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The Detection of BK Virus by Quantitative PCR of Plasma as Screening Test to Identify Renal Transplant Recipients at Risk of BK Virus Nephropathy

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Abstract

Background: BK virus infection and nephritis are increasing problems in renal transplant recipients (RTRs). Increasing prevalence of this infection and nephritis have been correlated with newer immunosuppressive agent in recent years. Routine post-transplant screening for BK viremia prior to the occurrence of nephritis and the reduction in immunosuppressive therapy for subjects with BKV infection appear to be attractive objectives.

Objectives: This study performed to screening for BK virus in blood to identify RTRs at risk of development BKV associated nephritis (BKVN) .

Methods: In this case-control study**,** a total of 75 renal transplant recipients as a patients group, with 75 healthy adults as a control group, were involved in this study. Quantitative detection of BKV viral load in blood by real time PCR assay was done for the both groups.

Results: This study revealed that 37% of the RTRs were positive for BK viremia by qPCR assay, While BKV has not detected in any one of the seventy five healthy persons of the control group, and about half (54%) of the recipients were identified as presumptive BKVN.

Conclusion: This study suggests that qPCR assay of blood is an important screening test for detection of BKV infection in RTRs.

Keywards BK virus , renal transplantation , viremia, BKVN.

Introduction

BK virus (BKV) is a small, non-enveloped, double-stranded DNA virus belonging to the Polyomaviridae family that was first isolated in 1971. During initial infection, virions infect urothelial cells and establish latent infection. BKV reactivation in RTRs is increasingly recognized (up to 60%) as an opportunistic infection particularly with the introduction of more potent immunosuppressive agents (1).

Once the virus has reactivated, there will be an ascending infection via cell-to-cell spread (2). The BK virus then multiplies in the interstitium and causing viruria then crosses into the peritubular capillaries, causing viremia and eventually invading the allograft, leading to various tubulointerstitial lesions and BKVN. Approximately one-third of patients with viruria will develop BK viremia and, without intervention, could progress to BKVN (rates ranging from 1 to 10%) (3) and leading to graft failure in 20 to 80% of these affected patients with nephropathy (4).

Thus, BKV infection is an important clinical problem in RTRs and this is most likely due to the enhanced immunosuppressive state and BKV-specific immune deficiency, especially when there are limited antiviral treatment options for BKV infection and there are no effective prophylaxis. Screening for viremia using diagnostic tools such as BKV DNA plasma can be used to identify early infection, and preemptive reduction in immunosuppression for patients with viremia can decrease the prevalence of BKVN.

BKV DNA quantification in plasma is used as an important diagnostic tool and BK viremia is detected in 15 to 30% of renal transplant recipients during the first posttransplantation year (5,6). BK detection by real-time PCR of plasma is very sensitive and specific for the development of BKVN. Therefore, BKV DNA quantification in plasma is the

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preferred screening method at most transplant centers. The cut-off to determine the clinical relevance of BKV viremia remains controversial (7), but retrospective studies have suggested that a BK viral load >4 log (10,000) copies/mL is strongly associated with finding BKVN on biopsy (8).

The aim of the current study is to perform a screening for BK virus by Quantitative detection of BKV viral load in plasma by real time PCR technique to identify renal transplant recipients at risk of BKVN development .

Methods

This study was carried out during the period from January 2016 to January 2017 in Iraq.

A total of 75 patients with renal transplantation (50 males and 25 females), whose age ranged from 25 to 55 years with mean of 38.48±9.54 years from two hospitals of two different Iraqi provinces:

1.Al-Khial Private Hospital in Baghdad province: 65 patients with renal transplantion

2.Al-Sader Teaching Hospital in Najaf province: 10 patients with renal transplantion

A total of 75 apparently healthy, non- renal transplant persons, were involved in this study, Whose age ranged from 25 to 55 years.

Both renal transplant recipients and healthy control group were matched regarding age, sex, residency and occupation and there were no significant differences in such factors.

Details regarding the age, gender, occupation, residency, duration of renal transplantation, race, smoking, past medical history of hypertension and diabetes were recorded.

- Specimens Collection

Samples of five milliliters (ml) of venous blood were collected from each patient as well as control group.

-Two ml. of blood was added to EDTA tube and centrifuged at 5000 rpm for 10 minutes to take plasma for viral DNA

-Viral Nucleic Acid Extraction

FavorPrep Viral Nucleic Acid Extraction Kit (Figure:1)was used in this study for extraction of BK Viral DNA from plasma and urine samples.

The procedure was applied according to the leaflet of the FavorPrep Viral Nucleic Acid Extraction commercial kit.

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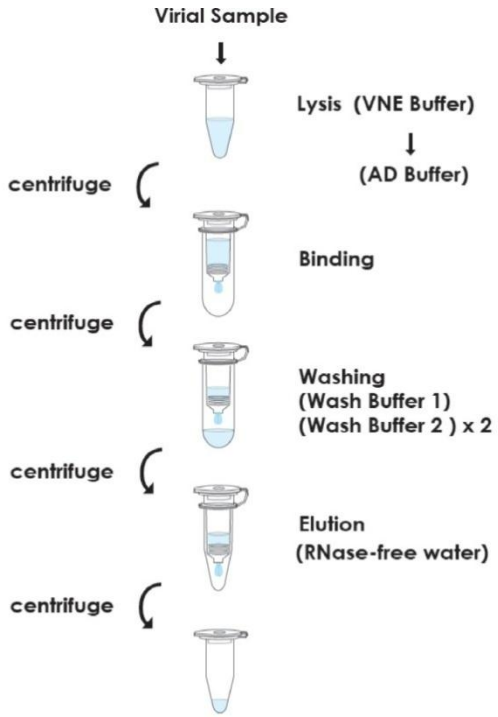

Figure (1): Brief Procedure of Viral Nucleic Acid Extraction.

- Polyomavirus BK Real Time PCR

Liferiver Polyomavirus BK real time PCR kit (Figure 3.3) was used in this study for the detection of Polyomavirus in serum, plasma or urine sample by using real time PCR systems.

Test procedure

The procedure was applied according to the leaflet of the commercial kit.

The Master Mix volume for each reaction was pipetted as follows (Figure: 3):

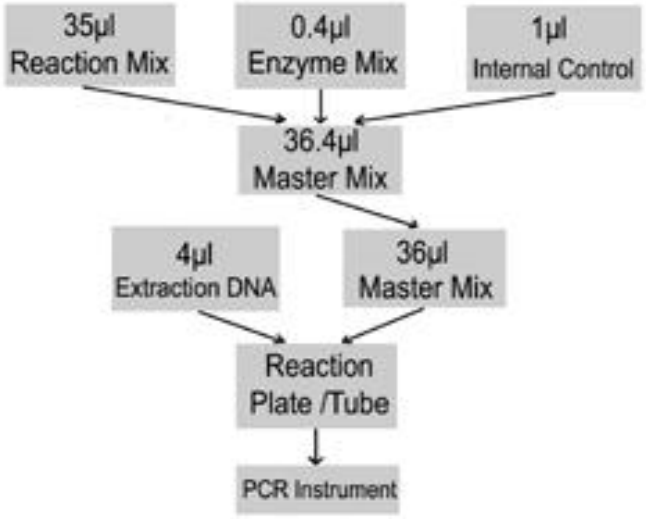

Figure (3): PCR Protocol.

- Statistical Analysis

Statistical analysis was done by using SPSS(statistical package for social sciences) version 20 in which the mean with standard deviation, numbers and percentages as descriptive statistics are used. For analysis of data chi square test, Yates corrected chi square, and independent sample t-test are used. P value ≤0.05 is regarded significant.

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- Ethical Issues:

Verbal consent from all patients had been approved in addition to explanatory information about the study during interviewing and questionnaire application.

Results

-Demographic and Relevant Features

Some features of such as age, gender, residency, showed differences but statistically not significant between the two groups. While renal transplant recipients with hypertension and diabetes show highly significant differences from the members of the healthy control group. (Table: 1).

Table (1): Demographic Characters of the Study Groups.

Variables	Patients (No.= 75)	Control (No.= 75)	Statistical values	
Age (Years)	38.48±9.54	38.49±7.88	P=0.993	t= -0.007
Gender: Male **Female**	50 (66.7%) 25 (33.3%)	50 (66.7%) 25 (33.3%)	P=1	χ^2=0
Residency: Urban **Rural**	55 (73%) 20 (27%)	57 (76%) 18 (24%)	P=0.707	χ^2=0.141
Hypertensive **normotensive**	30 (40%) 45(60%)	75 (100%) 0 (0%)	P<0.001	χ^2=64.286
Diabetic **Nondiabetic**	51 (68%) 24 (32 %)	75 (100%) 0 (0%)	P<0.001	χ^2=28.571

-Detection of BKV DNA Copies in Blood (Viremia) of RTRs and Healthy Control Group

The BKV DNA copies in the blood were detected in 28/75 (37%) of RTRs, but not detected in any one of the 75 members of the healthy control group. (Table: 2).

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Table (2): The Frequencies of BK Viral DNA Copies (Viremia) Positivity in Plasma of in Renal Transplant Recipient and Healthy Control Group.

Group	BK viral DNA Copies in Plasma (Viremia)					
	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Renal transplant recipients	28	37 %	47	63%	75	100%
Healthy control group	0	0 %	75	100%	75	100%
Statistical values	($P<0.001$)($\chi^2=34.426$)					

-Detection of BK Virus DNA Copies in Blood (Viremia) of RTRs in Relation to Age Group, Gender and Duration of Renal Transplantation.

Table (3) revealed that the highest viremia positivity was recorded in the RTRs of the 3rd age group (45-55y) [23/31 (74%)],also in RTRs with the 13-24 months duration of renal transplantation [7/13 /(54%)]. While the lowest positivity was recorded in the 1st (age group (25-34y) [1/35 (3%)], also in the first 12 months duration of renal transplantation [5/10 /(50%)]. In addition, male group, hypertensive and diabetic RTRs recorded higher positivity [21/50 (42%), 18/45 (40%) and 15/22 (68%) respectively] than that of the female group, normotensive and nondiabetic RTRs [7/25 (28%),10/30 (24%) and13/53 (15%) respectively].

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Table (3): The Frequencies of BK Viral DNA Copies Positivity in Plasma (Viremia) of Renal Transplant Recipients in Relation to Age Group, Gender, Duration of renal transplantation, hypertension and diabetes.

		BK viral DNA Copies in Blood (Viremia) of RTRs						
		Positive No.=28		Negative No. =47		Total No.=75		Statistical values
		No .	%	No .	%	No .	%	χ^2 and P
Age group/y	1st (25-34)	1	3 %	34	97%	35	47%	(χ^2=35.979) (P<0.001)
	2nd (35-44)	4	44 %	5	56%	9	12%	
	3rd (45-55)	23	74%	8	26 %	31	41%	
Gender	Male	21	42 %	29	58%	50	67%	(χ^2=1.396) (P=0.237)
	Female	7	28 %	18	72%	25	33%	
Duration of Transplantat.	1-12 m	16	31%	36	69%	52	69%	(χ^2=3.1586) (P=0.2061)
	13-24 m	7	54%	6	46%	13	17%	
	>24 m	5	50%	5	50%	10	14%	
Hypertensive		18	40%	27	60%	45	60%	(χ^2=0.341) (P=0.558)
Normotensive		10	33%	20	67%	30	40%	
Diabetic		15	68%	7	32%	22	29%	(χ^2=12.663) (P<0.001)
Non diabetic		13	15%	40	75%	53	71%	

-Identification of Viremic RTRs as Patients with a Presumptive BKVN

Table (44) shows the frequencies of viremic RTRs with BK DNA viral load ($\geq 10^4$/ml of blood) as recipients with a presumptive BKVN.

Fifteen out of thirty nine (54%) of the viremic RTRs with BK DNA viral load $\geq 10^4$/ml of blood was reported P value(= 0.415) (Table: 4) .

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Table (4): The Frequencies of Viremic RTRs with BK DNA Viral Load (≥ 10^4/ml of Blood) and (<10^4/ml of blood).

Viremic RTRs	BK Viral DNA Copies/ml of Blood (Viremia)					
	≥ 10^4		< 10^4		Total	
	No .	%	No .	%	No.	%
	15	54%	13	46%	28	100%
Statistical values	(P=0.415)(χ^2=0.425)					

-Identification of Viremic RTRs as Patients with a Presumptive BKVN Regarding to Age Group, Gender, Duration of renal transplantation, hypertension and diabetes.

The highest frequency of the viremic RTRs with BK DNA viral load ≥ 10^4/ml of urine was reported in RTRs of the 3rd age group (45-55 y) [13/31 (42%)], inRTRs of the 2nd duration of transplantation (23-24 m) [4/13 (31%)], while the lowest frequency was reported in the 1st age group (25-34 y) [0/35 (0%)] and in the 1st duration of transplantation (1-12 m) [8/52 (15%)]. (Table: 5).

Higher frequency of BK DNA viral load ≥ 10^4/ml of blood was reported in the male, hypertensive and diabetic viremic RTRs [12/50 (24%),[10/45 (22%) and7/22 (32%) respectively] than that of the female group, normotensive and nondiabetic viremic RTRs [3/25 (12%),5/30 (17%) and 8/53 (15%) respectively]. (Table: 5).

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Table (5): The Frequencies of Viremic RTRs with BK DNA Viral Load ≥ 10^4/ml of Blood in relation to Age Group, Gender, Duration of renal transplantation, hypertension and diabetes.

		BK viral DNA Copies in Blood (Viremia) of RTRs								
		≥ 10^4		< 10^4		Negative		Total		Statistical values
		No .	%	No .	%	No .	%	No .	%	χ^2 and P
Age group/y	1st (25-34)	12	24%	9	18%	29	58%	50	66%	(P< 0.001) (χ^2=36.252)
	2nd (35-44)	3	12%	4	16%	18	72%	25	34%	
	3rd (45-55)	15	20%	13	17%	37	63%	75	100%	
Gender	Male	12	24%	9	18%	29	58%	50	66%	(P=0.415)(χ^2=1.759)
	Female	3	12%	4	16%	18	72%	25	34%	
Duration of Transplantat.	1-12 m	8	15%	8	15%	36	70%	52	70%	(P=0.495) (χ^2=3.389)
	13-24 m	4	31%	3	23%	6	46%	13	17%	
	>24 m	3	30%	2	20%	5	50%	10	13%	
Hypertensive		18	40%	27	60%	45	60%			P=0.811 χ^2=0.4183
Normotensive		10	33%	20	67%	30	40%			
Diabetic		15	68%	7	32%	22	29%			(P=0.001) (χ^2=13.404)
Non diabetic		13	15%	40	75%	53	71%			

Discussion

BK viral infection has emerged as an important cause of progressive renal allograft dysfunction but in Iraq, the prevalence, potential risk factors and diagnostic modalities have been poorly elucidated. The current case-control study involved the analysis of renal allograft recipients for BKV infection.

In this study, BKV was detected in 28/75 (37%) of RTRs by the quantitative PCR assay of blood (viremia) [while the BKV was not detected in any one of the healthy control group by the three tests.

There are statistically significant differences between RTRs and healthy control group in detection of BKV by qPCR of blood ($p < 0.001$). Therefore, there is a relationship between renal transplantation and BKV infection in this study.

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Higher frequency of viremia in RTRs was reported in this study [28/75 (37%)] in comparison to what was reported by Hirsch *et al*. (9), 10; 6; 9, 11) and Randhawa *et al.*, (12) (13%, 8%, 11.5%, 12.4%, 12.2% and 7.7% of RTRs respectively). While in Brazil, higher frequency of viremia (43%) was reported by Montagner *et al.* (13), using qualitative PCR assay.

In the current study, the BKV was not detected in any healthy person of the control group which is incompatible with results of Hirsch (14) who detected the virus in 5% of the healthy individuals, and the findings of Randhawa *et al.* (15) which detected the virus in 8.7% of non-immunocompromised controls. This may be related to differences in sample size, type of diagnostic procedure, and type of patients.

It is possible, to some extent, that such difference may have been partially influenced by characteristics of the PCR assays used in each study, (type of the diagnosis procedure if qualitative or quantitative PCR assay and also according to the type of primer used in PCR kit). Beside that , it my be related to the type of immunosuppresion combination therapy (treatment protocol) used (16) .

In the current study, the finding of BK viremia in 37% of patients with renal transplantation, stresses the importance of screening for BKV with PCR assay of urine and blood.

Dall and Hariharan (17) focused on the importance of detection of BK viremia in improving the kidney graft survival as they stated that the actuarial kidney graft survival for patients with BKVN has improved in the past decade.

With a working terminology similar to that for invasive fungal disease, “possible” BKVN is defined as the presence of BKV viremia , “presumptive” BKVN is defined as a BKV viral load of 10,000 copies/mL of plasma, and “definitive” BKVN is defined as histological evidence of allograft involvement (Hirsch *et al.,* 2010). For patients with “presumptive” BKVN, the risks and benefits of intervention have not been conclusively evaluated. However, recent data suggest that progression to BKVN can be safely prevented if BKV viremia is used as a guide to reduce immunosuppression (Brennan *et al.,* 2010).

In this study, defining the threshold cut-off level (value) for viremia to predict tissue invasive disease (BKVN) was not determined because no renal biopsy was done to define the histopathologically proven BKVN.

Fifteen (54%) of the 28 RTRs with BK viremia considered with a a presumptive BKVN because they had BKV viral load of 10^4 copies/mL of plasma according to Hirsch *et al*. (9) threshold.

In the current study, higher frequencies of BKV positivity RTRs were reported with increasing in age. There is a statistically significant difference in the BKV positivity among the three age groups (P value < 0.001). So, there is a relationship between BKV renal infection in RTRs and age in this study.

In this study, the highest frequency of patients with a presumptive BKVN was detected in the 3rd age group (45-55 years and support to some extent the importance of being aged in developing BKVN, with high statistically significant difference (p values < 0.001) among the three age groups. Therefore, there is a relationship between increasing in age and presumptive BKVN in the current study. These findings increase the importance of monitoring of BKV reactivatin with increasing in the age of allograft renal transplant recipient.

These findings are in agreement with and support the studies that considered an old age is an important risk factors for BKV reactivation, replication and development of nephritis (BKVN).

As the study of Boan *et al.* (18) who found a difference in the mean age of the RTRs with BK nephropathy [58.3 y (47.8-61.6)] and those without BK nephropathy [51.2 y (41.6-58.1)], but with no statistically significant difference (p value = 0.113). Khamash *et al.* (19) reported

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that recipient age > 55 years is considered as a risk factor for BKVN when they found a relationship between this age group and BKVN in transplant recipients with a statically significant differences (p value < 0.001).

The increasing frequenting of BKV positivity with increasing in age may be related to the relative impairment of the immune response with increasing in age, which may be linked to the decrease in the development of T-cell oligoclonality together with a limited capacity of the thymus to generate naive T-cell and therefore reduced response to new antigen (20).

In this study, higher frequencies of BKV positivity were reported in males group of RTRs compared to females group. There is no statistically significant difference in the BKV positivity among the three age groups by any one of the three diagnostic tests (P values = 0.237).

The current study reported that the highest frequency of patients with a presumptive BKVN was detected in the males group and support to some extend the importance of male gender in developing of BKVN, and increase the importance of screening for BKV in transplant recipients of the male gender. But these finding of the current study with no statistically significant difference (p values = 0.415) among the three age groups. Therefore, there is no relationship between male gender and presumptive BKVN in the current study.

In this study, in spite of the fact that is no relationship between BKV renal infection in RTRs and presumptive BKVN with gender, these findings of higher frequencies of BKV detection and identification of presumpative BKVN in males group are in agreement with and support to some extend the findings of sudies that consider male gender is an important risk factors for BKV reactivation, replication and development of nephritis (BKVN).

As the study of Boan *et al.* (18) who found a higher frequency of BK nephropathy in male RTRs with [12 (70.6%)] than those without BK nephropathy [157 (60.4%)] but with no statistically significant difference (p value = 0.454). While Rocha *et al.* (21) reported that recipient gender (male) as a risk factor for BKVN when they found a relationship between male gender and BKVN in transplant recipients with a statically significant differences (p value < 0.04).

The higher frequencies of BKV infection presumptive the BKVN in males than females may be due to the life style differences which may effect the immunity as smoking and alcohol abuse which are higher in males than females (22).

Also, may be due to X chromosome diploidy in which female carry two X chromosomes (23).

In the present study, the highest frequencies of BKV positivity were reported within the two year of renal transplantation There is a statistically no significant difference in the BKV positivity among the three duration of renal transplantation by the diagnostic test (P value = 0.398). So, there is no relationship between BKV renal infection in RTRs and duration of kidney transplantation in this study.

There are higher frequencies of patients with a presumptive BKVN by in the 2nd and 3rd durations of transplantation compared to the frequency of 1st duration of transplantation but without statistically significant difference among the three durations of transplantation (p value = 0.495). Therefore, there is no relationship between increasing in duration of transplantation and presumptive BKVN by qPCR assay of blood in this study.

These findings are in agreement with and support the findings of many studies that reported that most BKV reactivation, replication and development of nephritis (BKVN) developed in the first two years of transplantation. As what is reported by Hirsch *et al.* (9) who said although relatively recently described, much has been learned about the epidemiology of BKVN which can guide practice. Most BKVN occurs in the first two years after transplant with only 5% of cases occurring between two and five years after transplant.

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Accordingly, screening should be most intense early after transplant with decreasing frequency as patients are further post-transplant (KDIGO Transplant Working Group, 22).

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Conflict of Interest

Authors declare no conflict of interest.

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