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Serological detection of Epstein-Barr virus in Hemodialytic patients associated with hematological parameters

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Abstract

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Background. Epstein-Barr virus is a herpes virus member infects many people across the world. In people of strong immune status, the infection mainly became asymptomatic and is well controlled, while in immunocompromised as hemodialysis patients, the EBV can cause severe illness due to loss of kidney function. **Objective.** This study objective was to detection of presence the IgG and IgM antibodies against EBV in HD patients and the suspected connection between produced antibodies and hematological parameters (CBC). **Methods.** During the period started from November 2025 to the end of January 2026, Blood samples obtained from 270 chronic kidney disease's patients were collected. As 137 samples were taken from hemodialytic men, and 133 samples are obtained from women patients. The age group of patients who were chosen in the study varied from 20 to 70. Serum samples were examined for EBV-IgG/IgM antibodies. Whole blood using for analysis complete blood count (CBC) by automated hematology analyzers. **Results.** Levels of specific viral IgG/IgM antibodies related to EBV genome was 54.8% and 10.4% respectively. The level IgM antibodies was statistically significant and were measured higher in the age group 20-30 years to 24% than what was detected in other age groups, and lowest among those aged 51–60 years (3.4%). The availability of IgM antibodies specific for EBV indicated to their correlation with the younger age group, duration of dialysis and frequent dialysis per week. **Conclusion.** No statistical stratified difference between patients with EBV-IgG and age and frequency dialysis.

Introduction

Epstein-Barr virus or HHV-4, is one of the herpes virus family, have a large dsDNA. The typical DNA genome of EBV is containing 172 kilobase pairs (kb) and 80 specific genes (1). EBV has ability to infect 90% of individual and it is causative agents to infectious mononucleosis, staying infection asymptomatic and controlled by strong immune system in obviously healthy individual, people with a weak immune status like those undergoing hemodialysis the EBV remaining latent in B lymphocyte and the reactivated infection in host with an immunosuppression, resulted in a risk life threating illnesses (2). Epstein-Barr virus is spread through saliva, contamination objects and blood transfusions, like other herpesviruses initial infection of epithelial cells and access to peripheral B cells and remains latent in B lymphocyte (3). Epstein-Barr virus genes produce proteins that include nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B and EBNA-3C), three latent membrane proteins (LMP-1, LMP-2A and LMP-2B), and two non-coding RNAs (EBER-1 and EBER-2), these

proteins induce the virus immortality in B lymphocytes (4). Kidney failure disease occurs when the kidney unable to eliminate metabolic end products from the blood and balance the fluid, balance PH and electrolyte of extracellular fluid, final-stage kidney failure patients require hemodialysis, patients with end-stage renal failure have a probability of affecting by some opportunistic viruses like Epstein-Barr virus (5). Life cycle of EBV complex, spread via saliva and entry the tonsils where it starts the lytic phase that included the replication of virus and infection various cells types, the EBV replication included the main five stages of infection, which represented by each of primary Lytic, latent III, Latent II, and Latency I/0, in addition to the reactivated infection. Four of these types of infections are related to EBV diseases (6). Mechanisms used by Epstein-Barr virus during the latent stages including each of epigenetic machinery and the cellular signaling pathways. The two pathways to latency phase also represented by (i) a direct infection of B lymphocytes, and (ii) the viral infection transmitted via a germinal center dependent process in the naïve B cells infected with Epstein-Barr virus cross through germinal center reactions and appear as memory cells containing the virus (7). Sporadically, Epstein-Barr virus is reactivating and then enter into the lytic replication cycle. Epstein-Barr virus expresses various of proteins based on the stage of infection such as BZLF1 and BRLF1 molecules act as transactivators (8).

Methods

1. Study design

This study is a cross sectional study

2. Sample collection

Blood samples were taken from the Al-Imam Al-sadiq Teaching Hospital in the Iraq city of Babylon, between August 2025 to January 2026, and were obtained from a research group consisted of 270 patients with chronic renal failure. The following variables were covered via a documented questionnaire including each of age, sex, duration of dialysis.

3. Serological detection

Five milliliters of blood were drawn from patients who participated in the recent study, then the 3mls part were centrifuged and then the separated serum sample were kept in -20 co, and then were tested for detection of the specific IgM and IgG antibodies for EBV using the ELISA method.

4. Hematological parameters estimation

Two milliliters of whole blood were taken into a tube coated with EDTA and used to measure complete blood count (CBC) by the Automated Hematology Analyzer. The CBC tests are mainly concentrated on includes whole and diffential count of white blood cells, hemoglobin and platelet counts.

5. Inclusion criteria

All collected data taken from the targeted hemodialytic patients by questionnaire submitted to the chronic renal failure patients who were undergoing with hemodialysis and record demographic information including age, sex, duration of dialysis and frequent of dialysis per week. Additionally, diagnostic confirmation was based on ELIZA kit (qualitative) for EBV-IgG/IgM antibodies.

6. Exclusion criteria

Exclusive criteria included patients those diagnosed with autoimmune conditions, diabetes mellitus, hypertension was excluded from the study.

7. Immunological assay

All taken samples were tested by ELISA procedure, using the kit of Human Epstein-Barr virus antibody (IgG) ELISA kit (Qualitative), Human Epstein-Barr virus antibody (IgM) ELISA Kit (Qualitative) provided commercially by the campany (SunLong, China).

8. Ethical approval

This study was followed the ethical considerations established and approved by the Ethics Committee for Research of the Health Service in the health institute of Babylon Province on November 4, 2025, under ethical number 1695. All patients included in this study provided the verbal consent which declaring the acceptance in going through the comprehensive

description of the study aim. oversaw The collection of patients's samples was supervised by Specialist physicians within safe procedures.

9. Statistical analysis

All data collected for this study were entered and analyzed using the program of SPSS version 26 (IBM Corp., Armonk, NY, USA) and Microsoft Excell version 2021. Continuous variables were assessed for normality using the Shapiro-Wilk test and visual inspection of histograms. Normally distributed variables were expressed as mean ± standard deviation (SD). Non-normally distributed variables were expressed as median and range. Categorical variables were presented as frequencies and percentages. For associations between categorical variables was used Pearson Chi-square test and Fisher's exact test, when more than 20% of expected cell counts were less than 5. For continuous variables was used independent samples t-test for normally distributed variables. Mann–Whitney U test was used for non-normally distributed variables. P-value ≤ 0.05 was considered statistically significant.

Results

Out of 270 hemodialytic patients targeted in the recent study, about 54.8% were positive for EBV IgG as show in figure 1, and (10.4%) was tested positive for EBV IgM as show in figure 2.

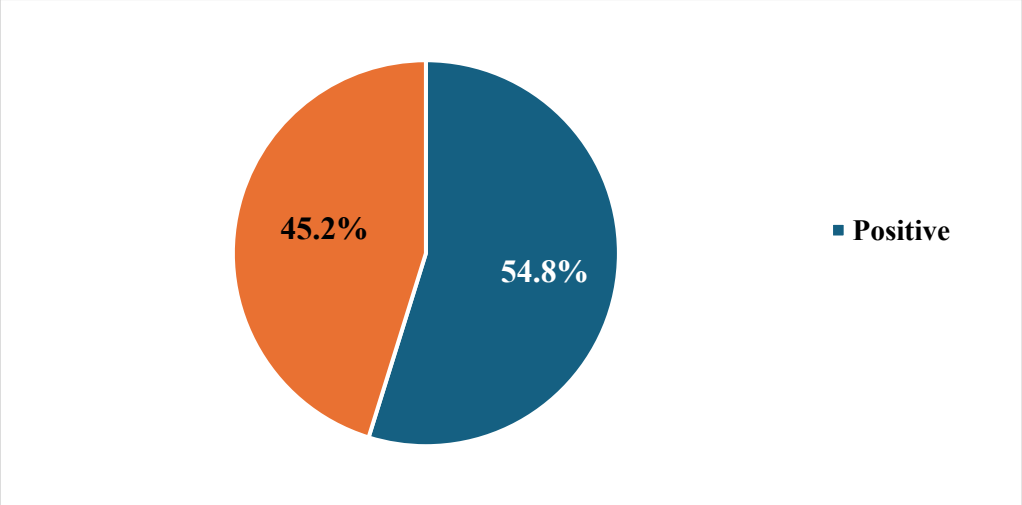

Figure 1: Overall Seroprevalence of EBV IgG Antibodies among study participants (N=270).

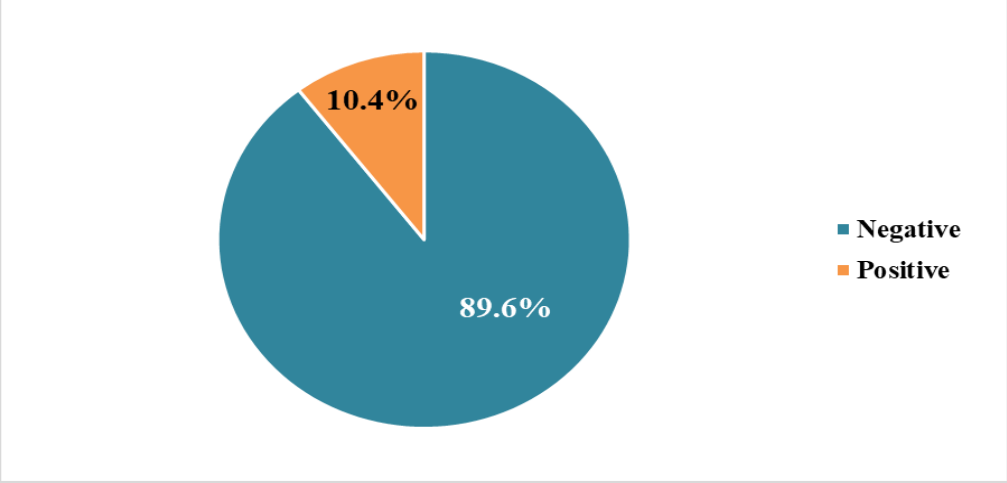

Figure 2: Overall Seroprevalence of EBV IgM Antibodies among study participants (N=270).

The majority of participants (25.2%) were in the age group 31-40 years. The mean age of participants was 45.2 ± 13.77 years. 50.7% were male and 49.3% female as shown in Table 1.

Table 1: Demographic characteristics of study sample (N=270).

Variable	Class	No.	%
Age	20-30	50	18.5
	31-40	68	25.2
	41-50	50	18.5
	51-60	58	21.5
	61-70	44	16.3
	Mean ± SD	45.2 ± 13.77	
Sex	Male	137	50.7
	Female	133	49.3

Table 2 describes the dialysis treatment characteristics of the study participants, more than half of the participants (54.1%) had been receiving dialysis for over two years. The median duration of dialysis was 36 months, with a range of 2 to 84 months. Regarding dialysis frequency, the majority of participants (71.9%) received dialysis 3 times per week. The median dialysis frequency was 3.1 ± 0.52 times.

Table 2: Distribution of study participants by Dialysis Duration and Dialysis Frequency (N=270).

Variable	Class	No.	%
Dialysis duration	<1 year	98	36.3
	1-2 years	26	9.6
	>2 years	146	54.1
	Median (range)	36 (2-84) months	
Dialysis frequency (per week)	2 times	33	12.2
	3 times	194	71.9
	4 times	43	15.9
	Mean ± SD	3.1 ± 0.52 times	

The mean of white blood cells count was 9.9 (± 5.20), with a median of 9 and a range of 2 to 29. The mean lymphocyte count was 3.8 (± 2.41), with a median of 4, ranging from 0.6 to 8.7. Hemoglobin levels showed a mean of 6.7 (±1.52), with a median of 6 and values ranging from 5 to 11. The mean platelet count was 167.3 (standard deviation = 56.04), and the median was 160, ranging from 70 to 370.

Table 3: Hematological parameters of study participants (N=270).

Parameter	Mean ± SD	Median	Minimum	Maximum

WBC **Normal value = 4 -10 × 10^9 /L**	**9.9 ± 5**	**9**	**2**	**29**
Lymphocytes **Normal value = 0.2-0.4%**	**3.8 ± 2.4**	**4**	**0.6**	**8.7**
Hemoglobin **Normal value = 11-16.5 g/dl**	**6.7 ± 1.52**	**6**	**5**	**11**
Platelets **Normal value = 100–300 × 10^9 platelets/L**	**16.3 ± 56**	**160**	**70**	**370**

Approximately half of the participants (44.4%) showed positive results for IgG and negative results for IgM. Recent or acute infection, as indicated by concurrent positive results for IgG and IgM, was observed in 10.4% of participants. No isolated IgM-positive cases were identified. In addition, 45.2% of participants were negative for both IgG and IgM antibodies as shown in Table 4.

Table 4: EBV Serology Pattern among study participants (N=270).

EBV Serology Pattern	No.	%	Interception
IgG^+ / IgM^-	120	44.4	Past EBV infection
IgG^+ / IgM^+	28	10.4	Recent / acute infection
IgG^- / IgM^+	0	0.0	Very early infection
IgG^- / IgM^-	122	45.2	No evidence of EBV infection
Total	270	100	

Table 5 showed that no statistically significant association between EBV IgG and age, sex ($p > 0.05$).

Table 5: Association Between Demographic Characteristics, and Epstein–Barr Virus IgG levels (N=270).

Variable	Class	EBV IgG		P-value
		Positive	Negative	
		No. (%)	No. (%)	
Age	**20–30**	**33 (66.0%)**	**17 (34.0%)**	**0.09**
	31–40	**38 (55.9%)**	**30 (44.1%)**	
	41–50	**22 (44.0%)**	**28 (56.0%)**	
	51–60	**27 (46.6%)**	**31 (53.4%)**	

	61-70	28 (63.6%)	16 (36.4%)	
Sex	Male	71 (51.8%)	66 (48.2%)	0.31
	Female	77 (57.9%)	56 (42.1%)	
Significant difference between percentages using Pearson Chi-square test (χ^2-test) at P-value ≤ 0.05.				

There was highly significant association between dialysis duration and EBV IgG levels at P-value <0.001and there is no association between dialysis frequency and EBV IgG levels as in Table 6.

Table 6: Association Between Dialysis Duration, Frequency and Epstein–Barr Virus IgG (N=270).

Variable	Group	IgG				P-value
		Positive		Negative		
		No.	%	No.	%	
Dialysis duration	< 1 years	7	7.1%	91	92.9%	0.001*
	1-2 years	18	69.2%	8	30.8%	
	> 2 years	123	84.2%	23	15.8%	
Dialysis frequency (per week)	2 time	13	39.4%	20	60.6%	0.12
	3 times	108	55.7%	86	44.3%	
	4 times	27	62.8%	16	37.2%	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at P-value ≤ 0.05.						

As shown in Table 7, there was a statistically significant difference in white blood cell count and lymphocyte between EBV IgG-positive and IgG-negative participants (WBC: Z = 2.68, p = 0.007; lymphocytes: Z = 13.97, p < 0.001). No statistically significant differences were observed in hemoglobin, or platelet counts (p > 0.05).

Table 7: Comparison of Hematological parameters according to EBV IgG levels (N=270).

Parameter	IgG	N	Mean Rank	Z value*	P-value
WBC Normal value = 4-10 × 10^9 /L	Positive	148	147.05	2.68	0.007*
	Negative	122	121.48		
	Total	270			
Lymphocyte Normal value = 0.2-0.4%	Positive	148	195.52	13.97	0.001*
	Negative	122	62.68		
	Total	270			

Hb **Normal value = 11-16.5 g/dl**	**Positive**	**148**	**138.11**	**0.61**	**0.53**
	Negative	**122**	**132.33**		
	Total	**270**			
Platelet **Normal value = 100–300 × 10^9 platelets/L**	**Positive**	**148**	**140.94**	**1.26**	**0.21**
	Negative	**122**	**128.90**		
	Total	**270**			

There was a statistically significant association between age group and EBV IgM positivity (P = 0.009). The highest seropositivity rate was observed among individuals aged 20–30 years, where 12 (24%) tested positive and 38 (76%) tested negative. There was no association between sex and IgM as shown in Table 8.

Table 8: Association Between Demographic Characteristics, and Epstein–Barr Virus IgM levels (N=270).

Variable	Class	EBV IgM		P-value
		Positive	Negative	
		No. (%)	No. (%)	
Age	20–30	12 (24%)	38 (76%)	0.009*
	31–40	4 (5.9%)	64 (94.1%)	
	41–50	7 (14%)	43 (86%)	
	51–60	2 (3.4%)	56 (96.6%)	
	61-70	3 (6.8%)	41 (93.2%)	
Sex	Male	14 (10.2%)	123 (89.8%)	0.93
	Female	14 (10.5%)	119 (89.5%)	
*Significant difference between percentages using Pearson Chi-square test (χ^2-test) at P-value ≤ 0.05. Fisher's exact test at P-value ≤ 0.05 used for cells have expected count less than 5.				

Table 9 show that significant association between dialysis duration, dialysis frequency and EBV IgM serostatus at P-value <0.001.

Table 9: Association Between Dialysis Duration, Dialysis Frequency and Epstein–Barr Virus IgM Serostatus (N=270)

Variable	Group	IgM				P-value
		Positive		Negative		
		No.	%	No.	%	

Dialysis duration	**< 1 years**	**0**	**0.0%**	**98**	**100.0%**	**0.001***
	1-2 years	**1**	**3.8%**	**25**	**96.2%**	
	> 2 years	**27**	**18.5%**	**119**	**81.5%**	
Dialysis frequency (per week)	**2 time**	**6**	**18.2%**	**27**	**81.8%**	**0.001***
	3 times	**10**	**5.2%**	**184**	**94.8%**	
	4 times	**12**	**27.9%**	**31**	**72.1%**	
***Significant difference between percentages using Pearson Chi-square test (χ^2-test) at P-value ≤ 0.05. Fisher's exact test at P-value ≤ 0.05 used for cells have expected count less than 5.**						

There was a statistically significant difference in lymphocyte between EBV IgM-positive and IgM-negative participants (lymphocytes: $Z = 6.15$, $p < 0.001$). No statistically significant differences were observed in WBC, hemoglobin, or platelet counts ($p > 0.05$) as shown in Table 10.

Table 10: Comparison of Hematological parameters according to EBV IgM serostatus (N=270).

Parameter	IgM	N	Mean Rank	Z value*	P-value
WBC **Normal value = 4-10 × 10^9 /L**	**Positive**	**28**	**151.86**	**1.17**	**0.24**
	Negative	**242**	**133.61**		
	Total	**270**			
Lymphocyte **Normal value = 0.2-0.4%**	**Positive**	**28**	**221.07**	**6.15**	**0.001***
	Negative	**242**	**125.60**		
	Total	**270**			
Hb **Normal value = 11-16.5 g/dl**	**Positive**	**28**	**132.36**	**0.22**	**0.82**
	Negative	**242**	**135.86**		
	Total	**270**			
Platelet **Normal value = 100–300 × 10^9 platelets/L**	**Positive**	**28**	**142.13**	**0.47**	**0.63**
	Negative	**242**	**134.73**		
	Total	**270**			

Discussion

Epstein-Barr virus is a major opportunistic viral pathogen frequently among immunocompetent and immunocompromised populations, particularly those undergoing hemodialysis. EBV high transmissibility in hemodialysis patients a trend consistent with the finding of the current study (12). The serological analysis in this study indicated that EBV-IgM antibodies were present in 10.4% of hemodialysis patients, this result agrees with the study from Kirkuk (9) that reported rate EBV-IgM was 7.6%, but disagree with study in Diyala (17) where EBV-IgM prevalence was reported at a lower rate of 4% among immunocompromised patients. While the seroprevalence of EBV-IgG reached 54.8%, this result agrees with study in Kirkuk (9) was reported rate EBV-IgG 42.8%, and study in Egypt (11) that reported rate EBV-IgG 56.3%, but disagree with study in Iran (13) where reported a 96% IgG positivity rate. The high prevalence of EBV-IgG and IgM antibodies among hemodialysis patients likely reflects both prior exposure and immunosuppressed. IgG indicates past infection and long-term dialysis. IgM reflects recent or reactivated infection that can occur due to immune impairment and frequent dialysis sessions, these factors together explain the elevated seroprevalence of EBV antibodies in this population. consequence due to a significant presence of reactivated EBV infection with a severe complication in the immunocompromised patients (19).

Epidemiologically, Epstein-Barr virus infection patterns exhibit geographical variation, in developing countries children under age 6 years' exposure typically to the infection this lead to long-term immunity. However, in industrialized nations the infection is often delayed until adolescence where around 50% of cases manifest as infectious mononucleosis (14). The current study indicated no statistically significant between sex and infection with EBV-IgG/IgM, this agree with study in Karbala (15), which reported that sex dose not significantly influence the rate of EBV infection among hemodialysis patients. On the other hand, this results disagree with the study in Denmark (16) observed a significantly higher seroprevalence in females, also in studies from Najaf (1), and Egypt (11) are reported a higher infection rate among females than male. These different between studies may be due to different in sample size, geographic distribution of patients across different regions.

The Current study observed a higher seroprevalence of EBV-IgG among all age groups patients, reach to rate of 66% in the 20-30 years age group, this results agree with study in Najaf (1) which reported 57.1% of EBV-IgG positive in age groups (17-26), and disagree with study in Kirkuk (9) reported only 33% of EBV-IgG positive in age group (20-35). The high of EBV-IgG in patients aged (20-30) years reflects prior exposure and long-term immunity, as seroconversion commonly occur during adolescence and early adulthood, persistent IgG influenced by healthcare interventions and immune modulation in this population .While observed this study rate of infection 24% in age group (20-30) for EBV-IgM, upon reviewing the previous studies, it was noted that there is a lack of published studies evaluating the of EBV-IgM antibodies among hemodialysis patients specificaliiy within the 20-30 year age groups. Most studies have investigated hemodialysis patients across broad age ranges without detailed age stratification. Consequently, the absence of comparable age specific data limits direct comparison with the current findings. This lack of targeted information highlights an important research gap and underscores the significance of the present study in providing age specific data on recent EBV-IgM infection among young hemodialysis patients.

In this study observed rate of distribution the infection in EBV-IgG/IgM increased with increase duration dialysis, in duration of dialysis more than 2 years found rate of EBV-IgG was 84.2%, and rate of EBV-IgM was 18.5%. In frequent dialysis per week (4 times) found rate of EBV-IgG was 62.8%, no statistical significantly difference between infection in EBV-IgG with frequent dialysis per week, and rate of EBV-IgM was 27.9% in patients have frequent dialysis per week (4 times). this results agree with study in kirkuk that showed the infection with EBV associated with duration dialysis and frequent dialysis per week (9). Because all patients who were undergone on hemodialysis were using the same dialysis machine, the risk of EBV transmission to these patients is at an extreme highly detected level. Therefore, it is very necessary to separate all patients who are positively detected for virus to avoid the risk of virus to be transmitted to hemodialytic patients not detected with virus. In addition, the sterile condition of hemodialysis devices should be cleaned and disinfected well after each usage time and then tested for EBV before next use for another patient, all these procedures would be followed the stringent laws (20, 21).

According to results, there was no statistically significant differences observed in hemoglobin, or platelet counts in EBV-IgG patients ($p > 0.05$), but show a statistically significant difference in WBC and lymphocyte. While in patients EBV-IgM observed a statistically significant in lymphocyte, and no statistically significant difference were observed in WBC, hemoglobin, or platelet counts ($p > 0.05$).

All participants have chronic renal failure thus were suffering from deficiency of hemoglobin align with study (23). All patients suffered anemia in hemodialysis patients and variation in platelet count. The platelet counts low slightly throughout the hemodialysis action, but mostly would be return to the baseline values at the end of hemodialysis. A high number of hemodialytic patients suffered of chronic renal failure were reported a marked low platelet count (50% or more) through hemodialysis in compared to patients with predialytic thrombocytopenia (22). In comparison to results of this study, a previous study indicated that each of WBC and platelet count were in normal value as detected in all patients of hemodialysis despite of EBV detection (23), also in study (24) platelet counts in chronic renal failure patients are normal but there is a functions change that causes chronic renal disease patients have a predisposition to bleeding. On the other hand, by using the heparin in hemodialysis patients suffered from chronic renal disease have side effects, such as bleeding and allergies, that leading to the occurrence of Heparin-Induced Thrombocytopenia. Also, chronic renal disease hemodialytic patients were requiring the periodic checkup of hemoglobin for determine anemia, and to check the leukocytes count to determine the presence of infection, as well as the platelet counts that can be normal or low and high. Total WBC counts had been reported to be increase in cases of acute renal failure and increase or normal in cases of chronic kidney disease (22). Previous research in agreement with study which found that most patients had normal or even decrease WBC. In addition, hemodialysis patients continue to vary the body's defenses WBC various counts, which can be inflammatory (24). In this study lymphocyte count show statistically significant differences between patients with EBV-IgG/IgM positive and patients non infection, in pateints with EBV was lymphocyte high but in non-infected patients was low, this result agrees with study (26) which observed EBV demonstrate a high degree of B-cell, but disagree with study (22).

Conclusions

According to the findings of this study, it is concluded that hemodialytic patients are exhibiting a s high levels of Epstein-Barr Virus-IgG antibodies in a significant difference across all age groups, no significantly statistical difference between male and female. Also no statistical significant difference according sex. EBV-IgG levels were associated to duration of dialysis, but no association between dialysis frequency and EBV-IgG. On the another hand, there was a significant association between dialysis duration, frequencies of dialysis and EBV-IgM levels. Hematological analysis revealed significantly in hemodialytic patients.

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