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# Sero-detection of Epstein-Barr Virus and Cytomegalovirus Antibodies among Patients with Systemic Lupus Erythematous in Sudan

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**Abstract**

Infection by Cytomegalovirus (CMV) and Epstein - Barr virus (EBV) has been implicated in the pathogenesis of Systemic lupus Erythematous (SLE). The current study aimed to determine the frequency of EBV and CMV IgG among Sudanese patients with SLE. A case-control analytical study was conducted on 82 participants in Khartoum State during the period from 2017 to 2018. Serum from patients with SLE (n=62), and controls participants (n=20) were tested for IgG antibodies against EBV and CMV using the Avidity ELISA assay. Pretested questionnaire data were analyzed by SPSS software V.20 (IBM, Chicago,

IL). Means and proportions of the demographic were calculated for SLE seropositive groups. Chi-square test and binary logistic regression analysis were used for SLE as a dependent factor while IgG seropositive groups of EBV and CMV as the independent variable and, SLE variables as dependent variables. Odds ratio (OR) with a 95% confidence interval was calculated and statistical significance was defined as a P value below 0.05. Eighty-two females with SLE volunteered to participate in this study, including 62 cases with systemic lupus erythematosus and 20 control participants. Average age means ± SD (34±1.4) for cases and (34±1.6) for controls. Original tribes in Sudan were screened Avidity assay for Western State 37(45.1%), Northern State 29(35.4%), Central State 17(20.7%), and Southern State1 (1.2%) respectively. Seropositivity of IgG by Avidity assay revealed high score for CMV 60 (96.8%) among cases vs 19(95%) among controls, (OR 1.579, CI -95% (0.136-18.4), P <0.05). Seropositivity for both EBV and Co-infection was 58 (98.3%) vs 16 (80.0%) among cases and controls, respectively (OR 3.625, CI -95% (0.227-2.708), P <0.05). Patients with SLE in Sudan have a high rate of infections by EBV and CMV as evidenced by the IgG avidity test indicating association.

**Keywords:** Cytomegalovirus, Epstein-Barr virus, Avidity index, SLE, Sudan.

## Introduction

Systemic Lupus Erythematous (SLE) is an autoimmune disease with an unknown etiology condition that is more common among women. The disease is associated with high morbidity and mortality [1], attributed to many complications associated with the disease [2]. Any organ can be involved but the prognosis is generally poor when the central nervous system and kidneys are involved [3]. The prevalence of SLE is estimated to be 1 to 7.6 per 100,000 individuals, with wide variations among geographic locations and ethnic groups [4].

It has been suggested that immune response in SLE can be mediated in part by infectious agents. Among these infectious agents are Epstein-Barr virus (EBV), Cytomegalovirus (CMV), and retroviruses are particularly relevant [5]. Importantly, CMV and EBV are known to cause complications and diseases flares for SLE patients and both are also part of the Herpesviridae family [6–14]. Interestingly, the two viruses were thought to induce SLE through molecular mimicry. For instance, for EBV this is likely due to cross-reaction of Epstein-Barr nuclear antigen 1 (EBNA-1) with self-antigens [15, 16], while for CMV this is likely due to epitope spreading, and an induced immune response to cryptic antigens [17]. Therefore, it's not surprising that SLE patients may have developed impaired immune responses during the latency or active phase of EBV. In recent years, serological methods to discriminate between low and high-avidity IgG have been developed. As it has shown that low-Avidity antibodies of specific IgG response will be produced immediately after the primary infections; while the avidity of these antibodies will mature and improves a few months after the primary infection [18]. The objective of this study was to determine the IgG antibody Avidity among

confirmed SLE cases and compare it to a control group.

**Methods:**

The selected cases and controls as defined above were interviewed by a trained interviewer using a pre-constructed questionnaire. A case-control analytical study was conducted on 62 SLE-proven cases and 20 normal controls participants from Khartoum State for two years (2017 to 2018). The samples cases were recruited from Rheumatology Clinics at the National Ribat University Hospital, Medical Military Hospital, and Private Clinics. Cases were females hospitalized with a diagnosis, established before discharge, of the first episode of SLE. Controls were females hospital admissions who have no history of SLE, and who were admitted with a disease not suspected of being related to SLE. The subjects had been currently female patients, aged 25-65 years. Sampling given the design of this study, the samples (or cases and controls) were selected by a nonrandomized method. The sample size was set at 62 cases and 20 controls by a sample size determination technique based on a reassigned significance level and power, and the level of relative risk to be detected. An attempt was made to avoid or diminish potential sources of bias and error that are frequently encountered in case-control studies. Misdiagnosis bias, recall bias, selection bias, and SLE reporting bias were of great importance to the validity of the study results. Patient characteristics: age, tribe.

**Sample size for independent case-control studies:**

the estimated sample size *n* for independent case-control study is calculated as

$$n = \frac{\left[Z_\alpha \sqrt{(1+m)\bar{p}'(1-\bar{p}')} + Z_\beta \sqrt{p_1(1-p_1) + m\,p_0(1-p_0)}\right]^2}{(p_1 - p_0)^2}$$

$$\bar{p}' = \frac{p_1 + p_0 / m}{1 + 1/m}$$

$$p_1 = \frac{p_0 \psi}{1 + p_0(\psi - 1)}$$

$$n_c = \frac{n}{4}\left(1 + \sqrt{1 \frac{2(m+1)}{n\,m\,|p_0 - p_1|}}\right)^2$$

Where α = alpha, β = 1 – power, ψ = odds ratio (odds ratio of exposures between cases and controls), *m* – number of control subjects per case subject, *p1* – probability of expo- sure in controls. *p0* can be estimated as the population prevalence of exposure, *nc* is the continuity corrected sample size and *Zp* is the standard normal deviate for probability *p*. If possible, choose a range of odds ratios that you want to detect the statistical power [19].

**IgG avidity test:** In this study we have used the IgG Avidity test for CMV and EBV was performed using kits (Euro Immune®, IgG avidity testing) which measured the avidity utilizing urea as a denaturing agent. Avidity index, which is a ratio of the relative fluorescence value obtained for the sample with a strip containing urea buffer to those without urea buffer. The Avidity index is a ratio of the relative fluorescence value obtained for the sample with a strip containing urea buffer to those without urea buffer. The IgG Avidity test for CMV and EBV was performed using kits (Euro immune, IgG Avidity testing) which measured the Avidity utilizing urea as a denaturing agent. The test was performed according to the manufacturer's instructions. Each diluted sample or control was pipetted into two adjacent wells and incubated for 30 min, followed

by washing for only one time, then each sample or control was treated with 200 µL of urea in one well and 200 µL of buffer into the parallel well and then incubated for 10 min and washed three times. Samples were incubated for 30 minutes by antihuman IgG antibody enzyme-conjugate, and 15 minutes by chromate substrate solution, and then the reaction was stopped by stop solution. The photometric measurement of the color reaction was made at 450 nm and a reference at 620 nm wavelength. The avidity index for each sample or control was calculated using the following formula: Relative avidity index (RAI) (%) = Extinction of sample with urea treatment/Extinction of sample without urea×100. The results were interpreted according to the manufacture in structure as follows: RAI < 40%, indicated low avidity antibodies, RAI 40%–60% indicated gray zone, and RAI > 60% indicated high avidity antibodies [20].

**Data analysis:**

Pretested questionnaire data were analyzed by SPSS software V.20 (IBM, Chicago, IL). Means and proportions of the demographic were calculated for SLE seropositive groups. Chi-test and Binary Logistic regression analysis was used for SLE as a dependent factor while IgG seropositive groups of EBV and CMV as the independent variable and, demographic and SLE variables as dependent variables. Odds ratio (OR) with a 95% confidence interval was calculated and statistical significance was defined as a P value below 0.05.

**Ethical considerations:** The ethical approval was obtained from the ethical board of the University of Science and Technology, National Centre for research, and tropical medicine center of research, and consents were obtained from all participants.

**Results:**

Eighty-two females volunteered to participate; 62 cases with systemic lupus erythematosus098 and 20 control participants. Mean age (± SD) of cases and control (34±1.4) vs (34±1.6). Original tribes in Sudan were screened Avidity assay for Western State 37(45.1%), Northern State 29(35.4%), Central State 17(20.7%), Southern State1(1.2%) respectively ( Table 1).Sero-positivity of IgG by Avidity assay for cases and controls revealed high score for CMV (60 (96.8%) vs 19(95%), (OR 1.579, CI -95% (0.136-18.4), P < 0.05), for both EBV and Co-infection (58 (98.3%) vs 16 (80.0%), (OR 3.625, CI -95% (0.227-2.708), P< 0.05 ) (Table 1). However, Sero-positivity of IgG by Avidity assay revealed disassociation with SLE of low score for CMV, EBV and Co-infection were recorded (2(3.2%) vs 1(5%), (4(6.5%) vs 4 (20 %))for CMV, EBV and Co-infection were recorded (2(3.2%) vs 1(5%), (4(6.5%) vs 4 (20 %)) respectively (Table 2).

**Discussion:**

Systemic lupus erythematosus is disease that can affect different organs and system in human body. Infection can be seen in patients with SLE and can be precipitated by the use of immunosuppressant medication. Several studies showed SLE in comparison with healthy controls can be associated with increased Seropositivity rate for CMV and EBV [21-24]. Furthermore, the two groups of participants' serum IgG avidity Seropositivity for both EBV and CMV in SLE were associated significantly. The most antibodies of IgG have revealed high score above 60% of total IgG, which indicated an old infection. Therefore, it possible to suggest that the administration of immunosuppressive medication in SLE, may be the main reason for the activation

or co-infection with latent CMV and EBV. This may also refute the possibility that Co-infection with these viruses being a trigger of SLE. Our result also in agreement with studies carried out by James et al and Chen. Who reported that SLE is associated with increased levels of anti-CMV IgG antibodies [25-26]. Importantly, the role of molecular mimicry mediated through EBNA1 protein and the SmB and Ro60 proteins was established [27].

Importantly, in the present study we observed that SLE patients of the young age group range between [18-40] years were higher in SLE than other group and this in concordance with fact that predominantly SLE affects young and middle-aged women [28].

It worth mentioning, the prevalence and pathogenesis of SLE among Sudanese population is not well researched. Further research in Sudanese population may reveal exciting result due to the fact that Sudan is inhabited by different tribes that have diverse origins like Nilotic, Western and Nubian tribes. Involvement in this study the increase of disease in a female group similar to that study reported by Nashwa et al. in

**Table 1: Demographic characteristics and IgG antibody Avidity score among cases and controls**

| Characteristic | Variable | SLE (N=62 ) | Control (N=20 ) | TOTAL (N=82 ) | P- Value |
|---|---|---|---|---|---|
| Age | 18-40 | 48(77.4%) | 16(80%) | 64(78.0%) | .027 |
| | 41-63 | 14(22.6%) | 4(20%) | 18(22.0%) | |
| Tribal origin in Sudan | Northern | 26 (41.9%) | 3(15%) | 29(35.4%) | 0.04 |
| | Centre | 13(20.9%) | 4(20%) | 17(20.7%) | |
| | Western | 24(38.7%) | 13(35.1%) | 37(45.1%) | |
| | Southern | 1(1.6%) | 0(00%) | 1(1.2%) | |
| IgG Avidity | | | | | |
| EBV | High-score | 58(93.5%) | 16(80.0%) | 74(95.1%) | 0.02 |
| | Low-scores | 4(6.5%) | 4(20%) | 8 (4.9%) | |
| CMV | High-score | 60(96.8%) | 19(95%) | 79 (96.3%) | 0.04 |
| | Low-scores | 2(3.2%) | 1(5%) | 3(3.7%) | |
| Co-infection | High-score | 58(98.3%) | 16(80.0%) | 74(95.1%) | 0.02 |
| | Low-scores | 4(6.5%) | 4(20%) | 8 (4.9%) | |

**Table 2: Statistical associations of IgG Avidity for SLE patients**

| Items | Binary logistic regression | | |
|---|---|---|---|
| | OR | CI-95% | P |
| High score EBV | 3.6 | .82-16.12 | <.05 |
| High score CMV | 1.6 | .14-18.4 | <.05 |
| Co-infection | 3.6 | .82-16.12 | <.05 |

Sudan [29].The increased frequency of SLE among women had been attributed in part to a postulated estrogen hormonal effect. A previous report from Sudan showed ethnic variation, with the disease being less frequently seen among Nilotic and Western Sudanese tribes [30]. This may in part explain the disease severity in different ethnic groups especially among Black, African-American, Hispanic and Asian patients [27].

This study has many limitations the small sample size, the lack of previous knowledge about seroconversion for the tested viruses, IgM and IgA of the viruses in this study. In additions, there were few recent infections, as determined by (IgG) avidity testing, to allow statistical interpretation between cases and controls. The IgG Seropositivity cannot be determined whether they are triggering factors or complications of the disease. A large cohort study needed for further investigation including molecular and genetic methods.

**Conclusion:**

Patients with SLE in Sudan have high rate of infections by EBV and CMV evidenced by the IgG avidity test indicating association.

**Declarations:**

**Ethical approval and consent to participate:**

Applicable

**Consent for publication:**

Applicable

Availability of data and material

The dataset generated during this study are available from the corresponding author on reasonable request.

**Competing interests:**

None declared

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