			
Mean ± SD	2.49 ± 1.8	2.58 ± 2.2	< 0.005
Median (Range)	0 (0 - 25)	0 (0 - 100)	
Urine Pus cells (/HPF)			
Mean ± SD	11.44 ± 10.1	1.53 ± 1.4	0.015
Median (Range)	0 (0 - 100)	0 (0 - 25)	
Urinary Casts			
Nil	27 (60%)	40 (88.9%)	0.001
Granular	5 (11.1%)	0 (0%)	
Hyaline	13 (28.9%)	5 (11.1%)	
HSV1/2 IgM			
Negative	18 (40%)	18 (40%)	NS
Positive	27 (60%)	27 (60%)	
HSV1/2 IgG			
Negative	2 (4.4%)	3 (6.7%)	NS
Positive	43 (95.6%)	42 (93.3%)	

SLE=Systemic Lupus Erythematosus, n=number, SD=Standard Deveiation, Hb= Hemoglobin, WBCs= White blood cells, Plt= Platelets, ESR= Erythrocyte sedimentation rate, anti-dsDNA= anti- double stranded DNA antibodies, HPF= High power field, HSV= Herpes simplex virus, IgM= Immunoglobulin M, IgG= Immunoglobulin G. *P* value>0.05 is not significant (NS).

Discussion

In current study, 81/83 of control subjects (97.6%) were positive for HSV1/2 IgG and 21/83 of them (25.3%) were positive for HSV1/2 IgM. A study from Croatia,[23] found that 84.5% of normal female population were positive for HSV1/2 IgG and 2.4% positive for HSV1/2 IgM. Also, another study from Bangladesh[24] found that 96% of normal females had positive HSV1/2 IgG and 3.5% of them had HSV1/2 IgM. In a study from Syria,[25] 52% of normal female population were positive for HSV1/2 IgG while 18% of them positive for HSV1/2 IgM. In a study from Yemen,[26] seroprevalence of HSV in normal population was as follows, 2.5% for HSV IgM, 99.4% for HSV-1 IgG and 5.1% for HSV-2 IgG.

The above mentioned studies together with two other studies [27, 28] reported high prevalence of HSV1/2 infection in normal female population. Sero-epidemiological surveys demonstrated that HSV1/2 is highly prevalent in humans. This prevalence has been shown to be

also increased in some immunodeficient states, and in some systemic autoimmune disorders.[29]

The presence of IgM antibody invariably suggests acute/recent infection, and the presence of IgG antibody suggests past or present active infection, while the presence of both IgM and IgG suggest that the patient had the infection in the past with reactivation of infection. This is due to the outstanding feature of herpes viruses to cause primary infections in addition to establish lifelong persistent infections in their hosts by undergoing periodic reactivation.[30] The immunity against HSV1/2 has probably been acquired from previous infection with the virus during childhood or adolescence.[31]

In the current study, there was no statistically significant difference in the frequency of HSV1/2 IgG positive tests between the SLE patients and study controls. This disagrees with a study from USA,[32] where IgG antibodies against HSV 1 and HSV 2 were significantly higher in SLE patients when compared to control subjects ($p = 0.03$ and 0.005, respectively). However, the observation of the current study agrees with several other studies. A study from USA[33] which reported no significant difference of HSV1/2 IgG between SLE patients and control subjects. Also, another study included 196 SLE patients and 392 female controls,[34] no significant difference in HSV1/2 IgG was reported between SLE patients and controls. A study in Iraq,[35] found no significant difference in the HSV1/2 IgG between 64 SLE patients and 32 control subjects. In a study in Turkey,[36] 50 SLE patients and 50 controls were tested for HSV1/2 antibodies and no significant difference was found in the positivity rates of anti-HSV1/2 IgG antibodies between the two groups.

In the current study, there was a statistically significant increase in frequency of HSV1/2 IgM positive tests in SLE patients when compared to controls. This disagree with findings of two studies in Iraq[35] and Turkey[36] which reported no significant difference in HSV1/2 IgM between SLE patients and control subjects. The differences between the current study which demonstrated a significant increase in frequency anti-HSV1/2 IgM positive tests in SLE patients and other studies with no difference may be related to the sensitivities of the assays used, the variable numbers and nature of individuals enrolled in the studies, different environments, and variable prevalence of HSV infection.

SLE patients may have an increased risk for infections due to dysregulation of the immune system. The usage of immunosuppressant, as steroids and cyclophosphamide, is another risk factor for increased infections in patients with SLE. IFN-a was found to be increased during a flare-up of SLE, which may reflect a viral infection or viral reactivation.[37] Various disease-modifying anti rheumatic drugs and steroids are reportedly associated with risk of severe HSV infection.[38] In the current study, all 54 SLE cases with HSV1/2 IgM positive were receiving immunosuppressive drugs (steroids ± cyclophosphamide) which might make them more susceptible for reactivation of HSV infection.

One of the drawbacks of the current study is the use of a combined HSV1/2 assay. Assessment of anti-HSV1 and anti-HSV2 antibodies separately would have been more informative.

In conclusion, there was a high prevalence of HSV1/2 IgG antibodies in Egyptian SLE patients. The lack of significant difference in frequency of HSV1/2 IgG between SLE patients and control subjects may indicate that HSV is not involved in SLE pathogenesis. The higher frequency of HSV1/2 IgM positive test in SLE patients compared to control subjects may be related to reactivation of latent HSV infection resulting from the occurrence of immune dysregulation in SLE and the use of immunosuppressive therapy. Also, the absence of significant difference in the frequency of HSV1/2 IgM and HSV1/2 IgG antibodies in SLE patients with higher disease activity compared to those with lower disease activity may indicate that HSV infection have no role in SLE disease exacerbation.

Author Contributions

HGR and TTHE proposed and designed the study. OMH clinically evaluated the patients and followed them up. AHR collected patients' information,

specimens and performed the laboratory work. TTHE and AHR interpreted the laboratory test results and analyzed the data. TTHE and AHR wrote the paper draft. HGR and OMH revised the paper draft. TTHE prepared the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by Ethical Committee, Faculty of Medicine, Assiut University (approval date: 8/11/2016).

Informed consent

Each subject or his parent **/**guardian gave an informed consent before being included in the study.

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