Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

Detection of Epstein Barr Virus in Renal Transplant Recipients: Two Centers Study

Sahar A. Shams-aldein[1] *MSc*, **Ahmed S. Abdlameer**[1] *PhD*, **Asmaa B. Al-Obaidi**[1] *PhD*, **Haider S. Kadhim**[1] *PhD*, **Ali J.Al-Saedi**[2] *CABM, FICMS*

[1]Dept. of Medical Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq. [2]Dept. of Medicine, College of Medicine, Baghdad University, Chief of Nephrology and Transplsnt Center Medical City Baghdad, Iraq

Abstract

Background Viruses are among the most common causes of opportunistic infections after transplantation. The risk for viral infection is a function of the specific virus encountered and the intensity of immune suppression used to prevent graft rejection.Epstein-Barr virus infection has also been implicated as co-factor in acute and chronic rejection syndromes.

Objective Detection of Epstein-Barr viremia in renal transplant recipients.

Methods Fifty seven (57) renal transplant recipients were enrolled in this study. Plasma samples were taken from all renal transplant subjects. Screening of Epstein-Barr virus was first done by serology viamono spot test, then, viral DNA of Epstein-Barr viruswas extracted from 200 µl plasma samples and Epstein-Barr virus DNA was detected and measured by Taqman quantitative real-time PCR.

Results 19/57 (33 %) of renal transplant subjects had Epstein-Barr virus viremia and the viral load ranged from 7100 to 16.165 copies/ml. Serology of all RT subjects showed negative heterophil antibody except for one patient had positive hetrophil antibody.

Conclusion The current study showed that Epstein-Barr virus might be considered as an important cause of renal impairment and allograft loss in renaltransplant subjects. And Epstein-Barr virus seems associated with post transplantation renal impairment and/or kidney rejection. Real-time PCR is a very sensitive and specific method for the detection of Epstein-Barr viremia in renal transplant subjects.

Key words Epstein-Barr virus, Renal transplantation, real-time PCR

List of abbreviation: EBV = Epstein-Barr virus, PTLD = post-transplant lymphoproliferative disease, RT = renal transplant, CSA = cyclosporine A, MMF = mycophenolate, TAC = tacrolimus.

Introduction

Epstein-Barr virus (EBV) is a double stranded DNA virus belonging to the family of herpes viruses. EBV causes a disease that can be intensified by the immunosuppressive agents used to prevent rejection of the allograft [1,2]. The virus persists long-term as a latent infection. EBV is capable of driving B cell proliferation *in vitro* to form immortalized cell lines and also *in vivo* when immune surveillance is inadequate [3,4].

In the setting of allogeneic transplantation when iatrogenic immunosuppressant is used to prevent graft rejection, an unintended consequence is failure to suppress active EBV infection, which is accompanied by a heightened risk of developing Post-transplant lymphoproliferative disease (PTLD) [5-7].

An EBV-negative renal transplant (RT) from an EBV-positive donor is at increased risk for developing PTLD [8]. EBV is one of the most prevalent viral infections of early reactivation occurring from the first week after the initiation of immunosuppressive therapy, suggesting that EBV reactivation may induce a

T cell response through the phenomenon of allo-cross-reactivity which could play a critical role in graft rejection [9].

The Kidney Disease Improving Global Outcomes (KDIGO) Transplant Work Group recommends that high-risk renal transplant patients should be tested for EBV nucleic acid once within the first week after transplant then at least monthly for 3 to 6 months, and then every 3 months for the rest of the first year. Additional EBV testing is recommended after treatment for acute rejection [10].

In Iraq, active kidney transplantation program was started in 1973 at Al-Rasheed Military Hospital; and since then, renal transplantation is being successfully done at several centers in Iraq [11-13]. Very few studies are conducted for the detection of viral infections or reactivation in Iraqi RT recipients using real time PCR [14,15], or urine cytology [16], however, to the best of our knowledge, this study is the first to investigate the incidence and the role of EBV viremia in RT subjects and its relationship to kidney impairment using quantitative RT-PCR.

Methods

Renal transplant subjects and blood sampling

This cross-sectional study was conducted from November 2013 to March 2014. A total of 57 RT recipients (including 42 males and 15 females) who attended the (Center of Kidney Diseases and Transplantation) in the Medical City of Baghdad and Al-Karama Teaching Hospital, were enrolled in the study. A consent letter was signed by each patient, and the study was approved by the ethical committees of the Ministry of Health and Al-Nahrain University.

The mean age of RT subjects was 35.95 year (ranging from 18-74 years), and the mean post-transplantation time of presentation was 161.4 days.

Renal function was decided according to the levels of serum creatinine that were measured in the hospital laboratories at the time of sampling, and accordingly, these RT subjects were divided into two groups. The first group is called control group where RT subjects with normal renal function (serum creatinine ≤ 1.2 mg/dl) [10,17]. The second group is the test group where RT subjects had biopsy proven either acute renal impairment and/or allograft rejection (biopsy results were taken from the patients' reports).

Relying on kidney transplantation specialists, the most suitable cut off time that separates between early and late presentation of RT subjects is 6 months (which was also considered in dividing the presentations into early and late renal impairment) [10,17].

3 ml blood samples were collected from these 57 RT subjects, plasma was then separated from blood and DNA was extracted from 200 µl of plasmain accordance to the manufacturer of DNA extraction kit, namely DNA-sorb-B (Sacace, Italy). DNA extraction steps included disruption/lysis of plasma sample, removal of the contaminants and recovery of the nucleic acid. The concentration and purity of the DNA were measured using the nucleic acid measuring instrument Analytica-Gena (USA) nanodrop.

Detection of EBV DNA and quantification of its DNA loadusing quantitative real-time PCR

The kit used was EBV Real-TM Quant Kit (Sacace, Italy) for the detection of LMP gene in EBV genome. The procedure was done according to the manufacturer guidelines. EBV DNA amplification was detected on FAM (Green) channel and exogenous internal control (IC) was detected on Rox (Orange)/Texas red channel.

The quantity of reactants for one reaction was 10 µL of PCR-mix-1 and 1.5 µL of PCR-mix-2 buffer and 0.5 µL of hot Start DNA polymerase. Then, DNA from sample/standard/positive or negative control was added to the mix. The final volume per reaction tube was 25 µL.

The RT-PCR instrument used in the study was STRATAGENE MxPro QPCR (Agilent Technologies, USA). The thermal protocol for Sacace Quantification Kit is composed of an initial denaturation for activation of the

HotStarTaq DNA Polymerase at 95 °C for 15 min; then, five cycles of thermal cycling 95 °C for 15 sec, and 60 °C for 20 sec, and 72 °C for 15 sec, and finally 40 cycles composed of 95 °C for 10 sec, and 60 °C for 40 sec, and 72 °C for 15 °C.

Statistical analysis

Data were analyzed using SPSS version 12.0.01 software. Qualitative frequency data were subjected to Chi square test for association while parametric quantitative data were subjected to ANOVA and t-test for measuring significance of difference. Relative risk (RR) and correlation coefficient (r) were also used in accordance to results, $P \leq 0.05$ was considered statistically significant.

Results

The results of this study are based on the analysis of fifty seven patients with renal transplantation. EBV viremia was detected in 19/57 (33 %) of RT subjects. The age of RT subjects ranged from 16 to 58 years with mean ± SD age of 35.95 ± 12.50 year. There was obvious predominance of males over females among RT subjects. Male: female ratio was 3.75: 1.

The findings showed that about three quarters of the RT subjects were studied early in this research, less than 6 months after kidney transplantation, while one quarter of RT subjects were studied late, more than 6 months after kidney transplantation (Figure 1).

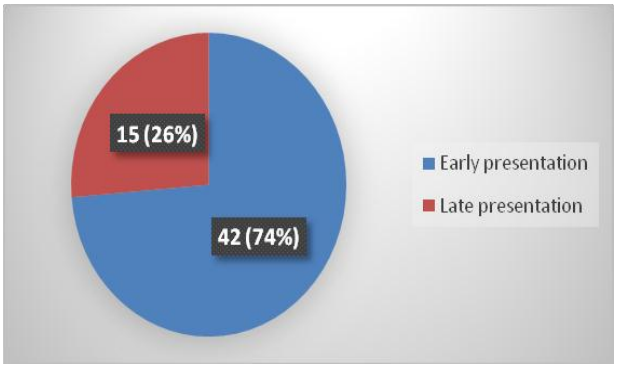

Fig. 1. Distribution of RT subjects according to the post-transplant period (cutoff 6 months) The association of positive EBV viremia with age and gender of RT subjects

Age distribution among RT subjects in relation with positive EBV viremia was non-significant ($P > 0.05$). However, the age group older than 40 years showed a bit higher percentage (40%) of EBV infection than others. The quantitative analysis of the load of EBV viremia in regard to age groups showed no significant difference ($P > 0.05$).

Positive EBV viremia was associated with gender of RT subjects involved in this study ($P > 0.05$). It was found that 18/45 males were shown to have positive EBV viremia with much higher percentage of positive EBV viremia, 40%, than that in females, 8.3% (Table 1). On the other hand, the quantitative analysis of the load of EBV viremia in regard to gender type showed no significant difference ($P > 0.05$), (Table 2).

Table 1. The association of gender of RT subjects with EBV viremia in real time PCR

Gender type		EBV		Total
		Negative	Positive	
Female	No. (%)	11 (91.7)	1 (8.3%)	12 (100.0)
Male	No. (%)	27 (60.0)	18 (40.0)	45 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value		0.036*		
RR for males as risk factor		4.8 : *P* = 0.1		

Table 2. The quantitative analysis of the load of EBV viremia in regard to gender type

Gender type	No.	Mean	Std. Deviation	Std. Error Mean	*P* value
Female	1	5025.00	1984.11	563.7	0.771
Male	18	5946.33	3012.22	709.99	

The association of positive EBV viremia with post-transplant period

The findings of this study indicated a high association between EBV positivity and late presentation (> 6 months) of RT subjects ($P < 0.05$) in that 66.7% of late presenters versus 21.4% of early presenters showed positive EBV viremia (Table 3). The quantitative analysis of the load of EBV viremia in regard to the time of presentation showed no significant difference ($P > 0.05$) Table (4). The mean ± SD of the post-transplantation time till presentation in this study was 161.40 ± 130.34 days.

Table 3. The association between time of presentation of RT subjects and EBV positivity

Post-transplant period		EBV		Total
		Negative	Positive	
Early-post- transplant*	No. (%)	33 (78.6)	9 (21.4)	42 (100.0)
Late-post- transplant	No. (%)	5 (33.3)	10 (66.7)	15 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value	0.002**			

* The cutoff is 6 months, ** = $P < 0.05$.

Table 4. The quantitative analysis of the load of EBV viremia in regard to the time of post-transplant period.

Presentation	No.	Mean	Std. Deviation	Std. Error	*P* value
Early post-transplant	9	5799.44	3118.31	1039.44	0.894
Late post-transplant	10	5986.40	2926.83	925.54	

The association of positive EBV viremia with the level of serum creatinine in RT subjects

It was found that 61% of RT subjects had abnormal high levels of serumcreatinine, namely renal impairment, versus 39% with normal levels of creatinine (Figure 2).

The association between creatinine levels and EBV viremia were remarkably significant. It was shown that 50% of RT subjects with abnormally high creatinine levels were with positive EBV viremia while none of the RT subjects with normal creatinine level showed EBV viremia.

This indicates the strong association between EBV viremia and renal impairment after kidney transplantation (Table 5).

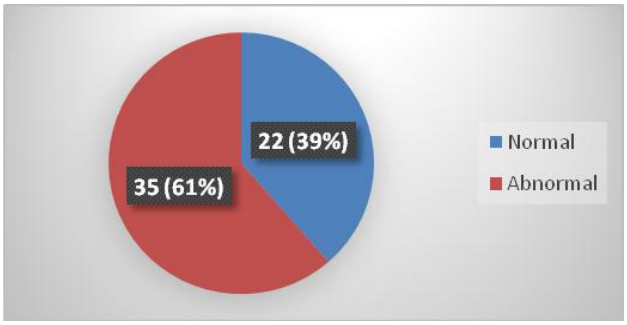

Fig. 2. The distribution of RT subjects according to the level of serum creatinine (cutoff normal serum creatinine ≤1.2 mg/dl)

Table 5. The association between positive EBV viremia and serum creatinine levels*

Blood creatinine level		EBV		Total
		Negative	Positive	
Normal	No. (%)	19 (100.0)	0 (0.0)	19 (100.0)
High	No. (%)	19 (50.0)	19 (50.0)	38 (100.0)
Total	No. (%)	38 (66.7)	19 (33.3)	57 (100.0)
P value	0.001**			
RR for high blood creatinine as a risk indicator			20 : *P* = 0.033**	
RR for positive EBV viremia as a risk factor for high blood creatinine			1.95 : *P* < 0.0001***	

* = cutoff normal serum creatinine ≤ 1.2 mg/dl, ** = *P* < 0.05, *** = *P* < 0.001.

As an interesting result, the high blood creatinine level was a grave risk indicator for the presence of EBV viremia with RR equal to 20 (*P* < 0.05) implying to the notion that RT subjects with abnormally high creatinine level are 20 times more prone to develop EBV viremia.

Moreover, considering EBV viremia as a risk factor for the development of abnormally high serum creatinine level, it was found that positive EBV viremia doubled the chances for RT subjects to have high serum creatinine; the interesting issue in this result, the confidence of EBV viremia as risk factor for high blood creatinine was too high (*P* < 0.0001) rendering EBV viremia as a remarkable risk for developing serious renal impairment (Table 5). However, the quantitative analysis of the load of EBV viremia in regard to the positivity of creatinine levels showed no significant difference (*P* > 0.05) (Table 6).

Table 6. The quantitative analysis of the load of EBV viremia in regard to the positivity of blood creatinine

Blood creatinine	No.	Mean	Std. Deviation	Std. Error	*P* value
Positive	18	5858.83	3014.99	710.64	0.814
Negative	1	6600.00	3543.38	737.18	

The association between EBV viremia and renal impairment in renal transplant recipients

The RT subjects were categorized into three groups: under control, acute renal impairment, and chronic renal impairment groups. The findings showed highly significant association between renal impairment, whether acute or chronic, and EBV viremia (P<0.05) (Table 7). Moreover, none of the control group had EBV viremia (*P* < 0.05). However, both chronic and acute renal impairment groups showed very close percentages of RT subjects with positive EBV viremia, 51.7 and 50.0 %, respectively (*P* > 0.05).

Table 7. The association between renal impairment and positive EBV viremia

Renal impairment		EBV		Total
		Negative	Positive	
Acute renal impairment	No. (%)	4 (50.0)	4 (50.0)	8 (100.0)
Chronic renal impairment	No. (%)	14 (48.3)	15 (51.7)	29 (100.0)
Total	No. (%)	18 (48.65)	19 (51.38)	37 (100.0)

Association between EBV viremia and the type of immuno-suppressive regimen used in RT recipients

Two main standard immunosuppressive regimes are mainly followed; the first regimen includes cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, the second regimen includes tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone (Figure 3).

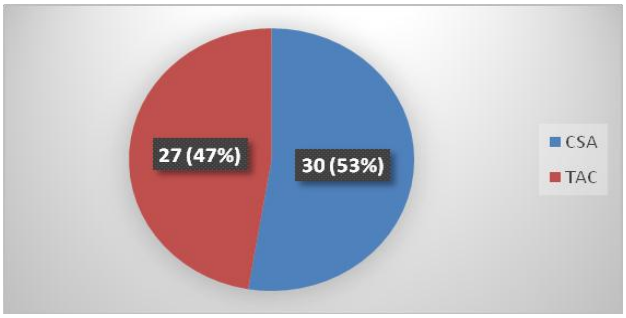

Fig. 3. Distribution pattern of the immuno-suppressive regimens among RT subjects

This study showed that CSA-based regimen is significantly associated with positive EBV viremia when compared to TAC-based regimen (P<0.05) (Table 8). Calculating the relative risk for RT subjects treated with CSA-based regimen showed that CSA acted as a significant risk factor for the development of EBV viremia (P<0.05) (Table 8).

Such results highlight that the potent immunosuppressive regimen using CSA might aggressively lead to extensive immuno-suppression which in turn favors EBV reactivation of latent infection or contraction more easily of external EBV infection. However, the quantitative analysis of the load of EBV viremia in regard to the type of immunosuppressive regimen showed no significant difference (*P* > 0.05) Table (9).

Table 8. The association between the type of immunosuppressive regimen and positive EBV viremia

Drugs		EBV		Total
		Negative	Positive	
CSA	No. (%)	16 (53.3)	14 (46.6)	30 (100.0)
TAC	No. (%)	22 (81.5)	5 (18.5)	27 (100.0)
Total	No. (%)	38 (66.7)	38 (66.7)	19 (33.3)
P value			0.023*	
RR for CSA as a risk factor			2.5 : *P* = 0.039	

CSA = cyclosporine A, TAC = tacrolimus * = p<0.05

Table 9. The quantitative analysis of the level of EBV viremia in regard to the immunosuppressive drugs used

Drug	No.	Mean	Std. Deviation	Std. Error	*P* value
CSA	14	5484.93	3091.18	826.15	0.318
TAC	5	7054.00	2332.53	1043.14	

CSA = cyclosporine A, TAC = tacrolimus

Discussion

In the current study, 57 Iraqi RT subjects were involved. EBV viremia was detected in 19/57 (33 %) of RT subjects. About 50 and 51.7% of RT subjects involved in the current study had acute and chronic renal impairment, respectively. Interestingly, in the current study, RT subjects with positive EBV viremia were commonly with high risk for developing both acute and chronic renal impairment (*P* = 0.0001) as well as high levels of serum

creatinine in RT subjects showed significant relative risk to have EBV viremia.

These findings agree with a study done earlier revealing that a higher rate of graft loss was observed in RT subjects had a positive EBV PCR during the first 6 months post-transplant [18]. Association of EBV viremia with acute/ chronic renal impairment indicates serious type of relationship.

EBV primary infection or reactivation in RT subjects might impose cause-effect relationship with renal impairment [1,4]. A study conducted in Germany found that EBV viremia is an underestimating cause of renal impairment and maybe rejection of transplanted kidneys [19]. In this instance, the exact driving cause for EBV infection to develop renal impairment is still not well known. However, several explanations were presented in the literature of the field and as follows:

First, EBV-induced cytotoxic T lymphocyte response contains clones that are reactive to self-MHC/peptide complexes that show strong allo-cross-reactivity against allo-MHC–presented peptides [5,10,20,21].

Second, EBV is implicated in counteracting immune suppression of T cells as EBV-driven induction of T cell immune response would be a limiting step for the immunosuppressive effect of drugs taken after kidney transplantation [22].

Third, EBV replicates mainly in B lymphocytes; this results in the induction of B cells' signaling pathway of immunoglobulins production which in turn results in excessive formation of heterophil antibodies. Heterophil antibodies are suspected to be another factor for targeting tissues of the transplanted kidney through complement activation leading to destruction of renal glomeruli [23].

The frequency and quantitative analysis of EBV viremia in regard to age groups showed no significant difference ($P > 0.05$). However, several previous studies showed a positive correlation between age and EBV viremia [24,25], but could be in agreement with previous reports demonstrated that recipient age did not affect the incidence and severity of acute rejection or graft survival [26,27].

In the current study, males were shown to have more positive EBV virema, 40%, than in females, 8.3 %. A previous study found that femalekidney transplant recipients have better 8 year graft and patient survival than male recipients [28].

A previous study found that EBV viremia is detected mainly in the first year of RT subjects [29]. However, the current study revealed high association observed between EBV positivity and late presentation (> 6 months) of RT subjects indicating that EBV viremia takes several months after kidneytransplantation to be detectable.

EBV load serves as a functional marker of the degree of immunosuppression and that undetectable EBV implies under immunosuppression and associated risk of rejection [30]. Unbalance between too little and too much immunosuppression given to RT subjects results in organ rejection and high risks of opportunistic infections, respectively [31].

The current study indicated a strong relationship between the type of immunosuppressive regimen with CSA and EBV positivity. Thisfinding is indirectly congruous with that of a previous study which found that cyclosporine levels after kidney transplantation were highly predictive of acute cellular rejection episodes [30,32]. Related to the point, another study showed that CSA, despite of its strong immunosuppression, results in significantly higher median creatinine compared to other drugs and long-term CSA use may cause glomerular sclerosis, arteriolar hyalinosis and tubular atrophy and interstitial fibrosis [32].

In conclusion, the findings of 33% positive EBV viremia among renal transplant recipient and all of them had impaired renal function; indicate a possible relationship between EBV positivity and impaired renal allograft function and may recommend good screening for this virus in these patients.

Acknowledgement

Authors would like to acknowledge the Center of Kidney Diseases and Transplantation in The Medical City of Baghdad and Al-Karama Teaching Hospital, for their cooperation in accomplishing this study

Author contributions

Sahar A. Shams-aldein, Ahmed S. Abdlameer did the DNA extraction and RT-PCR; Asmaa B Al-Obaidi and Haider S Kadhim collect the specimens; Ali J.Al-Saedi Providing patient's Data sheet

Conflict of Interest:

Authors declare no conflict of interest.

Funding

There is no funding source for this research

References

1. Sharifi MA. Epstein-Barr viral infection in renal allograft recipients. Saudi J Kid Dis. 2006; 17: 351-4.
2. Kharazy H. Post organ transplantation therapeutic cyclosporine dose significance determination. Saudi J kid Dis. 2006; 17: 351-4.
3. Martinez OM, de Gruijl FR**.** Molecular and immunologic mechanisms of cancer pathogenesis in solid organ transplant recipients. Am J Transplant. 2008; 8**:** 2205-11**.**
4. Snow AL, Martinez OM**.** Epstein-Barr virus: evasive maneuvers in the development of PTLD. Am J Transplant. 2007; 7**:** 271-7.
5. Hebert D, Weitzman S, Petric M, et al**.** Risk factors for post-transplant lymphoproliferative disorder in pediatric patients: a case-control study. Pediatr Transplant. 2005; 9**:** 450-5.
6. Frias, C, Lauzurica R, Bayes B, et al. Prospective follow-up of Epstein-Barr virus load in adult kidney transplant recipients by semi quantitative PCR in blood and saliva samples. Eur J Clin Microbiol Infect Dis. 2001; 20: 892-5.
7. Reusser P, Einsele H, Cordonnier C, et al. Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. Bone Marrow Transplant. 2009; 43**:** 757-70.
8. Cockfield SM, Preiksaitis JK, Jewell LD, et al. Post-transplant lymphoproliferative disorder in renal allograft recipients. Clinical experience and risk factor analysis in a single center Transplantation. 1993; 56: 88-96.
9. Thorley-Lawson DA. Epstein-Barr virus: exploiting the immune system. Nat Rev Immunol. 2001; 1: 75-82**.**
10. KDIGO Transplant Work Group, KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009; 9: S1-S155.
11. Al-Taee IS, Al-Shamaa I. Long term follow up of renal transplant patients-a single center experience in Iraq. Saudi J Kidney Dis Transplant. 2005; 16: 40-5.
12. Rifat UN. Kidney Transplantation in Iraq. Saudi J Kid Dis Transplant. 2006; 17: 399-400.
13. Muhsin ASA, Alnasiri US, Rifat UN: One-year (patient and renal allograft) survival following renal transplantation. Iraqi J Med Sci. 2007; 5: 7-12.
14. Al-Obaidi AB, Abd KH, Kadhim HS, et al. BK polyomavirus and Cytomegalovirus Co-infections in renal transplant recipients: a single center study. IJAR 2015; 3: 856-64.
15. Al-Obaidi AB, Abd KH, Habib MA, et al. Detection of BK polyomavirus using real time PCR in 99 renal transplant recipients. JIARM. 2015; 3: 131-41.
16. Al-Obaidi AB, Qasim BJ, Husain AG, et al. BK Polyomavirus-infected Decoy cells in urine cytology specimens of renal transplant recipients. Iraqi J Med Sci. 2015; 13: 70-5.
17. Kälble T, Alcaraz A, Budde K, et al. European Association of Urology: guidelines on renal transplantation. [http://www.uroweb.org/file admin/tx_eauguidelines/2009/Full/Renal_Transplant. pdf]. p. 1-90.
18. Li L, Chandhuri A, Weintraub LA, et al. Subclinical cytomegalovirus and Epstein Barr virus viremia are associated with adverse outcomes in pediatric renal transplantation. Pediatr Transplant. 2007; 11: 187-95.
19. Hornef MW, Bein G, Fricke L, et al. Coincidence of Epstein-Barr virus reactivation, cytomegalovirus infection, and rejection episodes in renal transplant recipients. Transplantation. 1995; 60: 474-8.
20. Burrows SR, Khanna R, Burrows JM, et al. An alloresponse in humans is dominated by cytotoxic T lymphocytes (CTL) cross-reactive with a single Epstein-Barr virus CTL epitope: implications for graftversus- host disease. J Exp Med. 1994; 179: 1155-61.
21. Burrows SR, Khanna R, Silins SL, et al. The influence of antiviral T-cell responses on the alloreactive repertoire. Immunol. 1999; 20: 203-7.
22. Bamoulid J, Courivaud C, Coaquette A, et al. Subclinical Epstein - Barr virus viremia among adult renal transplant recipients: incidence and consequences. Am J Transplant. 2013; 13: 656-62.
23. Shannon-Lowe CD, Neuhierl B, Baldwin, G. From the Cover: Resting B cells as a transfer vehicle for Epstein-Barr virus infection of epithelial cell. PNAS. 2006; 103: 7065-70.

24. Palomar R, Ruiz JC, Cotorruelo JG, et al. Effect of recipient age on the clinical course of renal transplantation. Nefrologia. 2001; 21: 386-91
25. Foss A, Tuvin D, Leivestad T. Should kidneys from older cadaveric donors by age-matched to the recipient? Transplant Proc. 2005; 3: 3280-2.
26. Meier-Kriesche HU, Cibrik DM, Ojo AO, et al. Interaction between donor and recipient age in determining the risk of chronic renal allograft failure. J Am Geriatr Soc. 2002; 50: 14-7.
27. De Fijter JW. The impact of age on rejection in kidney transplantation. Drugs Aging. 2005; 22: 433-49.
28. Meier-Kriesche HU, Ojo AO, Leavey SF, et al. Gender differences in the risk for chronic renal allograft failure. Transplantation. 2001; 71: 429-32.
29. Morales JM, Marcén R, del Castillo D, et al. Risk factors for graft loss and mortality after renal transplantation according to recipient age: a prospective multicentre study. Nephrol Dial Transplant. 2012; 27: 39-46.
30. Doesch, AO, Konstandin M, Celik S, et al. Epstein-Barr virus load in whole blood is associated with immunosuppression. Transpl Int. 2008; 21: 963-71.
31. El-Sabrout R, Delaney V, Butt F, et al. Improved freedom from rejection after a loading dose of sirolimus. Transplantation. 2003; 75: 86-91.
32. Naesens M, Kuypers DR, Sarwal M. Calcineurin inhibitor nephrotoxicity. Clin J Am Soc Nephrol. 2009; 4: 481-508.

Correspondence to Dr. Asmaa B. Al-Obaidi
E-mail: asmaa.viro@yahoo.com
Mobile: + 964 7901879348
Received 26th Mar. 2015: Accepted 14th Jun. 2015