ORIGINAL ARTICLE

Study of Epstein Barr Virus Infection in Kidney Transplant Recipients: One Egyptian Center Study

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ABSTRACT

Background: *Epstein–Barr virus (EBV) is the causative agent of infectious mononucleosis (IM). EBV infection is common in renal transplant recipients, due to the use of immunosuppressive drugs, and may lead to post-transplant lymphoproliferative disorders (PTLD). Thus, EBV accounts for high morbidity and mortality rates in solid organ transplant recipients.* ***Objectives:*** *The aim of this study was to examine the prevalence of EBV infection among Egyptian kidney transplant recipients and the relation between different immunosuppressive regimens, and EBV infection.* ***Methodology****: A total of one hundred kidney transplant recipients were included in this study (50 recent transplants and 50 late transplants). All cases were subjected to pre- and post-transplant evaluation, and post-operative immunosuppressive therapy. EBV infection was identified by serological testing for anti-EBV viral capsid antigen (VCA) IgG and IgM, and by molecular detection of Bacillus amyloliquefaciens H one (BamHI) region using conventional polymerase chain reaction (PCR).* ***Results:*** *BamHI region detection showed significant difference between the recent and late transplant groups (higher in late group) and was significantly associated with EBV VCA IgM; positive BamHI has 5.1 times higher odds of exhibiting positive IgM adjusted for the date of transplantation. Neither VCA IgM nor IgG were significantly different between the two studied groups.* ***Conclusion:*** *Despite the lack of association between EBV and immunosuppression drugs, hematological abnormalities, or graft function in this study, EBV monitoring in renal transplant recipients is required for early diagnosis of EBV infections and prevention of PTLD development. Serological diagnosis for EBV is of clinical importance as a simple, inexpensive tool for screening and follow-up of EBV infection. Molecular diagnosis of EBV by BamHI fragment detection had 5.1 times higher odds to exhibit positive IgM adjusted for the date of transplantation.*

INTRODUCTION

Epstein-Barr virus (EBV), a DNA herpesvirus, is recognized mainly as the etiological agent of infectious mononucleosis (IM). EBV primarily targets B lymphocytes, hence known as lymphotropic virus. There is a high prevalence of EBV among the population worldwide, commonly in the form of latent asymptomatic infection [1]. Epidemiological data suggest that EBV is expected to be positive in more than 90% of world population. Primary EBV infection occurs during early childhood and is asymptomatic. However, EBV infection in adults may lead to IM. In developing countries, most children become positive for EBV at the age of 5 years, but this primary infection is delayed in developed countries with high socioeconomic standards[2].

The principal mode of transmission of EBV is via oral route, mainly through saliva containing infected epithelial cells. Infection may also be transmitted via blood and body fluids by means of blood transfusion, organ transplantation, and sharing infected personal objects [3]. EBV infection after organ transplantation may occur by the transfer of seropositive donor leukocytes during organ donation or by exposure to EBV infection in immunocompetent individuals [4].

EBV serological testing is regularly used as a diagnostic test to evaluate viral infection status, but the immunosuppressive therapy administered to transplant recipients affects the interpretation of this test due to the alteration or inhibition of the immune response. Therefore, molecular techniques are used for quantification of EBV DNA, and continuous monitoring of the viral load after transplantation [5].

Solid organ transplantation (SOT), particularly renal transplantation, is the ultimate treatment for end stage renal disease. After transplantation, life-long immunosuppression is indicated to prevent immune-mediated allograft rejection. Such immunosuppression may lead to potentially fatal complications with a high risk of infection by BK virus (BKV), EBV and cytomegalovirus (CMV) [6]. Subclinical EBV infection occurs in more than one third of renal transplant recipients and may lead to development of post-transplant lymphoproliferative disorders (PTLD) in 1–5% of cases. Therefore, EBV infections play a major role in the high morbidity and mortality rate in SOT recipients [7].

In this study, we examined the prevalence of EBV infection among Egyptian kidney transplant recipients using two serological markers; VCA IgM and VCA IgG antibodies, and a molecular genetic marker; Bacillus amyloliquefaciens H one (BamHI) gene fragment. Also, we studied the relationship between different immunosuppressive regimens, and the occurrence of EBV infection.

METHODOLOGY

The sample size was calculated based on prevalence of EBV infection among renal transplant recipients from a previous study [8] using the G. power program with a margin of error = 5% and a confidence level of 95%.

A total number of one hundred kidney transplant recipients were included in this study. They were divided into two equal groups: group A included 50 recent transplant recipients (first year post-transplant) and group B included 50 late transplant recipients (more than 5 years up to 10 years post-transplant). All participants were selected from the Transplantation Department, Urology and Nephrology Center, Mansoura University, Egypt, from January 2021 to February 2022. The study was carried out at molecular biology laboratory of Urology and Nephrology center, Mansoura University. All selected cases were first-time kidney transplants, and had no previous malignancy, and no other organ transplantation. The study ethical review was performed according to Helsinki standards and written informed consent was obtained from each participant. Ethical approval was obtained from Ethical Committee of Mansoura Faculty of Medicine (R.24.03.2553), Egypt.

All participants in both groups (recent transplants and late transplants) were subjected to the followings:

Pre-transplant evaluation

The following data were collected: age, sex, previous blood transfusion, pretransplant hemodialysis, chronic comorbidities as diabetes mellitus, HLA-DR, HLA-A and HLA-B typing and previous exposure to any viral infections as CMV and Hepatitis C virus (HCV).

Post-transplant evaluation

This included the date of transplantation, full laboratory investigations including complete blood count (CBC), differential leucocytic count, serum creatinine, fasting blood sugar (FBS), estimated glomerular filtration rate (eGFR) using CKD EPI 2021 equation and MDRD equation, protocol of primary and secondary immunosuppression drugs, and histopathological examination of the graft biopsy in cases of graft dysfunction according to Banff classification[9] for diagnosis of renal allograft rejection.

Post-operative immunosuppression

Following transplantation, patients received induction therapy by Basilixmab, and only patients with high immunologic risk (patients with pre-transplant anti HLA antibodies class I and class II more than 10%) received anti-thymocyte globulin (ATG). Then, patients were maintained on maintenance protocol either double-(steroid-free) immunosuppressive therapy for low-risk patients or triple (steroid-based) immunosuppressive therapy consisting of a calcineurin inhibitor (CNI: cyclosporine A [CsA] or tacrolimus), a proliferation inhibitor (mycophenolate mofetil; MMF) and a steroid (methylprednisolone).

EBV laboratory studies

Serological evaluation:

Detection of EBV antibodies in the serum of all participants in both groups using commercial quantitative DRG EBV-VCA IgG (lot,110G/K100) and EBV-VCA IgM (lot,110M/K031) ELISA kits (DRG International, Inc., USA). Measuring viral capsid antigen (VCA) antibodies IgM and IgG by a solid phase enzyme linked immunosorbent assay (ELISA) was performed according to kit instructions.

Molecular detection of EBV DNA:

Genomic DNA was extracted from whole blood obtained from EDTA-treated samples by using QIAamp DSP Virus Spin Kit (61704) according to Kit instructions, extracts were stored at -80°C up to time of amplification. PCR was performed to amplify 175-bp fragment of the EBV BamHI region, the primers used were forward: 5'-AACATGCTGTATGCCTCGCAGCG- 3' and reverse: 5'-AATTACTGGCGTGAATTGTGCCCA- 3'[10]. The PCR reactions were made using 200 ng of DNA template in a total volume of 25µl [11]. As the reaction mixtures contained 1 U of Dream Taq polymerase (01026116), 1 PCR buffer, 0.25 mM of each dNTP and 0.2 mM of each primer. Thermal cycle settings were an initial denaturation step at 95°C for 5 min., followed by 25 cycles composed of denaturation 95°C, annealing at 55°C and extension at 72 °C, each for 30 seconds then the final extension step for 10 min. The amplification was done using a Thermal cycler (Gene Amp PCR System 9700, Applied Biosystems, USA). The

amplified DNA were separated by electrophoresis in 2% agarose gel stained with ethidium bromide, then the products were visualized in UV trans-illuminator. Samples with a single 175-bp band were recognized as positive samples.

Statistical analysis

Data was recorded and analyzed using IBM-SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. (Armonk, NY: IBM Corp). Qualitative data were expressed as number (N) and percentage (%), while quantitative data were expressed as median and interquartile range. The Chi-square test was used to test the association between two nominal variables and the expected count in all cells was ≥ 5, otherwise Fisher's exact test was used. The strength of association was examined by phi (φ) value. The Mann-Whitney U-test was used to compare non-normally distributed quantitative data between two groups. Binary logistic regression was used to ascertain the effect of predictor variables on the likelihood of occurrence in univariate analysis each predictor variable was tested alone, while in multivariate analysis all predictor variables were tested together.

RESULTS

A total of one hundred kidney transplant recipients were enrolled in this study. They were divided into two groups according to the time of transplantation; group A included 50 recent transplant (recent Tx) recipients (first year post-transplant), and group B included 50 late transplant (late Tx) recipients (more than 5 years up to 10 years post-transplant). The demographic and clinical characteristics of patients are listed in Table 1. There was a significant difference in age between recent and late transplant groups, with a higher proportion of adults in the recent transplant group (Table 1). Although there was no significant difference in the distribution of sex, and the degree of HLA-DRB1 matching between recipient and donor, our results showed a statistically significant difference in HLA-A and HLA-B matching (P=0.009) between the two studied groups. Other risk factors did not significantly differ between recent and late transplant groups, except for CMV and HCV infection (Table 1).

Also, while different protocols of immunosuppression and number of rejection episodes did not significantly vary, renal Banff classification showed statistically significant difference between recent and late transplant groups (Table 1).

Comparing hematologic abnormalities and graft function tests between recent and late transplant groups identified leukocytosis (P<0.001) and lymphocytosis (P=0.004) as the only two parameters that showed a statistically significant difference between the two studied groups (Table 1).

Table 1: Patient demographic and clinical characteristics in recent and late transplant groups

Parameter	RECENT TX	LATE TX	TOTAL	P VALUE
*Age (in years):	25 (17-35)	18 (14-28)	23 (16-30)	**0.041**
Pediatric	15 (30%)	25 (50%)	40 (40%)	**0.041**
Adult	35 (70%)	25 (50%)	60 (60%)	
Sex:				0.529
Male	34 (68%)	31 (62%)	65 (65%)	
Female	16 (32%)	19 (38%)	35 (35%)	
HLA-A & HLA-B matching:				**0.009**
0%	3 (6%)	15 (30%)	18 (18%)	
25%	3 (6%)	5 (10%)	8 (8%)	
50%	32 (64%)	19 (38%)	51 (51%)	
75%	9 (18%)	6 (12%)	15 (15%)	
100%	3 (6%)	5 (10%)	8 (8%)	
HLA DRB1 matching:				0.424
50%	43 (86%)	40 (80%)	83 (83%)	
100%	7 (14%)	10 (20%)	17 (17%)	
Risk Factors:				
Blood transfusion	15 (30%)	16 (32%)	31 (31%)	0.829
Pretransplant hemodialysis	42 (84%)	40 (80%)	82 (82%)	0.603
Diabetes	1 (2%)	2 (4%)	3 (3%)	1.000
CMV infection	18 (36%)	9 (18%)	27 (27%)	**0.043**
HCV infection	4 (8%)	1 (2%)	5 (5%)	**0.043**
Induction Immunosuppression Protocol				
Basilxumab	48(96%)	50(100%)	98(98%)	0.495
ATG	2(4%)	0(0%)	2(2%)	
Maintenance Immunosuppression Protocol				

Parameter	RECENT TX	LATE TX	TOTAL	P VALUE
Steroid-based regimen	20(40%)	19(38%)	39(39%)	1.000
Steroid-free regimen	30(60%)	31(62%)	61(61%)	
Histopathological findings in recipients subjected to graft biopsy				
Borderline change	5(71.4%)	2(28.6%)	7(7%)	**<0.001**
Acute cellular rejection	5(16.7%)	25(83.3%)	30(30%)	
Antibody-mediated rejection	2(66.7%)	1(33.3%)	3(3%)	
Chronic-allograft nephropathy	11(78.6%)	3(21.4%)	14(14%)	
Number of rejection episodes				
Zero	46 (92%)	44 (88%)	90 (90%)	0.487
1	3 (6%)	6 (12%)	9 (9%)	
≥ 2	1 (2%)	0 (0%)	1 (1%)	
Hematological abnormalities and graft function				
Anemia* (<13.5gm/dl in male & <12gm/dl in females)	32 (64%)	27 (54%)	59 (59%)	0.309
Leukocytosis* (WBCs >11000 /µl)	8(16%)	27 (54%)	35 (35%)	**<0.001**
Lymphocytosis	11 (22%)	26 (52%)	37 (37%)	**0.004**
Thrombocytopenia	2 (4%)	2 (4%)	4 (4%)	0.502
Serum creatinine(mg/dl)	1.2 (1-1.5)	1.4 (0.9-1.8)	1.3 (1-1.7)	0.341
eGFR (CKD-EPI 2021)	62 (83-109)	77 (55-116)	79 (57-113)	0.608
eGFR (MDRD)	65 (82-107)	77 (55-115)	79 (57-111)	0.677
Creatinine clearance	61 (55-66)	60 (45-90)	61 (50-82)	0.872

Assessment of the viral markers demonstrated that both serologic markers EBV VCA IgG (P=0.461) and EBV VCA IgM (P=0.683) did not significantly differ between the recent and late transplant groups (Table 2). In contrast, there was a significant difference between the two groups in BamHI gene (P=0.027) (Table 2).

Table 2: Serological and molecular EBV markers in the study groups

Parameter	Recent Tx	Late Tx	Total	P value
EBV VCA IgG				
Positive	38 (76%)	41 (82%)	79 (79%)	0.461
Negative	12 (14%)	9 (18%)	21 (21%)	
EBV VCA IgM				
Positive	29 (58%)	31 (62%)	60 (60%)	0.683
Negative	21 (42%)	19 (38%)	40 (40%)	
BamHI by conventional PCR				
Positive	6(12%)	15(30%)	21(21%)	**0.027**
Negative	44(88%)	35(70%)	79(79%)	

The association between EBV molecular (BamHI gene) and serological markers (EBV VCA IgG and IgM) was examined (Table 3). There was no significant association between BamHI gene and EBV VCA IgG (P=0.551). However, the analysis showed a significant association of low strength (P=0.027, phi [φ] = 0.221) between BamHI gene and EBV VCA IgM. In addition, the only statistically significant independent predictor of positive IgM was positive BamHI, where positive BamHI had 5.1 times higher odds to exhibit positive IgM adjusted for the groups.

Table 3: Association of BamHI gene with EBV serological markers

BamHI gene and EBV VCA					
		EBV VCA IgG			
		Negative	***Positive***	**Total**	**P value**
BamHI gene	***Negative***	18 (85.7%)	61 (77.2%)	79 (79%)	0.551
	Positive	3 (14.3%)	18 (22.8%)	21 (21%)	
		EBV VCA IgM			
		Negative	***Positive***	**Total**	**P value**
BamHI gene	***Negative***	36 (90%)	43 (71.7%)	79 (79%)	**0.027**
	Positive	4 (10%)	17 (28.3%)	21 (21%)	

Predictors of Positive IgM				
Predictors	**Univariate**		**Multivariate**	
	P	**COR (95% CI)**	**P**	**AOR (95% CI)**
Groups: Recent Tx Late Tx	0.683	r (1) 1.2 (0.53-2.6)	0.716	r (1) 0.85 (0.35-2)
BamHI: Positive Negative	**0.034**	r (1) 3.6 (1.1-11.5)	**0.027**	r (1) 5.1 (1.2-22)

Finally, there was statistically significant correlation between the impact of EBV positive cases and the different immunosuppression protocols (either induction or maintenance protocols), histopathological findings in recipients subjected to graft biopsy, number of rejections episodes, hematological abnormalities and graft function among the two studied recent- and late-transplant groups (Table 4).

Table 4: Correlation between different EBV risk factors and the impact of EBV diagnosis on hematological abnormalities and graft function among the two study groups

Parameter	**Recent Tx**			**Late Tx**		
	Positive EBV	**Negative EBV**	**P**	**Positive EBV**	**Negative EBV**	**P**
Age: *Pediatric* *Adult*	 26 (20-36) 7 (46.7%) 23(65.7%)	 23(14-31) 8(53.3%) 12(34.3%)	 0.172	 20(15-25) 15(60%) 19(76%)	 17(14-32) 10(40%) 6(24%)	 0.182
Sex: • *Male* • *Female*	 20(58.8%) 10(62.5%)	 14(41.2%) 6(37.5%)	0.528	 23(74.2%) 11(57.9%)	 8(25.8%) 8(42.1%)	0.187
HLA-A & HLA-B matching: *Zero%* *25%* *50%* *75%* *100%*	 2(66.7%) 2(66.7%) 20(52.5%) 5(55.6) 1(33.3%)	 1(33.3%) 1(33.3%) 12(37.5%) 4(44.4%) 2(66.7%)	0.885	 9(60%) 2(40%) 16(84.2%) 4(66.7%) 3(60%)	 6(40%) 3(60%) 3(15.8%) 2(33.3%) 2(40%)	0.321
HLA DRB1 matching: *50%* *100%*	 25(58.1%) 5(71.4%)	 18(41.9%) 2(28.6%)	0.410	 27(67.5%) 7(70%)	 13(32.5%) 3(30%)	0.600
Risk Factors: *Blood transfusion* *Pretransplant hemodialysis* *Diabetes* *CMV infection* *HCV infection*	 13(86.7%) 29(69%) 0(0%) 13(72.2%) 2(50%)	 2(13.3%) 13(31%) 1(100%) 5(27.8%) 2(50%)	 0.060 0.505 0.400 0.153 0.528	 10(62.5%) 28(70%) 2(100%) 4(44.4%) 1(100%)	 6(37.5%) 12(30%) 0(0%) 5(55.6%) 0(0%)	 0.398 0.400 0.458 0.103 0.680

Parameter	Recent Tx			Late Tx		
	Positive EBV	**Negative EBV**	**P**	**Positive EBV**	**Negative EBV**	**P**
Induction Immunosuppression Protocols			0.645			------
Basiliximab	29(60.4%)	19(39.6%)		34(68%)	16(32%)	
ATG	1(50%)	1(50%)		0(0%)	0(0%)	
Maintenance Immunosuppression Protocols			0.386			
Steroid based regimen	13(65%)	7(35%)		12(63.2%)	7(36.8%)	0.394
Steroid free regimen	17(56.7%)	13(43.3%)		22(71%)	9(29%)	
Graft histopathology			0.859			0.460
Borderline change	3(60%)	2(40%)		2(100%)	0(0%)	
Acute cellular rejection	4(80%)	1(20%)		17(68%)	8(32%)	
Antibody mediated rejection	1(50%)	1(50%)		1(100%)	0(0%)	
Chronic allograft nephropathy	7(63.3%)	4(36.4%)		3(100%)	0(0%)	
Number of rejections:			0.686			0.635
Zero	27(58.7%)	19(41.3%)		30(68.2%)	14(31.8%)	
1	2(66.7%)	1(33.3%)		4(66.7%)	2(33.3%)	
≥2	1(100%)	0(0%)		0(0%)	0(0%)	
Hematological abnormalities:						
Anemia (Hg <13.5gm/dl in male & <12gm/dl in females)	12.4 (11.3-13)	12.1 (10.9-12.4)	0.154	12.6 (11.1-14.5)	11.9 (11.3-13.5)	0.236
Leukocytosis (WBCs >11000 /µl)	4 (50%)	4 (50%)	0.697	20 (74.1%)	7(25.9%)	0.244
Lymphocytosis	4(36.4%)	7(63.6%)	0.171	18(69.2%)	8(30.8%)	0.338
Thrombocytopenia	1(50%)	1(50%)	0.536	1(50%)	1(50%)	0.728
Graft function:						
Serum creatinine	1.3(1-1.7)	1.1(0.9-1.3)	0.119	1.4(1.1-1.9)	1.1(0.8-1.7)	0.160
eGFR (CKD-EPI 2021)	75.6 (50.8-99)	88.9 (75.9-123.7)	0.065	72.4 (54.8-94.1)	101.9 (59.1-128.1)	0.236
eGFR (MDRD)	75.9(51.1-97)	86.3 (76.4-121.5)	0.059	73.3 (54.8-92.8)	99.4 (59.8-125.6)	0.220
Creatinine clearance	63 (53.5-66.7)	61 (56-66)	0.968	60.5 (44.7-89.2)	61 (47-94)	0.779

The frequency of concordance results for the markers of EBV diagnosis by VCA IgG, VCA IgM and BamHI gene were plotted as a Venn diagram (Figure 1). Fourteen cases (n=14) were triple positive diagnosis (VCA IgG, VCA IgM and BamHI), thirty-nine cases (n=39) were double VCA IgG and VCA IgM, three cases (n=3) were double VCA IgM and BamHI, three cases (n=3) were double positive VCA IgG and BamHI. In addition, twenty-two cases (n=22) were VCA IgG only, five cases (n=5) were VCA IgM only and one case (n=1) was BamHI only.

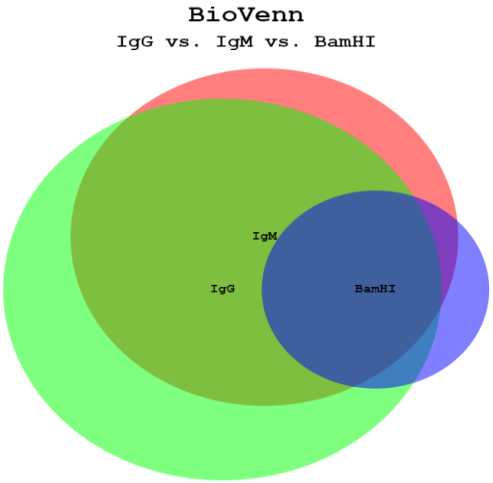

Fig. 1: Venn diagram showing the frequency of concordance results for the markers of EBV diagnosis by VCA IgG, VCA IgM and BamHI gene.

DISCUSSION

Viral infection is one of the potentially fatal complications that may occur following solid organ transplantation, particularly renal transplantation. The immunosuppressive therapy used post-transplantation increases the risk of contracting viral infections, especially EBV, BKV and CMV. A major complication of EBV infection following renal transplantation is the development of PTLD, a severe complication occurring in about 1% of patients mainly after one year post transplant [12].

In Egypt, previous studies have examined EBV infection in breast cancer, Burkitt's lymphoma, acute lymphoblastic leukemia, multiple sclerosis and nasopharyngeal carcinoma[13, 14]. However, none of these studies has evaluated the occurrence of EBV post-organ transplantation in general or post-kidney transplantation in particular.

One of the important factors in contracting infections is the time of transplantation. During the first month post-transplantation, most complications are related to surgery. While after 6 months post-transplantation, there is high risk for viral infections, such as EBV and CMV, because of the post-transplant immunosuppressive drugs [15].

Similar to our center's protocol, most immunosuppressive protocols depend on the combination of more than one agent with different actions. They aim to reduce the incidence of early rejection, prolonging the duration of graft survival and decreasing the side effects. Almost half of transplant recipients received basiliximab as induction therapy. Steroid-free regimen was used when the induction protocol was a lymphocyte depleting antibody as ATG or Alemtuzumab. Most centers use maintenance immunosuppressive regimens composed of tacrolimus, MMF and steroids. In case of allograft dysfunction whether acute or chronic, graft biopsy is the gold standard to establish an accurate diagnosis [16].

Serological diagnosis of EBV by detection of EBV antibodies is used to assess the infection status. VCA IgG antibodies appear at acute infection with the onset of the disease, and remain positive for life, whereas VCA IgM antibodies appear with VCA IgG and disappear after few weeks [5]. In this study VCA IgG antibodies were positive in 38 cases (76%) in the recent transplant group and in 41 cases (82%) in late transplant group. Meanwhile, VCA IgM antibodies were positive in 29 cases (58%) in the recent transplant group and in 31 cases (62%) in late group.

Previous studies have examined the diagnosis of EBV infection after renal transplantation by serologic testing for EBV antibodies. Byrne et al. [17] found that VCA IgM and VCA IgG antibodies were negative at time of transplantation, then became positive at 5 months and peaked up at 9 months post-transplantation. Heldman et al.[18] reported that 93% of cases were positive for VCA IgG. Also, Beader et al.[19] demonstrated that 91.4% of cases were positive for VCA IgG while 9.0% were positive for VCA IgM indicating acute EBV infection.

Nevertheless, antibody detection is unreliable in immunocompromised patients as in case of transplant recipients, due to the impairment of their immune system resulting from the immunosuppressive drug therapy. Thus, the use of molecular techniques for diagnosis of EBV by the detection of EBV DNA such as BamHI[5]. BamHI is expressed in EBV lytic phase to help cell survival which enhances sensitive detection of EBV as it targets the repeat region of the viral genome. Molecular detection of EBV by PCR is affected by the type of sample and the time of collection [20]. In this study, BamHI gene was positive in 6 cases (12%) in the recent transplant group and in 15 cases (30%) in the late group.

Previous studies have emphasized the role of molecular diagnosis of EBV in renal transplant recipients. Braz-Silva et al. [21] found that 80% of cases were identified as positive EBV by PCR detection of BamHI in their buccal mucosa. Moreover, Chan et al. [22] demonstrated that EBV DNA detection by targeting BamHI had a sensitivity and specificity of 63% and 95%, respectively). In contrast, serological diagnosis of EBV by VCA IgM and IgG antibodies had poor sensitivity and specificity (54% and 57%, respectively). In addition, Lay et al. [23] examined the correlation between EBV DNA viral load directed toward BamHI fragement and serological diagnosis. They reported BamHI fragement in 21.6% of cases, and weak correlation between VCA IgG antibodies and BamHI DNA load.

Also, VCA IgM antibodies began to appear alone in patients' serum from the first day of infection and persisted for 2-3 months (indicating acute infection) before declining. Meanwhile, molecular EBV DNA started to increase within 2 weeks of primary infection then declined when kept under the control of immune system. The co-incidence of serological VCA IgM antibodies and molecular EBV DNA ranged from 2 weeks to approximately 100 days [1]. In another study by She et al. [24], only 70% of documented VCA IgM cases were EBV DNA positive, therefore serological testing should be considered as an initial diagnosis and molecular assay by PCR is of great significance particularly in immunocompromised patients. Depending on these criteria for EBV-related diseases, EBV infection was identified by positive VCA IgM and/or positive EBV-DNA in peripheral blood [25].

In agreement with our findings, previous studies by Sobouti et al. and You et al. [26, 27] reported no association between positive EBV and transplant recipients' gender, original kidney disease, initial medications, and the type of donor. In contrast, Beader et al.[19] reported that VCA

IgG had higher association with adult female cases, but no significant association between VCA IgM and gender. Also, Shams-Aldein et al. [28] demonstrated an association with males than females (gender), and with late transplantation (duration of transplantation). Moreover, Laurent et al. [29] suggested an increased risk for EBV infection with age <5 years, ≥5 HLA mismatches, graft from a deceased donor, EBV-seronegative status of the recipient at the time of transplantation.

Regarding blood transfusion, in contrast to our data, Beader et al. [19] demonstrated that EBV is highly prevalent among hemodialysis patients (97.7%), mainly adults (95.9%). Similar to our findings, Naraqi et al. [30] reported that incidence of EBV infection in hemodialysis patients was not greater than before dialysis. Despite the lack of data on the impact of diabetes on EBV infection, a study by Dworzanski et al. [31] reported a high incidence of EBV infection (35.9%) in diabetic patients. Like our study, Bamoulid et al. [32] reported that the incidence of EBV in HBV and HCV cases was 1.63% and 17.39%, respectively, but no statistically significant difference was recorded. On the other hand, Blazquez-Navarro et al. [12] demonstrated a significant association between CMV and EBV infection.

Hocker et al.[33] found that type of CNI did not influence the incidence of EBV infection and no difference between patients received basiliximab or not. In contrast to our study, Blazquez-Navarro et al. and Bamoulid et al. [12, 32] reported that EBV positive cases were significantly associated with ATG and rapid steroid withdrawal, while the lowest EBV positive prevalence was documented with basiliximab and rapid steroid withdrawal. Also, Morton et al. [34] reported an association between low viral DNA levels and the use of MMF. In addition, Shams-Aldein et al. [28] revealed a significant association between EBV positive cases and CsA-based regimen. However, Hocker et al. [33] reported that the rate of biopsy-proven acute rejections was not significantly different in EBV positive and negative cases. Also, Kotton et al. [35] showed that EBV infection resulted in a mononucleosis-like syndrome presented with lymphocytosis due to the high doses of immunosuppressive drugs.

Similar to this study, Morton et al.[34] did not find any significant association between EBV DNA and the graft outcome, eGFR and rate of kidney function. In contrast, Shams-Aldein et al.[28] reported a strong association between EBV positive cases and renal impairment where transplant recipients with abnormally elevated serum creatinine were 20-times more liable to develop EBV infection.

Interestingly, conflicting results are common among different studies. Such discrepancies in the reported findings may be attributed to many factors including differences in genetic and ethnic background, clinical characteristics, sample size, and environmental factors. Nevertheless, this study provides valuable data on the prevalence of EBV infection among Egyptian live donor renal transplant recipients using molecular assay for more accurate detection, in addition to serological markers. However, the current study had few limitations including the need for pre-transplant evaluation of EBV state, to differentiate between primary infection and EBV reactivation, as well as post transplantation serial monitoring of EBV infection. Also, positive cases by either VCA IgM or EBV DNA need to be quantitated by qRT-PCR to monitor viral load.

CONCLUSIONS

Although examining the risk factors for development of EBV infection, recipients' immunosuppressive protocols and its impact on hematological abnormalities and graft function in this study revealed no association, continuous EBV monitoring in renal transplant recipients was imperative for early diagnosis of EBV infections and prevention of PTLD development as EBV infection in both recent and late transplantation groups was detected by both serological and molecular assays. Serological diagnosis of EBV infection was a valuable simple and inexpensive tool for screening and follow up of EBV infection. Meanwhile, BamHI fragment detection by molecular techniques had 5.1 times higher odds to exhibit positive IgM adjusted for the date of transplantation.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

Declaration

Ethics approval and consent to participate.

All methods were performed by the ethical standards as laid down in the Declaration of Helsinki and its later amendments or comparable ethical standards. The ethical approval of the study was obtained from the ethical committee of Mansoura Faculty of Medicine (R.24.03.2553) and written informed consent was obtained from each participant.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed during the current study are available in the fig share repository at https://doi.org/10.6084/m9.figshare.25465846.v1

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Authors' contributions

Mary S. Karras shared in the laboratory study the draft preparation of the article, data analysis of the study, and revision of the draft. Noha El Mashad shared in the

laboratory study, the draft preparation of the article, data analysis of the study and revision of the draft of the article Ayman Refaie shared in the clinical data collection, and draft preparation of the article. Mohamed Mofreh shared in the laboratory study, the draft preparation of the article, and data analysis of the study. Dina F. Badr shared in the laboratory study and draft preparation of the article. Hazem H. Salah shared in the laboratory study and draft preparation of the article. ' Raghda W. Magar shared in the laboratory study and draft preparation of the article. Mayssa Elsayeed Zaki shared in the laboratory study and draft preparation of the article. Mohamed Anies Rizk shared in the laboratory study and draft preparation of the article. All authors have read and approved the final manuscript.

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