

Seroprevalence and newly isolated strains of LT-Ag gene (MMGHA2 and MM GHA5) of BK Polyomavirus in A Sample of Iraqi renal transplant recipients

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Abstract

Background: BK virus (BKV) opportunistic viral infection causes nephropathy and allograft loss in renal transplant recipients (RTRs).

Aims: Investigating BKV seropositivity, viremia rate and renal function were the primary goals of this study, in addition to the molecular study of the Iraqi BKV strains (LT antigen gene).

Settings and Design: Case-control study.

material and methods: Serum and plasma specimens were collected from 106 RTR and 100 non-RTR. Serum samples were analyzed for anti-BK IgG antibodies by BK-IgG ELISA kit. Plasma samples were used for detection of large tumor (LT-Ag) gene region of BKV by RT-PCR technique and then sequenced by Sanger's method.

Statistical analysis: frequencies, percentages, and Chi square-test by SPSS v.28

Results: The Study showed that 114 (55.3%) have a positive result for BK-IgG, so there was no significant difference between seropositivity of BKV IgG antibody among the studied groups, $p = 0.191$. On the other hand, a total of 206 subjects, 31 (15.0%) had positive viremia and 175 (85.0%) were negative. Renal function showed highly significant differences ($p \leq 0.000$) between seropositive and negative RTR patients. 2 sequences of Iraqi local strains were deposited in the GenBank with the accession numbers LC718551.1 and LC718552.1

Conclusions: The viremic state of BKV in RTR has similarly no impact on renal function. While the highly significant association between seropositivity of BK-IgG with high levels of serum creatinine. New strains of BKV were found in the Iraqi RTR patients with 98% similarity with the reference gene.

Keywords: Bk polyomavirus, Renal Transplant Recipient, immunosuppressive drugs, and Real-time polymerase chain reaction.

INTRODUCTION

The present study is considered one of the first reports on serological detection and level estimation of anti-BK-IgG in Iraqi RTRs and healthy blood donors as control. According to recent research, BKV co-evolved with humans, which explains the high prevalence and low morbidity among healthy individuals(1). Babies are protected against BKV infection by maternal antibodies during the first few months of life; but, once these antibodies begin to wear off, BKV infection may begin to develop, as indicated by 10 to 30 percent seropositivity in newborns and 65 to 90 percent seropositivity between the ages of 5 and 10 years old.(1)

In up to 10% of kidney transplant recipients (KTRs), BKV produces nephropathy (BKVN). Researchers have shown a correlation between BKV genotypes Ib2 and IV and BKVN. It appears that the virus in the donor's kidney can "travel" and

proliferate at high levels in recipients who initially lack neutralising antibody responses to BKV genotypes present in the graft.(2)

After the first infection in immunocompetent individuals, the BKV may be found in the kidney, in the leukocytes of the peripheral blood, and perhaps in the brain. The initial infection is typically asymptomatic or is characterised by mild nonspecific symptoms. (1,2) Similar to earlier studies, Hirsch et al., found 80% seropositivity in a prospective study of patients with kidney transplantation.(3)

Investigating BKV seropositivity, viremia rate and renal function were the primary goals of this study, in addition to the molecular study of the Iraqi BKV strain (LT antigen gene).

Material and Methods:

This Case-control study was conducted from November 2020-to June 2022. Samples were collected from the (Center of Kidney Diseases and Transplantation) and the (Iraqi blood donation center) in the Medical City of Baghdad. This study was conducted in the Medical Research Unit at the College of Medicine-Al-Nahrain University.

A total of (206) subjects were collected from (106) renal transplant recipients (RTR) patients within the first two years post-transplantation and (100) non-renal transplant samples (healthy blood donors). Inclusion criteria include RTR within the first two years post-transplant.

And RTRs who develop renal impairment without apparent rejection. Whereas exclusion criteria included RTR who had biopsy-proven renal allograft rejection, RTR on anti-thymocytes globulin (ATG), and CMV IgM positive patients or CMV IgG raising titers.

The five ml whole blood of RTR and non-renal transplants were divided into two parts (serum and plasma).

serum samples were analyzed for anti-BK IgG antibodies by using quantitative and qualitative Human BK Virus IgG (BK-IgG) ELISA Kit and according to the manufacturer (ABBKINE, China), with the cut of value=66pg/ml to estimate the positivity of BK-IgG and titration.

Viral DNA was extracted from 100µl plasma specimens via the procedure of silica membrane column separation method using a WizPrep™ Viral DNA/RNA Mini Kit (V2) according to the manufacturer's protocol (Wizbiosolutions, Korea).

QRT-PCR was performed using the Qiagen Real-time PCR System (Corbett RotGeneene Technologies) with qPCR soft software. The virus detection was quantified by measuring the threshold cycle (Ct) employing the 2×EasyTaq qPCR Master Mix Kits components. Every reaction was done in a duplicate and included a non-template control (NTC), non-amplification control (NAC) and non-primer control (NPC) as negative controls (for sequences of primers and prob see the supplementary materials).

The positive samples in the reaction of QRT-PCR underwent a conventional PCR reaction (using the rest of extracted DNA) in order to perform sequencing.

The conventional PCR reaction was conducted according to the manufacturer using EasyTaq® PCR SuperMix (Cat. No.: ABIN5519404) to amplify the BK polyomavirus large T antigen using specific primers (see supplementary materials).

Samples of amplified PCR-products for (LT) antigen from all group patients and control were subjected to direct sequencing (Sanger's sequencing) using an ABI3730XL automated DNA sequencer (Macrogen Company/ Korea) for detecting mutation within these sequences responsible for a gain of function mutation. The sequences were compared to a reference sequence of the LT antigen gene in the national center biotechnology information (NCBI) Gene Bank online and processed by using BioEdit software (BioEdit Pro. version: 7.2.5). The number of nucleotides from the gene of gene bank results as Sequence (NCBI Reference Sequence. To identify the genetic detection of the (LT) antigen gene in the sample of the Iraqi population, It was screened the region sequences with reference BK virus for the large T antigen gene Human polyomavirus 1 genome assembly,

chromosome: BKPyV_complete_genome Sequence ID: LR216264.1 Length: 5141 Number of Matches: 1 (see supplementary materials).

Results:

The positive result for BK-IgG was 114/206 (55.3%). Seropositivity was detected in 54(50.9%) of 106 RTR patients and 60 (60.0%) in the 100 control group, so there was no significant difference in seropositivity of BKV IgG antibody among the studied groups, p-value =0.191.

The patients' group was subdivided according to serum creatinine into two subgroups the first one equal to or less than 1.3mg/dl and the second more than 1.3mg/dl. After the distribution of these subgroups according to seropositivity was carried out, the finding showed seropositive BK-IgG were 27 (38.0%) in the first subgroup and 27(77.1%) in the second one, with highly significant differences (p-value ≤ 0.000) as shown in figure (2).

The real-time-PCR run was carried out for detection of BKV in the blood, and out of the total 206 subjects, gave positive viremia in 31 (15.0%) and 175 (85.0%) were negative. The mean of BKV Ct values was 20.99 ± 2.737 . The positivity of BKV DNA by RT-PCR was 23 (21.7%) of RTR patients and 83 (78.3%) were negative, while 8 (8.0%) from control were positive and 92 (92.0%) were negative, and there was a statistically significant p =0.005. Figure (1).

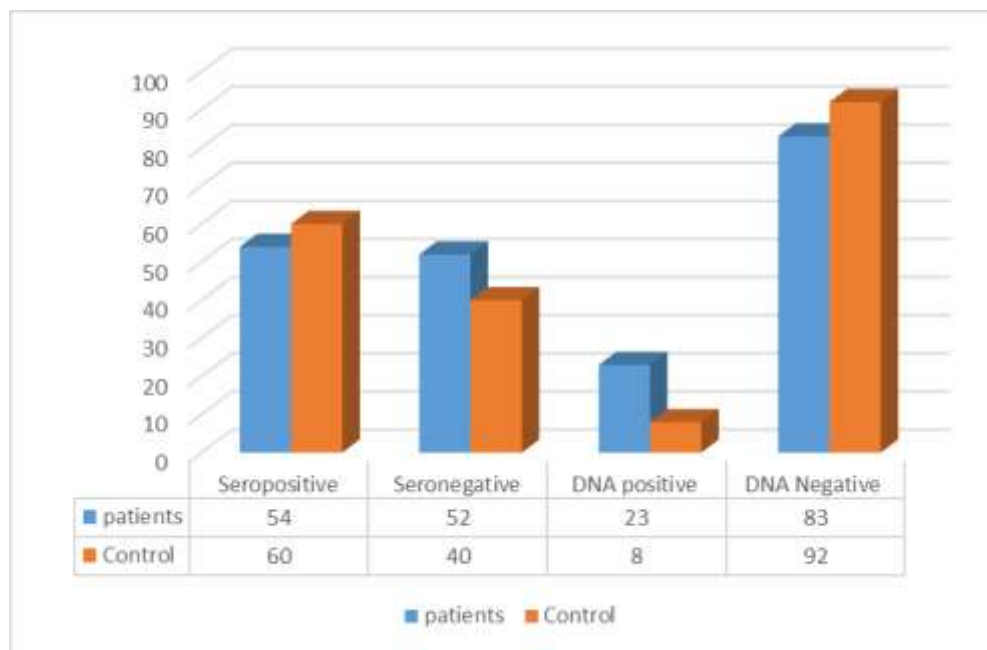


Figure (1): Seroprevalence and DNA positivity of BKV in RTR and control groups

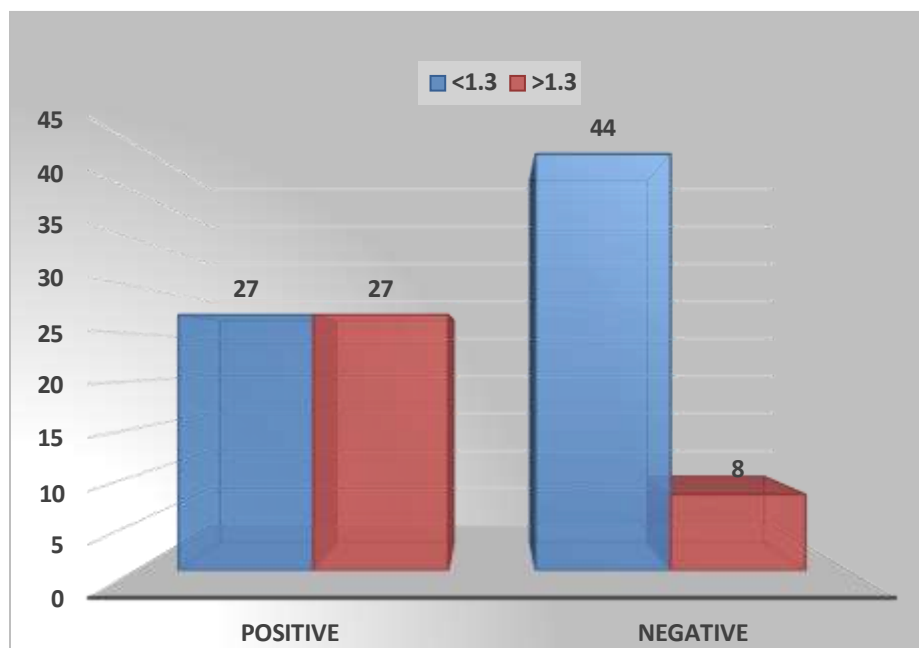


Figure (2) Distribution of seropositive BKV-IgG according to serum creatinine.

The association between qualitative measurements of anti- BKV IgG and BKV DNA In the current study 13 (41.9%) were positive for both BKV IgG and DNA, there was statistically no significant $P=0.103$, Table (1).

Table (1): Distribution of RT_PCR positivity for BKV DNA according to seropositivity of BKV IgG in study groups.

			Anti-BK-IgG		Total
			Positive	Negative	
BKV-DNA	Positive	Count	13	18	31
		% within BKV-DNA	41.9%	58.1%	100.0%
		% within anti-BK-IgG	11.4%	19.6%	15.0%
	Negative	Count	101	74	175
		% within BKV-DNA	57.7%	42.3%	100.0%
		% within anti-BK-IgG	88.6%	80.4%	85.0%
Total		Count	114	92	206
		% within BKV-DNA	55.3%	44.7%	100.0%
		% within anti-BK-IgG	100.0%	100.0%	100.0%
Chi-Square test		0.103			

The data sequence of samples revealed compatibility with the reference BK virus for the LT antigen gene table. 2 sequences then were deposited in the GenBank with the accession numbers LC718551.1 and LC718552.1. Local BKV isolates were assigned as (5BKF and 6BKF).

Discussion:

Based on serological studies, The overall IgG seroprevalence of BKV is approximately 82%. Significantly, high antibody levels correlated with higher BKV-specific CD4 T-cell activity. The primary infections caused by BKV have not yet been adequately defined(5-7). BKV can remain active throughout an individual's lifetime, and the cells of the proximal renal tubule and mononuclear blood cells may serve as tissue sanctuaries for the virus.(4) In this study, BKV-IgG was investigated in serum of both RTRs and control using ELISA and the frequencies of 114(55.3%) have a positive result for IgG and there was no significant difference between seropositivity of BKV IgG antibody among the studied groups, p-value =0.191. Recipients' seropositive rates drop due to BKV replication early after transplantation and after chemotherapy for rejection, when immunosuppression is high and immune control is low. However, the relative contributions of the humoral, cellular, and innate immune compartments to immune control are not well understood.(5) Children who test negative for BKV seropositivity are more likely to develop BKV viremia and nephropathy. Shah found that seropositive donors and seronegative recipients were both 43% more likely to develop a BKV infection that could be defined by its antibody response.(6)

According to Bohl et al. BKV viremia was 50% more likely to be acquired by donors and recipients who tested positive for antibodies to the virus. Both sets of researchers found that 10% of seronegative donors and recipients contracted BKV throughout the course of their research.(5)

In our finding result, there were no significant differences of seronegative BK-IgG in recipients groups was 56.5% whereas in control groups 43.5% Consequently of this, Although BKV-specific antibodies may aid in the immune response, they may also signal the possibility of the virus becoming active again, by decreasing the levels of immune suppressants, BKV-specific IgG antibody titers rise, BKV-specific cellular immunity is developed, viremia is cleared, and graft function is maintained.(7) There does not appear to be much of an effect from the prevalence of BKV antibody. According to our research, the explanation provided by Chen et al. supports the hypothesis that there is a correlation between a positive serology for BK-IgG and elevated levels of serum creatinine, individuals with BKV nephropathy who had high BKV antibody titers but inadequate cytotoxic T cell responses had persistent viremia and elevated creatinine levels. Even though these individuals had healthy cytotoxic T cell responses, this was nonetheless the case.(8) Repeated BKV viremia was linked to a low frequency of IFN-producing cells, as found by Comoli et al. despite repeatedly increased BKV antibody titers. This was the case even when BKV antibody titers had been high for a long time.(9)

In contrast, a high cytotoxic T cell response but modest antibody titers resulted in viremia clearing and creatinine levels returning to normal in the recipients. And BKV nephropathy has already affected these patients.(8&9)

BK virus which is the etiological agent for BKVN, currently, there is no specific treatment for BKVN except for reducing immunosuppression, risking the reoccurrence of rejection. PCR was used only in a very small number of studies to investigate the identification of viral infections or reactivations in Iraqi kidney transplant recipients.(10) The detection of BKV in the blood, which can be quantified by measuring the threshold cycle (Ct), is a valuable tool not only for BKV nephropathy diagnosis but also for monitoring the patient's response to treatment. However, regardless of the amount of virus present, real-time PCR is a very sensitive and specific approach for identifying BKV in blood components (plasma or serum). Utilizing Real-time PCR kit that can identify moderately low levels of viral antigen is critical for screening tests.(11)

In current study the results on the finding of BKV DNA from plasma samples examined of the total 206 were 31(15.0%) positive BK-viremia, This falls about in the middle of the overall incidence of BK viremia, which may be anywhere from 11 and 29 percent.(12)

This conclusion is consistent with the findings of (Al-Obaidi et al.,2015), in which BK viremia was shown to be present (12.12 %) in 12 out of 99 Iraqi RTRs. BK viremia is considered to be the outcome of a more widespread infection that led to significant tubular damage Once the tubular basement membranes burst and the virus enters the circulation through the blood capillaries.(13) But there was controversy in the results of two groups of local research articles. The first group that of (Mohammed et al.,2016 reported a very low percentage of Bk-viremia was 2/72(2.8%), and the second group (Shamran et al.,2016) showed a high percentage like our finding (11/50 (22%) in Iraqi.(11&13) In contrast to our finding, where there was a positive result of RT-PCR for BKV- DNA in the control group (8.0%), several local researchers reported no detection of BKV_DNA in their control group such as (Al-Obaidi et al.,2015)and (Shamran et al.,2016). The analytical sensitivity of the kit utilised for the viral DNA quantification will be responsible for this variation. The outcome can also be influenced by the target gene. However, Shamran et al. (2016) utilised a kit that targets small T-antigen, whereas the kit we and Al-Obaidi et al.

employed targets the large tumor-antigen gene (LTA). In a global cohort examination of patients with Greek RTRs, the incidence of BK viremia was as high as 31 percent. (11&14)

In general, variations in sample type, size, DNA extraction procedures, primers, probe sequences, and BKV strain DNA used to generate the standard curve might influence quantification findings and introduce clinically meaningful variability. The low prevalence of BKV in samples from the renal transplant and control groups was probably due to the small number of samples size, conclusions about these results are premature and may represent an epidemiological feature of the virus in Iraq, which may be related to the population density and environmental conditions, which are of paramount importance for BKV transmission. (15)

To explain the relation between the prevalence of BK IgG and BK viremia by investigate whether the intensity of infection is associated with the humoral immune response. In the current study, we evaluated BKV-specific IgG levels in renal transplant recipients with positive and negative BK viremia and correlate it with the overall severity of infection, there was statistical no significant $p=0.103$. These results disagree with a study reported by Randhawa et al, the cross-sectional study showed that renal transplant recipients with BKV viremia had higher IgG BKV-specific antibody levels than those without infection.(16) The present study adds to these previous findings and shows that recipient seropositivity may not prevent infection but may mitigate the severity of the infection. The presence of BKV-specific antibodies as measured by ELISA was not associated with prompt clearance of viremia or viruria. The reason for the small rise in BKV-antibody titer in the recipients negative a BKV infection is unclear. One might expect antibody titers to be reduced due to immunosuppressive drugs that showed to suppress the humoral immune response. (17&18)

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