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Detection of Epstein-Barr virus among chronic kidney disease patients in Najaf, Iraq

Zahraa Basem[1*], Huda Jameel[2]

[1] Department of Medical Laboratories Techniques, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Kufa, Iraq
[2] Department of Biology, College of Science, University of Kufa, Najaf, Iraq

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ABSTRACT

Many people across the world have been infected with Epstein Barr virus (EBV), a kind of a human herpes virus. Loss of kidney function happens progressively with time in those with chronic kidney disease (CKD). Individuals with CKD may be more susceptible to viral infections such as EBV due to their compromised immune systems. The purpose of this research is to quantify the frequency with which EBV infection occurs in patients with CKD in the Al-Najaf governorate. Blood samples from 50 people with CKD and 50 healthy controls were collected for the study at the Specialized Centre for Diseases and Kidney Transplant at Al-Sadder Medical City in Al-Najaf city. Nineteen of the CKD patients were men, whereas 31 were women. The age range of individuals who took part in the research varied from 17 to 47. The time frame for this study's data gathering was from January 2022 to December 2022. Fifty hemodialysis patients gave blood samples for analysis. Researchers measured anti-EBV IgG antibodies using the ELISA method to evaluate the efficacy of humoral immunity. Eleven (22% of all samples) were found to have positive results for Epstein Barr nuclear antigen2 (EBNA2) IgG. This number was 43.1% of all males and 38.4% of all females. However, the serum concentration was found to be statistically significantly higher in the age range of 17-26 years (p0.05) than in any other age group. The RT-qPCR method was also applied to detect EBV infection in the study. Only 18 (36% of the sample) of the 50 patients had positive RT-qPCR results for EBV. This number was 15.7% of all males and 48.4% of all females; however, the positivity rate increased to 71.4% in the (17-26) age group and decreased to 28.5% in those older than 57. Results from the control group were negative for EBNA2 IgG antibodies and EBV DNA. Findings of this research demonstrate that the prevalence of EBV antibodies is considerably higher among patients undergoing hemodialysis than in the group serving as a control, regardless of age. In addition, the results of the study support the use of real-time PCR for detecting EBV among patients with CKD, due to its high sensitivity and specificity.

INTRODUCTION

Epstein Barr virus or HHV4, is a large DNA-copying human herpes virus [1]. The EBV genome is about 172 kilobase pairs (kb) in size and consists of 80 genes These genes produce multifunctional proteins that include six nuclear antigens (EBNA1, EBNA2, EBNA3A, EBNA3B, and EBNA3C), three latent membrane proteins (LMP-1, LMP-2A, and LMP-2B), and two non-coding RNAs (EBER1 and EBER2). These proteins are expressed by infected B lymphocytes. They support the virus's existence in the host cell's DNA and provide the virus with a fighting chance against the host's immune system [2,3].

EBNA2, a protein with crucial function in cellular conversion, consists of repeat of 18 amino acids from Arg-Gly and an acidic C-terminal [4]. Its function is thought to be crucial for promoting cell growth, aiding the proliferation of virus-infected cells inside the host within vivo, and effectively establishing viral latency in a significant percentage of B cells [5]. The primary nuclear transactivator of the virus, EBNA2, controls host cell regulatory genes and latent viral transcription [6]. In addition, EBNA2 serves as vital

* **Corresponding author**
e-mail: zahra.shakerckm@atu.edu.iq

for the Epstein Barr virus induced immortality of B lymphocytes [7,8].

Epstein Barr virus (EBV) lies dormant after initial infection, just like other herpesviruses. During this stage, the virus infects the epithelial cells, gains access to the peripheral B lymphocyte, and remains dormant [9]. According to epidemiological statistics, roughly ninety percent of the world's population [10]. It is estimated that 79.8% of all Iraqi people are infected with Epstein-Barr virus (EBV) [11].

EBV is spread through oral contact with food items or contaminated objects. Additionally, it can be spread by blood transfusions, childbirth, solid organ transplants, blood cell transplants and sexual interaction. It is worth noting that over 90% of individuals infected with EBV are intermittently intermittently contagious throughout their lives, even if they do not display any symptoms [12].

End-stage kidney failure patients might require hemodialysis (HD) to eliminate waste products from their blood circulation [13]. Individual with end-stage renal failure, who experience significant deficiencies in cell-mediated immunity, have a higher chance of acquiring opportunistic viral infections such as EBV infection [14,15].

MATERIALS AND METHODS

Samples collection

Blood samples were taken at the Specialised Centre for Diseases and Kidney Transplant in the Iraqi city of Al-Najaf, from November of 2022 to May of 2023, and were drawn from a research group consisted of fifty patients with CKD (31 women and 19 men), while 50 non-CKD individuals formed the control group. Before usage, the blood samples were kept at – 20 degrees.

Immunological assay

All the samples were diagnosed by applying the ELISA technique, using the Human Epstein-Barr virus nuclear antigen2 (EBNA2) IgG Kit provided commercially by the company (SunLong, China).

RT-qPCR technique

DNA was obtained from the peripheral blood specimens from those with CKD using an extraction kit purchased from Favorgen (Taiwan), following the instructions provided by the manufacturer. Subsequently, the target gene was amplified using specific primers in an RT-qPCR technique. The forward primer sequence used was 5'-CTT GGA GAC AGG CTT AAC CAG ACT CA-3', and the reverse primer sequence was 5'-CCA TGG CTG CAC CGA TGA AAG TTA T-3'.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 17 was employed to accomplish the computer-assisted statistical tests. The means of the various variables in each of the four groups were analysed via one-way (ANOVA) analysis of variance. Parametric statistical tests of significance were then established via the T-test in the study groups, while least standard error (SE) was applied to identify significant differences between parameters. Statistical significance was defined as a P-value at 0.05.

RESULTS AND DISCUSSION

Out of the 50 hemodialysis patients included in the study, 11 (22%) tested positive for EBV using the ELISA technique. The diagnosis was subsequently confirmed through molecular detection by way of RT-qPCR, which identified 18 (36%) positive cases. In contrast, in the healthy group consisting of 50 individuals, none tested positive for EBV either by way of ELISA technique or RT-qPCR, as illustrated in Figure 1.

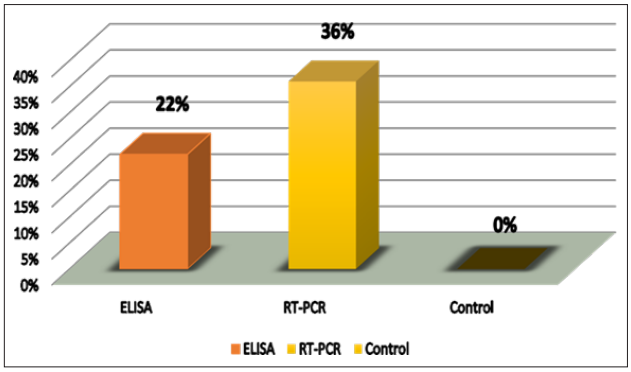

Figure 1. The distribution of EBV among renal failure patients

This finding closely matches that of the researchers' study [16], which revealed that 57.6% of the total of 118 patient samples had tested positive for anti-EBV IgG antibodies, while [17] demonstrated that 11.49% of all HD cases are affected by EBV infection. The findings of this study's RT-qPCR are consistent with those of [18], who discovered that roughly 10 (18.51%) of the samples examined produced positive results for the EBV viral genome. Even though the immunological detection of EBV reactivation is not always certain, it is frequently associated with a seroprofile that includes high levels of antibody against the early antigen (EA) of the virus, along with identifiable IgG, or immunoglobulin G, antibodies towards EBNA and undetected immunoglobulin M (IgM) antibodies towards VCA. While serological diagnosis for EBV infection is a straightforward tool for screening and follow-up for EBV infection, molecular diagnosis, however, appears to be a more precise diagnostic procedure [19]. These quantitative techniques have recently been used to manage patients with high viral loads and to investigate the pathophysiology of disorders linked to the EBV [20].

EBV detection in the Sample and Control Groups Using the ELISA technique

Table 1 shows the percentage of EBV antibodies (EBNA2 IgG positivity) seen for the age groups of (17-26), (27-36), (37-46), and (47) – 57.1%, 16.7%, 15.8%, and 16.7%, respectively.

The EBNA2 IgG levels in all patient groups tested by the ELISA were significantly higher (p 0.05) when contrasted to the negative control group, with the patient group aged (17-26) years recording the highest mean level (0.48125 IU/ml) in contrast to other patient groups. Our research closely

resembles that of researcher [21] from Taiwan, who discovered that higher age and educational level were associated with an increased risk of EBV seropositivity, which may reach a rate of 93% throughout adolescence at the age of 14 to 16 years and 88.5% at an early age with rising antibodies against the virus. Additionally, the study conducted in Iraq by [22] indicated that there was a reduced distribution of EBV-NA IgG Ab. EBV has a greater impact on patients with renal failure who are 20 years of age or younger because of a combination of immunosuppressive drugs, a longer period of disease, compromised immune systems and social circumstances.

Table 1. The Levels of EBNA2 IgG among CKD patients according to age group

Age groups	No.	EBV		Mean of Positive Sample	Mean of Positive Control	Mean of Negative Control	P value
		No.	%				
17-26	7	4	57.1*	0.48125	1.076	0.006	0.0113
27-36	12	2	16.7	0.818			
37-46	19	3	15.8	0.229667			
≥47	12	2	16.7	0.592			
Total	50	11	22				
X2 c			11.2				
X2 t			7.81				

Detection of EBV-DNA by RT-qPCR according to age group

In our work, we established the outcomes of real-time PCR amplification to detect EBV DNA in serum samples. In 18 (36%) of the 50 blood samples analyzed from patients with chronic renal disease, the EBV genome was found as illustrated in Figure 2. According to the findings, the ratio peaked between the ages of 17 and 26 at 71.4% and dropped to 28.5% by the time a person reached 57.

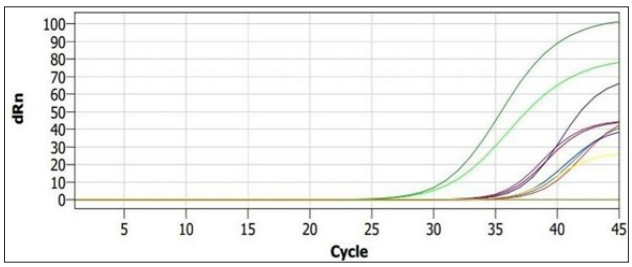

Figure 2. Diagram plot amplification RT-qPCR of EBV gene

The results of our investigation do not agree with those given by [23]. Age distribution among RT individuals with positive EBV viremia was non-significant, per their findings (P >0.05). However, compared to other age groups, those over 40 had a somewhat greater percentage (40%) of EBV infection. There were no statistically noteworthy variation among the ages when using the quantitative measurement of an EBV viremia load (P >0.05).

Quantitative PCR is extremely useful for detecting viral infections and keeping track of them, especially in those with weakened immune systems. This method works well as a diagnostic method for identifying cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections. Due to its speed and accuracy, real-time PCR enables the immediate and precise measurement of PCR results. As a result, it is now a crucial tool for diagnosing and keeping track of viral infections, especially in those with impaired immune systems [24-26].

Sensitivity and Specificity between ELISA and RT-qPCR

According to the findings listed in Table 2, the ELISA technique had sensitivity of 29.4%, while the specificity of RT-qPCR was 78.7%. This ratio shows that ELISA displays a true positive EBV infection, while RT-qPCR with the specificity of 78.7% shows a true negative EBV infection. The current study demonstrates good results for relying on RT-qPCR for the screening of EBV antibodies. RT-qPCR is regarded as the gold standard in the detection and identification of the infection with a true positive EBV infection, whereas ELISA is useful for preliminary assessment.

Table 2. Sensitivity and Specificity for ELISA and RT-qPCR Techniques

	Disease No	None Disease No	Total No
Positive NO.	5 A, true positive	7 B, false positive	12
Negative NO.	12 C, false negative	26 D, true negative	38
	Sensitivity= a/a+c)×100 29.4%	Specificity=d/b+d)×100 78.7%	

The research [27] verified that, with a specificity and sensitivity of 100% and 84.5%, respectively, ELISA is greater in sensitivity but less specific than PCR in identifying Epstein Barr virus. IgG and IgM serological tests are frequently used to identify viral infections [28,29]. However, [30] discovered that due to frequent viral reactivation and variable antibody levels, this test may not offer an accurate diagnosis of infection in immunosuppressed patient populations. In their work, the researchers of [30], compared real-time PCR to a serological test for the cytomegalovirus (CMV). Real-time PCR results revealed that 32 out of 98 patients (32.7%) had CMV, which was a higher rate than the IgM result of 6.1%. This substantial discrepancy in outcomes can be attributed to real-time PCR's superior sensitivity and specificity in detecting viral DNA when compared to serological assays.

Real-time PCR testing has a number of benefits, such as fewer time-consuming processes, enhanced accuracy, quicker amplification and measurement of viral loads, and more effectiveness in detecting infections in transplant patients. Real-time PCR is also more effective than serological testing at detecting viral infections, especially in populations of immunosuppressed patients [31,32].

Detection of EBV by ELISA test and RT-qPCR according to sex

Our study demonstrates that the ELISA test for the detection of EBV antibodies in 50 renal failure patients revealed positive results for EBNA-2 IgG antibody in 5 (26.3%) from a total of 19 males and 6 (19.3%) females from a total of 31 females (Figure 3). In contrast, the results of RT-qPCR revealed that 15 (48.4%) females from a total of 31 females tested positive.

The results of our current investigation, which used the ELISA test, did not agree with those reported by [33] who observed that anti-EBV IgG prevalence was 100% (32 out

of 32) in females and 94.23% (49 out of 52) in males, but in a study [34] that took into account the RT-qPCR results, the researchers discovered a statistically significant difference in the mean EBV viral load between the hemodialysis group's male and female participants (p = 0.019). The study found that the mean EBV viral load was larger in females than in males (431.6297.0 *vs*. 39.754.1). It is significant to note that the control group did not exhibit the reported difference.

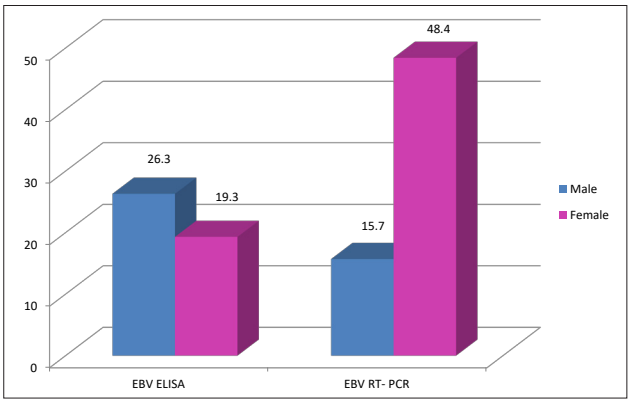

Figure 3. EBV by ELISA test and RT-qPCR according to sex

Our results did not agree with those of [16], who reported no significant differences between genders in their investigation. In the [16] investigation, 39 out of 66 samples (43.9%) and 29 out of 66 samples (38.4%) of the male and female samples, respectively, tested positive for EBV IgG. Similarly, when employing the PCR method, EBV was detected in 36 samples (54.5%) from men and 27 samples (51.9%) from women. Contrary to our findings, these data imply that there were no appreciable differences in the prevalence of EBV between males and females according to the researchers of [16].

The demographics, sample size, and underlying medical conditions of the study group may potentially have an impact on the disparities in EBV detection between males and females. Disparities in the reported prevalence of EBV among males and females can result from variations in these parameters across different studies.

CONCLUSION

The study's findings lead to the conclusion that patients undergoing hemodialysis exhibit a significantly higher prevalence of EBV antibodies across all age groups compared to the control group. Additionally, the study suggests that real-time PCR is a highly sensitive and specific method for detecting Epstein-Barr viremia in subjects with chronic kidney disease (CKD).

ORCID iDs

Zahraa Basem https://orcid.org/0009-0000-4752-1194
Huda Jameel https://orcid.org/0000-0002-0918-4278

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