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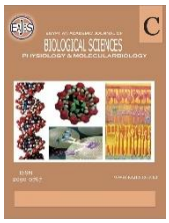

The Association between Epstein Barr virus and Systemic Lupus Erythematosus among Hemodialysis Patients

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ABSTRACT

The Epstein-Barr virus (EBV) is a prevalent herpesvirus that commonly infects a substantial proportion of the global population. Infection with the Epstein-Barr virus has been hypothesized to be one of the environmental factors that contribute to the development of systemic lupus erythematosus. Our objective was to investigate the correlation between Epstein-Barr virus (EBV) reactivation and the prognosis of systemic lupus erythematosus (SLE). The study conducted Specialized Center for Diseases and Kidney Transplant in AL-Sadder Medical City in Al-Najaf Governorate involved obtaining blood samples from 50 hemodialysis patients and 50 healthy control. The data collection period for the study was from January to December 2022. The study collected blood samples from 50 hemodialysis cases. The researchers used RT-qPCR technique to detect EBV infection. Among the 50 patients, 18 (36%) tested positive for EBV using RT-qPCR. The ELISA technique was also used in the study to detect systemic lupus erythematosus by detecting the level of Anti-ds-DNA in all samples. The results showed that 19 (36%) of the samples tested positive for anti-dsDNA. Remarkably, among those who tested positive for EBV, 13 individuals (72.2%) were simultaneously positive for both EBV and SLE. We also examined serum levels of IL-10 and IL-18 in hemodialysis patients with both EBV and SLE. IL-10 and IL-18 concentrations in co-positive samples (EBV+ SLE) in hemodialysis patients showed significant differences, with IL-10 increasing by (379±55.2) and IL-18 increasing by (26.4±8.86), respectively, in comparison to the control group's levels of (207±5.9) for IL-10 and (11.7±0.72) for IL-18. The study's findings lead to the conclusion that patients undergoing hemodialysis exhibit demonstrate a greater incidence of both Epstein-Barr virus (EBV) and systemic lupus erythematosus (SLE) in comparison to the control group. Furthermore, the study indicates a significant increase in IL10 and IL18 levels in comparison to the control group.

INTRODUCTION

Epstein–Barr Virus (EBV), also called human herpesvirus 4, is a lymphotropic herpesvirus and the causative agent of infectious mononucleosis (IM). It was first discovered in cells isolated from African Burkitt's lymphoma, later, it was recognized that it is highly prevalent worldwide (Epstein and Barr, 1964; Smatti *et al*., 2018).

The EBV has a double strand DNA enclosed by a protein membrane called the capsid, which is enclosed by a protein cap called th tegument protein and envelope (Oda *et al*., 2016). A protein tegument, outer envelope, and 162 capsomers make up the nucleocapsid. Three major tubular and several minor virion proteins make up the icosahedral viral capsid. The envelope is dominated by the viral 350 or 220kDa glycoprotein (gp350/220), while the tegument contains both cellular and viral proteins (Johannsen *et al*., 2004; George, 2016). EBV contains various nuclear proteins on its surface, including LMP-1, EBNA-2, EBER, v-snoRNA1, EBV-sisRNA, EBNA-3, and miRNAs (Kang & Kieff, 2015). The transmission of EBV occurs mainly via contact with saliva. However, the virus can be transmitted through sexual contact, blood transfusions, and organ transplantations. Hemodialysis (HD) is the process of purifying the blood of a person whose kidneys are not working normally, especially patients in with end-stage renal failure. Individuals with HD indicate that patients are at risk of developing an EBV infection. (Yasir& Marzoq, 2022).

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with multisystem involvement. It is multifactorial and involves epigenetic, genetic, ecological, and environmental factors (Ameer *et al*., 2022). SLE prevalence varies from 20 to 150 per 100,000, higher in Afro-Americans and AsiansIn the past 40 years, the incidence has tripled since mild disorders are now more accurately identified (Pons-Estel *et al*., 2010). The condition affects females nine to one ratio more often than males. (Tanaka, 2020). Most SLE patients develop nephritis. Lupus nephritis (LN) is a major cause of SLE-related morbidity and death. Lupus nephritis has many clinical symptoms and morphologic features. Immune complexes can accumulate everywhere in the kidney (Giannico and Fogo, 2013).

IL-10 is an immunosuppressive cytokine that regulates immune responses by inhibiting the ability of APCs to present antigens to T cells in a variety of ways. It is produced by Th1, Th2, and the secretion of IL-10 is delayed and always follows that of pro-inflammatory factors with a latency of a few hours (Stenvinkel *et al.*, 2005; Mittal, & Roche, 2015). IL-10 is crucial to renal physiology, acute kidney injury, and chronic renal failure (Sinuani *et al*., 2013). Numerous studies suggest IL-10 may perform various and competing roles in murine lupus. IL-10 promotes SLE progression by proliferating and differentiating autoreactive B cells into plasma cells (Caielli, 2019; Facciotti *et al*., 2020).

Interleukin-18 (IL-18), a potent pro-inflammatory cytokine, affects the innate and acquired immune response and host defense against infections. IL-18 is produced by hematopoietic and non-hematopoietic cells like monocytes, macrophages, keratinocytes, and mesenchymal cells. IL-18 may cause autoimmunity by activating inflammatory and cytotoxic immune cells. Its blood levels are higher in patients with immune-related disorders like rheumatoid arthritis, systemic lupus erythematosus, type I diabetes mellitus, atopic dermatitis, psoriasis, and inflammatory bowel disease (Ihim *et al*., 2022). In viral infections such as type 1 HIV, rotavirus, human papillomavirus, and dengue virus, serum IL-18 levels are raised. IL-18 is significantly enhanced in acute EBV infections and EBV-associated illnesses (Watanabe *et al*., 2010).

MATERIALS AND METHODS

Samples Collection:

Blood samples were collected from January to December 2022 at Sadar Medical City in Najaf Governorate, Iraq. The patient group included fifty patients who suffered from hemodialysis, and among them were some patients experiencing systemic lupus erythematosus that were diagnosed by a specialist physician as well as laboratory findings. The control group consisted of fifty healthy people.

Immunological Assay:

All patient samples were tested for anti-double-stranded DNA (anti-dsDNA)

antibody levels to diagnose SLE. The assay was performed according to the manufacturer's instructions for the kit (Aeskulisa, Germany). Interleukin-10 (IL-10) and interleukin-18 (IL-18) were measured according to the directions of the manufacturing protocol of the kit (BT-Lab, China). All the tests were accomplished via the ELISA technique

RT-PCR Technique:

The DNA was extracted from the blood of hemodialysis patients according to the manufacturer's instructions using an extraction kit (Favorgen, Taiwan). Then the target gene was amplification using detection using unique primers, Forward: '5 -CTT GGA GAC AGG CTT AAC CAG ACT CA-3', Reverse: '5 -CCA TGG CTG CAC CGA TGA AAG TTA T-3' (Uphoff *et al*., 2010) by use RT-qPCR technique.

Statistical Analysis:

Statistical tests were conducted using the computer-assisted Statistical Package for Social Sciences (SPSS) version 17. The comparison between the patients and the healthy group has been analysed using a T-test. A P-value of less than 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

Detection of EBV-DNA by RT-qPCR:

The results of real-time PCR amplification for the presence of EBV DNA in serum samples. The EBV genome was detected in 18 (36%) of the 50 blood samples tested from patients suffering from hemodialysis Figure (1).

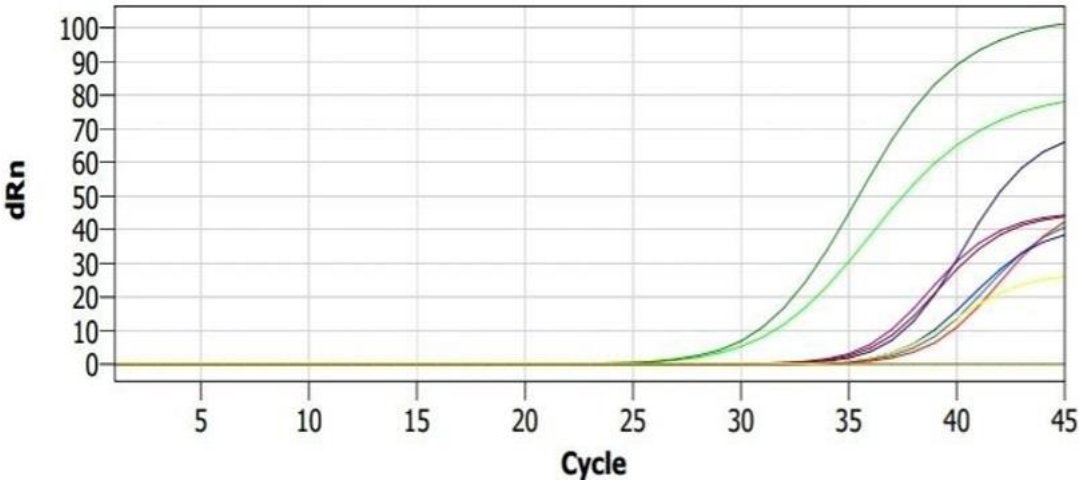

Fig.1: Diagram plot amplification RT-qPCR of EBV gene.

Our study is close to the result of the study by Martins *et al*., 2023 which found in the hemodialysis group, 10 patients (18.51%) were positive for EBV, with a mean viral load of 196.4 copies/ml.

End-stage renal failure patients have severe changes in cell-mediated immunity, which increases their risk of contracting opportunistic viral infections like EBV. When the immune system is suppressed by disease, EBV is more likely to cause problems (Kato *et al*., 2008, Merlo *et al*., 2010). Also, patients undergoing hemodialysis often have weakened immune systems due to their underlying kidney disease and the medical treatments they receive. This weakened immune response could make them more susceptible to viral infections like EBV. Additionally, as you mentioned, repeated blood transfusions during hemodialysis can also play a role in transmitting infectious agents like EBV.

Detection of Systemic Lupus Erythematous Among Hemodialysis Patients:

Anti-double-stranded DNA (anti-dsDNA) antibody levels in all patient samples were used to diagnose systemic lupus erythematosus. This was achieved using the ELISA technique. Among the collected samples, a subset displayed observable symptoms of SLE, and these cases were

diagnosed by expert physicians specializing in dialysis centers, where the samples were obtained.

Within the provided Figure (2), it was noted that 19 individuals (equivalent to 36% of the sample) exhibited a positive result for anti-dsDNA when the index value exceeded 20. Conversely, negative anti-dsDNA was observed in 31 individuals (representing 64% of the sample) when the index value was below 20.

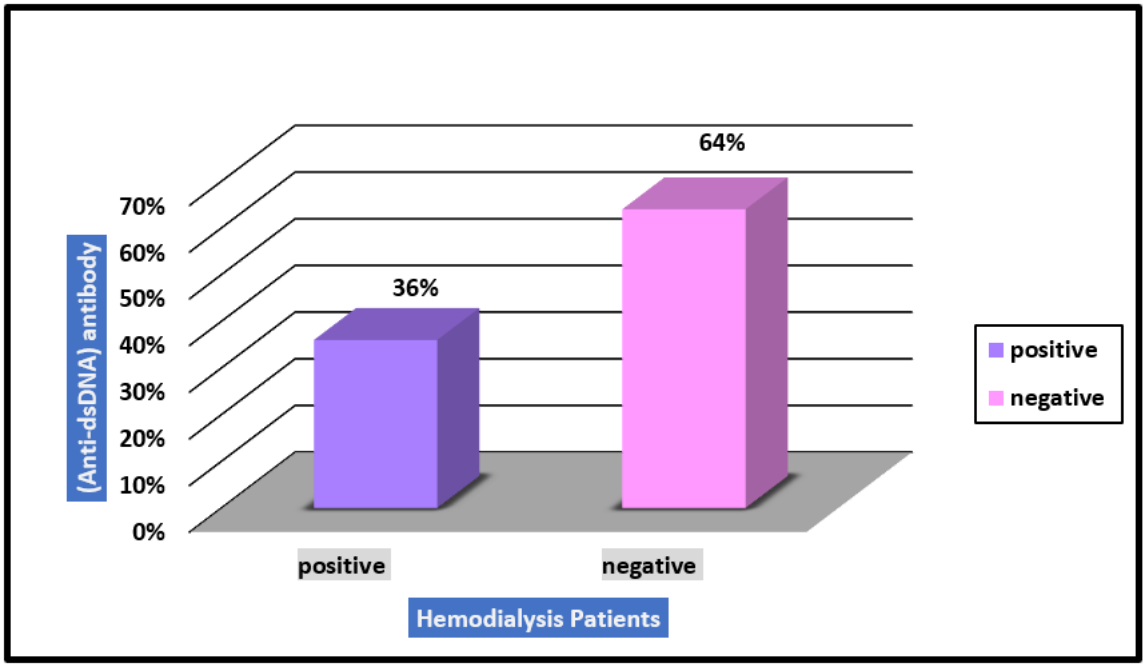

Fig. 2: Detection of systemic lupus erythematous among hemodialysis patients**.**

Our findings are consistent with the results reported by Noori *et al*., 2013 regarding Iraqi patients with Systemic lupus erythematosus (SLE), where the anti-dsDNA test showed a prevalence of 56%. The results also align with the study conducted by (Al Shibli, 2016 which found that the level of Anti-dsDNA in patients with lupus nephritis was 26.79. Furthermore, the prevalence of anti-dsDNA observed in our study is in line with the findings reported by AL-Hashimi, 2017, which indicated that approximately 75% of SLE patients exhibit anti-dsDNA reactivity.

Several studies have assessed that anti-DNA antibodies have been reported to correlate with renal activity (but not chronicity) index and to have some correlation with WHO type IV glomerulonephritis (Kavanaugh *et al*., 2002; Sui *et al*., 2013). Anti-dsDNA antibodies have also been recovered from the glomeruli of active lupus nephritis patients, and the various histological patterns seen in lupus patients are due to their deposition in different sites in the glomerulus (Waldman *et al*., 2005; Yung & Chan, 2015).

Anti-dsDNA antibodies are specific to SLE and can be detected in patients at least 2 years before diagnosis of clinical disease. Serum anti-dsDNA antibody levels often reflect disease activity in lupus nephritis patients. For many years, the anti-dsDNA antibody assay was considered the gold standard in the diagnosis and assessment of disease activity in SLE patients (Isenberg et al., 2007; Yung & Chan, 2015) .

Extensive research has established the involvement of anti-dsDNA antibodies in causing damage to the kidneys, skin, and brain in individuals with SLE. The production of these antibodies is influenced by various factors, including abnormalities in dendritic cells, B cells, or T cells, as well as a

deficiency of a DNase enzyme responsible for clearing released nuclear materials. However, further studies are required to fully understand these mechanisms. The detrimental effects of penetrating autoantibodies in initiating complex inflammatory and fibrotic processes underscore the role of anti-DNA antibodies in harming target cells and organs (Wang and Xia, 2019).

The Relationship between EBV and Systemic Lupus Erythematosus In Hemodialysis Patients:

Out of the 50 hemodialysis patients, 18 individuals (36%) were found to have positive Epstein-Barr virus (EBV) results according to RT-qPCR analysis. Furthermore, the ELISA test showed that 19 individuals (38%) had positive results for Systemic lupus erythematosus (SLE). Remarkably, among those who tested positive for EBV, 13 individuals (72.2%) were simultaneously positive for both EBV and SLE.

Our result was near with result of research conducted by Yu *et al.,* 2014 revealed a noteworthy disparity in the rate of Epstein-Barr virus (EBV) positivity between renal tissue samples with lupus nephritis (LN) and those with minimal change nephropathy or non-nephropathy. The findings of the study revealed a statistically significant increase in the rate of Epstein-Barr virus (EBV) positivity in renal tissue samples affected by lupus nephritis (LN). This suggests that EBV infection may play a role in the pathogenesis of LN, maybe through the stimulation of autoantibody synthesis. According to the study conducted by Ding *et al.,* 2015, it was suggested that EBV infection might be an exacerbating factor in some lupus patients via promoting anti-Sm production. In the investigation conducted by Zaki *et al.,* 2018 regarding autoantibodies in individuals with lupus nephropathy, it was observed that indicated that a significant proportion of the patients had positive results for specific autoantibodies. The highest prevalence was observed for anti-dsDNA antibodies (73.7%), followed by anti-Sm antibodies (57.8%), and then anti-RNP antibodies (31.6%).

The association of EBV with SLE has been continuously investigated, with considerable evidence suggesting the participation of EBV in the pathogenesis of SLE. A link between Epstein-Barr virus (EBV) and lupus has been suggested through serologic, molecular, and experimental studies (Chen *et al.,* 2005; Barzilai *et al.,* 2007; Miskovic *et al*., 2023). EBV infection or reactivation can act as an environmental trigger in the induction or promotion of the development of SLE. The aberrant production of autoantibodies in SLE can target various nuclear antigens leading to the development of lupus nephropathy (Lu *et al*., 2007, Draborg *et al*., 2012).

Variability in our study designs, patient populations, and diagnostic methods may contribute to the reverse correlation. Differences in sample collection, antibody assays, and study criteria could influence the detection and interpretation of EBV infection in SLE patients. It is possible that the immune dysregulation characteristic of SLE could affect the body's response to EBV. Altered immune function in SLE patients may result in impaired control or clearance of EBV infection, leading to a reduced prevalence of detectable EBV markers.

Previous studies have shown conflicting results regarding the relationship between SLE and EBV, which may be attributable to a number of factors, including differences in EBV activity or replication between SLE patients and healthy controls, altered immune responses and cytokine profiles in SLE patients that may influence the replication and persistence of EBV, resulting in lower viral loads or reduced EBV-associated markers, and the variability in study designs, patient populations, and other factors. Possibly also part of Variations in sample collection, antibody testing, and study requirements may alter the detection and interpretation of EBV infection in SLE patients; Previous studies have found inconsistent results about the association between Epstein-Barr virus and systemic lupus erythematosus. This may be due to a

number of factors.

Immunological Marker Detection:

Estimation of Interleukin 10 in A Co-Positive Sample (EBV+ SLE) in Hemodialysis Patients:

Figure 3 shows that the mean serum levels of interleukin 10 were elevated in hemodialysis patients with (EBV+ SLE) (379±55.2 pg/ml) compared to healthy controls (207±5.9 pg/ml). The association was found to be statistically significant.

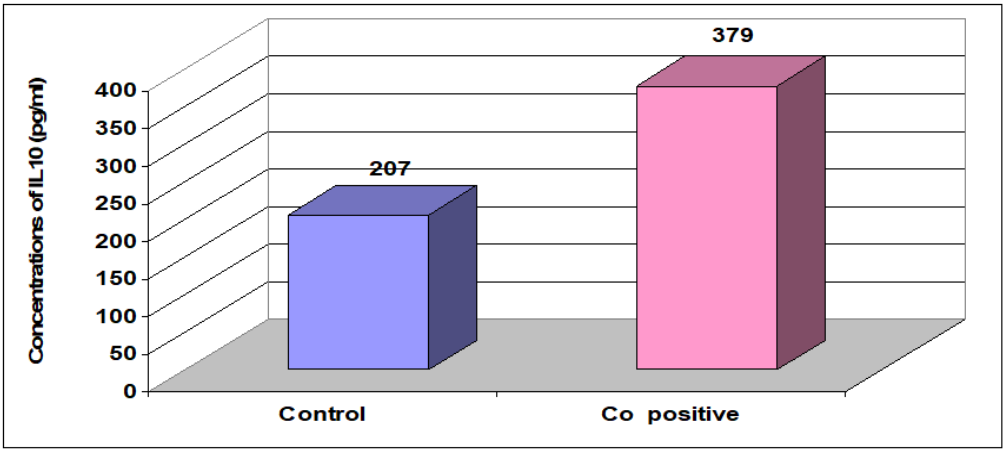

Fig. 3: Estimation of interleukin 10 in a co - positive sample (EBV+SLE) in hemodialysis patients.

The results of our investigation align with those of Dorego et al. (2016), who also observed that the activation of IL10, SLE patients and healthy controls exhibited a significant increase in the levels of these cytokines upon EBNA1 stimulation, with a highly significant p-value of 0.008** for the comparison between the two groups.

Epstein-Barr virus (EBV) is especially associated with SLE and numerous studies have suggested a link including abnormally elevated viral load, notable antibody responses directed towards EBV, and deficient EBV-specific T-cell responses in SLE patients compared to healthy controls (HCs) (Berkun *et al.,* 2009; Csuka *et al.,* 2013),

IL-10 is a potent in vitro inducer of B lymphocyte differentiation as well as an inhibitor of T helper lymphocyte and antigen-presenting cell function. Thus, the immunological imbalance of SLE may be related to an abnormally high production of IL-10, or to a hypersensitivity of immune cells to this cytokine (André *et al*., 2009).

Estimation of Interleukin 18 in Co-positive sample (EBV+ SLE) in hemodialysis patients:

Figure 4 shows that the mean serum levels of interleukin 18 were elevated in hemodialysis patients with (EBV+ SLE) (26.4±8.86ng/ml) compared to healthy controls (11.7±0.72 ng/ml). The association was found to be statistically significant.

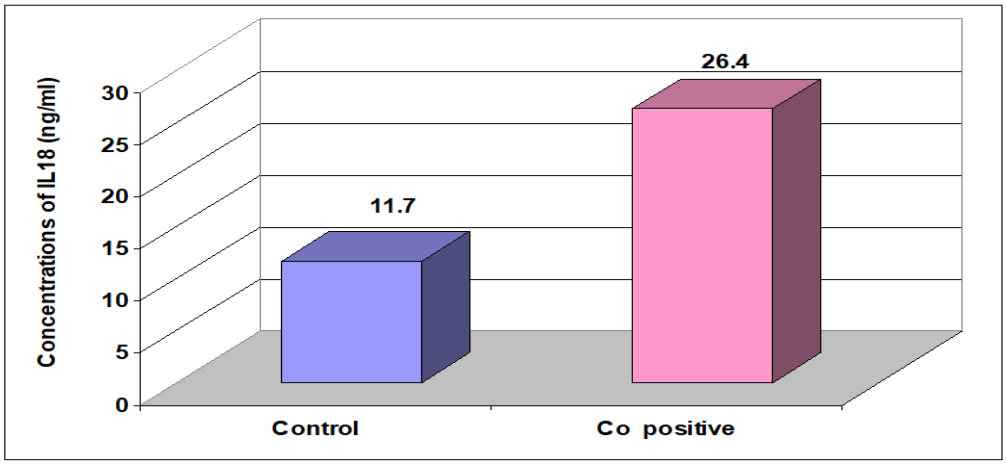

Fig.4: Estimation of Interleukin 18 in a Co - positive sample (EBV+SLE) in hemodialysis patients.

Reviewing the relevant literature revealed that no statistical studies had previously been filed to analyse Interleukin 18 levels in the serum of patients with Co - positive sample (EBV+SLE) in hemodialysis patients, making the current study the first of its kind.

In our opinion about that is that individuals with SLE, EBV infection, and hemodialysis are at increased risk for immunological dysregulation due to all three conditions. The immunological response may be further intensified in the presence of coexisting SLE and EBV infection, which may result in increased production of IL-18. genetic predisposition and environmental factors can both have a role in the development and progression of SLE, EBV infection, and renal failure. Individual genetic predisposition plays a larger role in the development of SLE than environmental factors do. These variables may have an interaction with IL-18 and/or other cytokines, which may contribute to the clinical symptoms that have been reported.

CONCLUSION

The study's findings lead to the conclusion that patients undergoing hemodialysisn exhibit demonstrate a greater incidence of both Epstein-Barr virus (EBV) and systemic lupus erythematosus (SLE) in comparison to the control group. Furthermore, the study indicates a significant increase in IL10 and IL18 levels in comparison to the control group.

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