.Assessment of Systemic Lupus Erythematosus in Term of Serum Anti-nucleosome and Interleukin 12 Markers in Relation to Epstein–Barr virus and Cytomegalovirus

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Abstract

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, more dominant in female, associated with high risk of life threating complication and increase morbidity and mortality. the exact cause of SLE is not yet known with many suggestions about a possible role of viral infection in the development of the disease. Epstein –Barr virus is the most commonly studied virus in an attempt to prove its presumed association with development of SLE.The objectives of this study are the measurement of the effect of CMV and EBV seropositivity in the auto immune status and severity of disease SLE measured by estimating the level of immunological markers anti-nucleosome and IL-12 with viral marker.

Materials and Method: Forty newly diagnosed female with SLE were randomly selected from patient attended rheumatology clinic at medical city in Baghdad during the period between December 2018 to May 2019. In addition, with 40 apparently healthy females, as, a control groups. Both group were investigated for IL12, anti-nucleosome, EA, VCA, EBNA and CMV by ELISA and the results were statistically evaluated.

Results: The studied viral markers in the current study which included EBV markers EA, VCA, EBNA, have shown significantly higher level compared to the control group. Similarly, anti-nucleosome and IL12 have shown significantly higher level compared to the control group.

Conclusions: The possible defect in controlling viral infection and increased number of latent infected cell in SLE may enhance production of auto-antibodies

Keywords: *SLE, EBV, VCA, IL-12; Erythematosus*

Introduction

SLE represents an autoimmune disease with chronic clinical course that is associated with a wide range of clinical features and variable types of autoantibodies including anti-double strand DNA (dsDNA) and antinuclear antibodies (ANA) involving multiple organs in the body such as joints, skin, respiratory system. As most of the body organs are affected by SLE, the clinical features sound extremely variable and involve the affected organs, of course, the most commonly encountered clinical features include photosensitivity, recurrent oral ulcers, malar rash, discoid lesions, arthritis, serositis, hemolytic anemia, neuropsychiatric and poor vascularization. Different infectious agents are implicated in SLE, and were shown by serology to be more prevalent in SLE patients[(1)].

Because SLE is a multisystem disease, there is no obviously validated or standardized "gold standard" biomarker that can cover the whole aspects and the variable phenotypes of the disease. The many traditional laboratory assays commonly used for diagnosis and monitoring of SLE, such as complement Factors in serum, anti-double-stranded DNA (Anti dsDNA) and anti-nuclear antibodies (ANA) have never been validated or standardized themselves [(14)].

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Despite that anti-dsDNA antibodies are significantly specific for SLE, the high majority of patients might be negative for anti-dsDNA for these antibodies during the disease course. These are present in 70% to 100% of SLE patients and have a very high specificity (around 97%) for the disease. Amongst SLE patients, the anti-nucleosome antibodies are significantly more prevalent in patients with lupus nephritis and can serve as an important biomarker for the diagnosis of patients with active lupus nephritis, at the same time it has a significant correlation with disease activity in general [(2)] .

EBV is a lymph trophic double stranded DNA virus . It is one of a human herpes virus belongs to the gamma herpes family and infects more than 95% of the adult population worldwide.[(3)]

EBV infect B-cell, and persist in latent form in memory cell, and the virus used many mechanisms that lead to developed SLE like (molecular mimicry, epitope spreading, Infection and Immortalization of Autoreactive B-cells).[(4)] So when the virus infection occurs, EBV invades the B cells reprogramming them, and control of their functions. A limited number of studies have implicated another herpes virus, cytomegalovirus (CMV), in SLE, although the effects of CMV on SLE development and autoantibody production remain unclear [(5).]

Materials and Methods

The current study has included 40 young female patients with SLE on no specific treatment for SLE with at least four of the American College of Rheumatology (ACR) criteria for the diagnosis of SLE. Patients other chronic autoimmune diseases were excluded. Patients were recruited between December 2018 to May 2019 as they consulted at the Departments of rheumatology clinic of Baghdad Teaching Hospital at medical city. The control subjects were apparently healthy and age-, sex- matched volunteers; written informed consents were obtained from subjects.

Venus blood was drawn from patients and control to measurement EBV protein EA, VCA, EBNA, CMV IgG, IL12 and Antinucleosome by enzyme linked immunosorbent assay and the results were statistically evaluated using SPSS software version 23.

Results

The results of the current study have shown that the titer of IL-12, was increased in 40 (100%) of patients group while it was increased in only 13 (32.5%) of control group and the difference was statistically significant ($p<0.001$). The titer of anti-nucleosome, was increase in (62.5%) of patients group while it was increased in (2.5%) the control group, this difference was statistically significant ($p<0.001$).

In respect to EBV, the findings have shown that the titer of VCA was increased in ___ (92.5%) of patients group while it was increased in only (55.0%) of control group which was significant statistically ($p<0.002$). In addition, mean titer of EA was increased in (90.0%) of patients group while it was increased in (22.5%) of control group, a difference that was statistically significant ($p<0.001$). Regarding EBNA level, the current study has shown that the mean titer of EBNA was elevated in (75.0%) of patients group while it was elevated in (22.5%) of control group which was statistically significant as well ($p<0.002$).

Regarding CMV IgG, the current study has shown that the mean titer of CMV IgG, was increased in (97.5%) of patients group while it was increased in (27.5%) of control group ($p<0.002$).

Discussion

The role of immunological markers in the development of SLE has been thoroughly studied. The current study revealed that IL-12 was significantly higher in SLE patient compared to the control group and surprisingly, 100% of SLE patients revealed high IL-12 concentration indicating the role of dendritic cell in development of SLE as these cells represent the main source of IL-12 [(6)]. These findings are consistent with results revealed by similar studies; CK Wong for instance has revealed a similar result to the current study in respect to IL12 during his study of various proinflammatory cytokines in SLE patients[(7)]. Similarly, E. R. CAPPER in his study that discussed IL12 in both active and quiescent SLE has also revealed a significantly higher concentration of IL12 in patients with SLE compared to control group(8)including interleukin (IL. Many other studies have shown similar results despite being different from the current study in study design and aim[(8)(9)]. The current study has shown a significantly higher serum anti-nucleosome antibody concentration in SLE patients compared to control group This increase in anti-nucleosome may reflect the extent of cell damage caused by SLE which explain its

flare up shown in the current study as well , these result consistent with that revealed in studies of Ahemd Ali Abid and P Cairns where anti-nucleosome concentration was significantly higher in SLE patients compared to the control group[10][11]. In simon study, a similar result has been also obtained. Many studies have discussed the association between various infectious factors and the development of SLE at the top of which is EBV infection. In the current study, 92.5% of patients with SLE were positive for anti- EBV-VCA, while 45% of the control group were seropositive for the same antibody, a result clarifies the significant association between EBV and SLE. Chougule, for instance has revealed a similar result to the current study in respect to VCA during his study of EBV antibody profile in SLE patients[12]. Similarly, Alaa Y. Al-Hamadany, in his study that discussed EBV in Systemic Autoimmune Patients has also revealed a significantly higher concentration of VCA in patients with SLE compared to control group[13]. Many other studies have shown similar results despite being different from the current study in study design and aim[14][15].

At the same time, 90% of patient with SLE were anti- EBV-EA positive while only 22.5% of the control group were seropositive for the same antibody. In fact, the difference of EA seropositivity between SLE and control group was more significant than VCA; this might indicate the role of increased EBV reactivation in patients of SLE and this reactivation could be secondary to decreased cytotoxic T cell response to EBV with subsequent impaired control of replication of EBV in SLE patient[16]. This finding is consistent with the results revealed by many similar studies ; NS Rasmussen, for instance has revealed a similar result to the current study in respect to EA during his study of antibodies to early CMV, EBV, and HHV6 antigens in SLE patients [17]. Similarly, Evan S Vista, in his study discussed viral associations with SLE in Filipinos and this study also revealed a significantly higher concentration of EA in patients with SLE compared to control group [18]. Many other studies have shown similar results despite being different from the current study in study design and aim [19]. In addition, the current study has shown a significantly higher concentration of EBNA in patients with SLE compared to control group, a result that is consistent with the findings revealed by many similar studies. Chougule, for instance has revealed a similar result to the current study in respect to EBNA during his study of EBV antibody profile in SLE patients [20]. Similarly, Evan S Vista, in his study that discussed viral associations with SLE in Filipinos has also revealed a significantly higher concentration of EBNA in patients with SLE compared to control group[18]. Another study has shown similar results despite being different from the current study in study design and aim [21].

Regarding CMV infection, it was noticed that 97.5% of SLE patients were seropositive for CMV IgG while only 27.5% of control group were seropositive for the same antibody; a result is statistically significant and was consistent with similar studies which revealed a similar association , BARZILAI, for instance revealed a similar result to the current study in respect to CMV during his study of EBV and CMV in autoimmune diseases [1]. Similarly, Muhsin, in his study, he discussed the association of various viral infections with the development, in his study, Muhsin revealed a significantly higher concentration of CMV in patients with SLE compared to control group [14].

Taken together, this study revealed an essential association of all EBV serological markers studied, namely VCA, EA, and EBNA proteins, when compared to control group. And a threshold values were statistically assigned in order to differentiate between SLE and normal population which might help in diagnosis. This association revealed in this study was also confirmed by previous reports done elsewhere in the world. This indicates several things. First, the association between SLE and EBV can be expressed as a scientific fact. Second, it was found that EBV markers of reactivation, namely EA protein, and markers for latency, EBNA proteins, are remarkably increased in SLE when compared to control group; this highlights that EBV is highly reactivated in SLE patients as well as ssEBV latency is an active ongoing process where EBNA proteins are abundantly synthesized. A recent study found that EBNA 1 and 2 work as transcriptional activators for about half of high risk genes associated with SLE occurrence[15]. Therefore, EBV is related to SLE in different ways including both active ongoing latency and reactivation of latent infection.

Actually, this poses a question, is EBV a consequence or an etiology for SLE. The research to answer this question is underway; however, it is thought that the most probable nature of relationship between EBV and SLE is bidirectional in which EBV is reactivated in SLE patients due to deregulated immune system and this in turn can further promote and/or propagate SLE disease [22]. It is noteworthy to mention that SLE as such is not

considered an immune suppression condition; moreover, the SLE patients selected in the current study were those not receiving immunosuppressive drugs. Hence, it is believed that the significance of this study might issue from comparing a non-immunosuppressed patient of SLE with control group. Therefore, any significant association shown in the current study was not due to immune suppression. This accordingly, shed light on a probable role of EBV in promoting/ propagating SLE disease in susceptible individuals. Nevertheless, this requires a far deeper research in terms of molecular and immunological levels.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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