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ORIGINAL ARTICLE

Transplant failure in relation to BK viremia status among kidney transplant recipients in Jordan

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ABSTRACT

Background and Objectives: BK polyomavirus (BKPyV) poses a significant threat to kidney transplant (KT) recipients due to immunosuppression, leading to BK-associated nephropathy (BKVN) and reduced transplant survival. This study aimed to determine the prevalence of BKPyV among kidney transplant recipients in Jordan and to evaluate the association between BKPyV activity and kidney transplant outcomes.

Materials and Methods: A retrospective observational study was conducted at the Jordanian Royal Medical Services Hospital (JRMS) from 2021 to 2024. Blood samples (n=157) from kidney transplant recipients were collected, and quantitative real-time PCR was performed to detect BKPyV DNA.

Results: The prevalence of BKPyV infection among kidney transplant recipients was 40.8% (n=64). Transplant failure occurred in 36% of cases (n=57), with BKPyV-DNA viremia observed in 74% of those with transplant failure (n=42). The prevalence of infection was significantly higher in patients under 18 years of age (81%, $p<0.001$) and in males (72%, $p<0.001$). BKPyV infection increased the odds of transplant failure tenfold.

Conclusion: In Jordan, the prevalence of BKPyV among kidney transplant recipients is high, particularly in males and younger patients. BKPyV significantly increases the risk of kidney transplant failure. Other studies are needed to further elucidate the impact of BKPyV on kidney transplant rejection and complications.

Keywords: BK virus; Polyomavirus infections; Kidney transplantation; Immunosuppression; Prevention

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INTRODUCTION

BK polyomavirus (BKPyV) is a ubiquitous virus that infects most individuals during childhood and forms persistent infections in the kidney or the urinary tract. In normal adults, the sero-prevalence of BKPyV is very high, as previous studies show that approximately 80% to 90% of adults are seropositive for the virus (1-3). This high sero-prevalence shows that the majority get exposed to BKPyV sometime during their lifetime, usually without any harmful clinical effects (3). First reported in 1971 from a kidney transplant recipient, BKPyV is a member of the family *Polyomaviridae* and is noted for inducing latent infection, which can be reactivated, most notably in immunocompromised individuals, such as organ transplant recipients (4, 5).

In kidney transplant patients, there is a high risk of BKPyV reactivation due to immunosuppressive therapy that is employed to prevent graft rejection. BK viremia can develop in about 30% of them, and BK virus-associated nephropathy (BKVN) can occur in about 1-10%, which may have a significant loss of graft function unless treated optimally (1, 3).

BKVN development is controlled by the interaction between the virus and the host immune system. The cellular immune response, especially CD4+ and CD8+ T cells, plays a pivotal role in BKPYV suppression of replication (4, 6, 7). The immunosuppressive treatment provided to transplant recipients can impair this immune response, leading to viral replication and nephropathy (4, 7).

BKVN is increasingly recognized as an important cause of kidney transplant loss. Clinical presentation usually begins with asymptomatic viremia, which may progress to nephritis and renal failure (4, 7, 8). Routine screening for BKPyV in urine and blood is essential for detection. Current treatment approaches aim to decrease immunosuppression levels and utilize antiviral medications, though achieving effective prevention continues to be a challenge (4, 7, 8).

To the best of our knowledge, there is limited data about the prevalence of BKPyV among kidney transplant recipients in Jordan. Therefore, this study aimed to investigate the prevalence of BKPyV and to determine the association between BKPyV activity and kidney transplant outcomes in Jordan.

MATERIALS AND METHODS

Study design and population. A retrospective observational study was conducted from 2021 to 2024 at the Jordanian Royal Medical Services Hospital (JRMS). Samples and demographic data were collected from the virology department. The study included all patients aged one year and older who had received KT, regardless of gender. However, patients with active Cytomegalovirus (CMV) infection at the time of BKPyV diagnosis or within a defined period (e.g., 3 months), patients who had received a multi-organ transplant (e.g., kidney and liver), and patients undergoing re-transplantation during the study period, were excluded from the analysis.

Sample collection. Blood samples (n=157) were collected in EDTA tubes, centrifuged for 10 minutes at 3000 rpm, and stored as plasma at -20°C for further analysis.

DNA extraction. Following the manufacturer's protocol, DNA was isolated from 400 µL of plasma using EZ1&2™ Virus Handbook EZ1&2 Virus Mini Kit v2.0(Qiagen, Hilden, Germany). Extracted DNA samples were measured for purity and concentration using the NanoDrop-2000 (Thermo Fisher Scientific, Waltham, Massachusetts) spectrophotometry. Purity was evaluated by the absorbance ratio at 260/280 nm, with values between (1.8 and 2.0) considered indicative of high purity. DNA concentrations were quantified at 260 nm and recorded in ng/µL. Isolated DNA samples were subsequently stored at-20°C until further use (9).

BKPyV DNA detection by Real-time PCR. The BKPyV DNA detection and viral load quantitation were detected using the Artus® BK RG PCR Kit (Qiagen, Hilden, Germany) on the Rotor-Gene Q instrument. Following the manufacturer's instructions, 15 µL of DNA was used to identify BKPyV DNA and quantify the viral load. This detection technique uses 15 µL BKPyV for real-time PCR amplification. The final extraction volumes were 25 µl, which was added to 10 µl of the BK viral RG Master, containing 27 enzymes and reagents for the selective amplification of a 274 bp section of the BK viral genome. The fluorescence reporter dyes were used for direct detection of the amplified product. Thermal cycling conditions were as follows: initial denaturation at 95°C for 10

minutes, followed by 45 cycles of amplification with denaturation at 95°C for 15 seconds, lowered to 65°C annealing temperature for 30 seconds, extended at 72°C for 20 seconds. The BKPyV copy number was calculated for viral load measurement by comparing each sample's fluorescence intensity to a standard curve created using the quantitative standards included in the kit (BKV RG QS1-QS4). Results are reported as copies/ml thanks to these standards, which enable accurate quantification (10).

Statistical analysis. Statistical analysis was performed using IBM SPSS statistical software version 25. Categorical variables were presented as frequencies and percentages. Numeric variables were expressed as mean ± standard deviation (SD). The association between categorical variables was assessed using chi-square test. P-values < 0.05 were considered statistically significant. The association between BKPyV activity and kidney transplant outcomes are presented as odd ratios (OR) and their 95% confidence interval (95% CI).

Ethical consideration. All methods were performed in accordance with the Declaration of Helsinki. The study followed all ethical considerations. It was approved by the Institutional Review Board (IRB) of Zarqa University (IRB/ZU/2024/10).

RESULTS

Patients characteristics. The study included 157 samples from kidney transplant patients admitted to Jordanian Royal Medical Services between 2021 and 2024, divided into seven age groups, with a mean age of 13.1 ± 6 years. The distribution of patients by age showed that, ages 11-15: The largest group, with 84 patients (54% of total), ages 26-30: The lowest group, 3 patients (2% of total) as summarized in Table 1.

The prevalence of BKPyV among kidney transplant recipients. Out of the 157 patients enrolled in the study, about 64 (40.8%) tested positive for BKPyV, while approximately 93 (59.2%) had negative results for the virus, as illustrated in Fig. 1.

Distribution of BKPyV according to kidney transplant outcomes. Among the 157 kidney transplant recipients admitted to JRMS between 2021 and 2024, 100 (64%) had successful kidney transplants, while 57 (36%) experienced graft rejection or returned to dialysis, indicating transplant failure. Of the successful transplant recipients, 22 (22%) tested positive for BKPyV, while 78 (78%) were BKPyV-negative, demonstrating a significantly higher prevalence of BKPyV negativity in this group ($p < 0.001$). In contrast, among the 57 failed transplants, 42 (74%) had active BKPyV viremia, a significantly higher prevalence ($p < 0.0001$), as detailed in Tables 2 and 3.

Table 1. Distribution of enrolled Patients by age groups

Patient age group	Total sample (N=157) N (%)
1-5	8 (5)
6-10	38 (24)
11-15	84 (54)
16-20	12 (8)
21-25	5 (3)
26-30	3 (2)
>30	7 (5)
Patient age (Mean ± sd)	13.24 ± 6

sd: standard deviation; N: Number; %: percentage

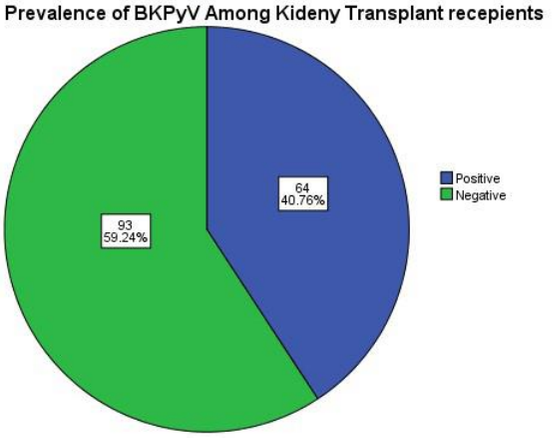

Fig. 1. The prevalence of BKPyV among 157 kidney transplant recipients

Distribution of BKPyV according to gender. Among the 157 kidney transplant recipients, 103 (66%) were males and 54 (34%) were females showing a higher significance of prevalence in males ($P<0.001$). While 57 out of the 157 kidney transplants failed, with a statistically significant difference between males (41,72%) and females (16,28%) ($P <$

Table 2. kidney transplant outcomes among 157 kidney transplant recipients

Kidney transplant recipients		P value
Failed (N,%)	Success (N,%)	
(57,36%)	(100,64%)	0.001

N: Number, %: Percentage, BKPyV: BK polyomavirus

Table 3. BKPyV status and kidney transplant outcomes

Kidney transplant outcomes	BKPYV status		P value
	BKPYV +ve (N,%)	BKPYV -ve (N, %)	
Failed	(42,74%)	(15,26%)	< 0.0001
Success	(22,22%)	(78,78%)	< 0.001

N: Number, %: Percentage, BKPyV: BK polyomavirus

0.001). Among the 42 patients with failed transplants and active BKPyV viremia, 29 (69%) were males and 13 (31%) were females (P = 0.014), indicating a higher risk of dialysis or graft loss after the kidney transplant operation in male patients, as shown in Table 4.

Association between BKPyV (Viral Load) and kidney transplant outcomes. The BKPyV-DNA load in patients ranged 8*10^3 copies/ml. Analysis revealed no statistically significant association between viral load and kidney transplant outcomes (success or failure), as detailed in Table 5.

Distribution of kidney transplant failure associated with BKPyV across age. According to the kidney transplant failure linked to BKPYV, there is a notably significantly high prevalence of infection among patients under 18 years of age (81%)(p<0.001), as shown in Table 6.

Association between kidney transplant failure and BKPyV status. Among the BKPyV-positive patients, 65.63% (42 out of 64) experienced transplant failure. In BKPyV-negative patients, 16.13% (15 out of 93) experienced transplant failure.

To evaluate the association between BKPyV status and kidney transplant outcomes, we calculated the odds ratio (OR) as shown in Table 7. The total number of patients was 157, with 64 BKPyV-positive patients (22 successes, 42 failures) and 93 BKPyV-negative patients (78 successes, 15 failures).

The odds of transplant failure for BKPyV-positive patients are 42/22 = 1.91. The odds of transplant fail-

Table 5. Association between BKPyV (Viral Load) and kidney transplant outcomes in 64 cases

Viral Load	Kidney Transplant		P value
	Success (N,%)	Failed (N,%)	
1*10^3-5*10^3	(20,37.7%)	(33,62.3%)	0.074
5.1*10^3-9.1*10^3	(2,18%)	(9,82%)	0.564

N: Number, %: Percentage

Table 6. Distribution of kidney transplant failure among 42 patients with positive BKPyV across age

Kidney transplant failure with positive BKPyV, (42 cases)		P value
<18 years (N,%)	>18 Years (N,%)	
34,81%	8,19%	< 0.001

N: Number, %: Percentage, BKPyV: BK polyomavirus

Table 4. Prevalence of BKPyV according to sex in 157 kidney transplant recipients

Kidney transplant recipients (N=157)		P value	Kidney transplant (Failed, N=57)		P value	Kidney transplant (Failed with positive BKPyV, N=42)		P value
Male (N,%)	Female (N,%)		Male (N,%)	Female (N,%)		Male (N,%)	Female (N,%)	
(103,66%)	(54,34%)	>0.001	(41,72%)	(16,28%)	0.001	(29,69%)	(13,31%)	0.014

N: Number, %: Percentage, BKPyV: BK polyomavirus

Table 7. Cross-tabulation of BKPyV status and kidney transplant success/failure

BKPYV Status	Kidney transplant (Success)	Kidney transplant Failure	Total
Positive	22	42	64
Negative	78	15	93
Total	100	47	157

BKPyV: BK polyomavirus

ure for BKPyV-negative patients are 15/78 = 0.19. The OR for transplant failure in BKPyV-positive patients compared to BKPyV-negative patients is 1.91 / 0.19 = 10.05.

Thus, the odds of transplant failure are approximately 10.05 times higher for BKPyV-positive patients compared to BKPyV-negative patients. This suggests a strong association between BKPyV positivity and transplant failure.

DISCUSSION

BKPyV is notably prevalent among kidney transplant recipients. Research suggests that in the first year following transplantation, about 30% of these individuals may develop BK viremia (4, 11). Approximately 1-10% of these individuals may develop clinically significant BKV N, a leading cause of transplant failure (4, 11).

The major finding of our study showed a BKPyV prevalence of 40.8% among kidney transplant recipients, which coincides with previous studies that indicated a prevalence of 49.3% (12).

The odds of transplant failure are approximately 10.05 times higher for BKPyV-positive patients compared to BKPyV-negative patients. Numerous studies have indicated that BKVN has been linked with a 2-3-fold risk of graft loss. For example, a systematic review demonstrated that patients with BKVN had a high risk of graft loss compared to non-infected patients (13). Furthermore, earlier studies have indicated different rates of BKPyV infection among kidney transplant recipients. For instance, some studies showed that the BKVN develops in about 1% to 10% of renal transplant recipients, with others reporting that as many as 50% of untreated BKPyV infection patients develop graft loss (11). This study illustrates the ongoing importance of BKPyV infection among kidney transplant recipients and the necessity for ongoing monitoring and treatment to minimize this complication.

Based on our evidence, male recipient kidney transplant failure is significantly prevalent when BKPyV is positive. This finding was in agreement with a previous study that reported that male gender was independently linked with increased rates of BKPyV viremia, which indicated that males are more pathologically sensitive to BKPyV because of biological or immunological reasons (14).

A significantly elevated prevalence of BKPyV infection in kidney transplant recipients under 18 years old was seen. This result is consistent with other research; for instance, one study found that 19% of pediatric renal transplant recipients had BKPyV in their urine, and a significant portion of them had BKPyV nephropathy (15). Conversely, some studies have found that older recipient age is associated with a higher risk of developing BKVN and subsequent graft failure. For instance, a study indicated that recipient age was an independent risk factor for BK viruria progressing to BK viremia, suggesting that older patients may have a diminished immune response to BK virus reactivation, leading to worse outcomes (12).

Moreover, the results show no statistically significant correlation between kidney transplant outcomes and viral load.

The results of previous studies on the correlation between BKPyV DNA load and transplant success or failure are conflicting. While some studies find no significant correlation, others indicate that higher viral loads are linked to an increased risk of BKVN and graft failure. For instance, a study showed that larger BKPyV-DNA copy numbers are connected to both the severity of the disease and an elevated likelihood of developing nephritis (7).

Other studies utilized BKPyV DNA levels larger than 7,000 or 10,000 copies/ml in plasma as a threshold for substantial infection, which is consistent with

BKVN (7). However, nephritis can occur with BKPyV DNA levels less than 7,000 copies/ml (7). While these thresholds indicate an increased likelihood of developing BKVN, it is crucial to be aware that nephritis may develop with BKPyV DNA levels under 7,000 copies/ml (7). Conversely, other studies have reported that even within a range of viral loads, the outcomes can vary significantly based on individual patient factors, including immune response and the intensity of immunosuppression (16).

These findings highlight the complexities of controlling BKPyV in kidney transplant recipients. A more complete strategy, including monitoring the patient's immunological status and adjusting immunosuppressive medicine may be required to enhance outcomes.

This finding further supports the contention that there is a strong association between BKPyV positivity and an increased risk of graft loss. Previous Studies have shown that BKVN occurs in 1% to 10% of kidney transplant recipients and is a significant cause of graft loss (17). Additionally, the presence of BKPyV has been linked to poorer graft and patient survival rates, highlighting the critical need for monitoring and managing BKPyV in kidney transplant recipients (17, 18). This correlation emphasizes the importance of proactive management strategies in kidney transplant recipients to address the challenges posed by BKPyV.

Study limitations are typically related to those of retrospective observational studies, in which routinely collected electronic health record data are used, with some missing data, residual confounders, and potential biases. Additionally, the small sample size drawn from only one hospital limits the generalizability of our study findings. We recommend further studies with a broader range of data and a larger sample size to make more correlations, and to perform whole genome sequencing to provide a clear picture of the diversity of BKPYV strains in kidney transplant patients.

CONCLUSION

This study represents the first investigation into the prevalence of BKPyV among kidney transplant recipients in Jordan. The BKPyV plays a critical role in kidney injury risk, such as BKVN. In Jordan, the prevalence of BKPyV among kidney transplant recipients was 40.8%, with a high frequency in males and in patients under 18 years of age. BKVN increases the risk of kidney transplant failure. Future studies with larger samples are needed to confirm these findings and explore prevention strategies, in addition to the standard practice of screening kidney transplant recipients for BKPyV.

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