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DETECTION OF THE BK VIRUS IN POST-TRANSPLANT PATIENTS IN A TERTIARY CARE HOSPITAL

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ABSTRACT
Background: Polyomaviruses are small (45 nm) non-enveloped circular double-stranded DNA and belong to the Polyomaviridae family, with Polyomavirus as the only genus. The polyomaviruses are omnipresent. The primary sites of BK virus appearance are the kidney tubular epithelial cells and urinary bladder surface transitional cells. To detect the BK virus in post-transplant patients by molecular methods. **Materials and methods:** Specimens of 88 patients were collected aseptically in Vials. Nucleic acid was extracted manually and processed in real-time PCR for identification of the BK virus. **Result**: This study analyzed 88 samples from suspected BK virus patients from January 2022 to January 2023.There were 88 samples tested, 24(27.27%) were positive and 64(72.72%) were negative. **Conclusion**: The prevalence pattern of BK virus presented in post-transplant patients 24(27.27%) were positive. BKV induces both difficult diagnosis and treatment; transplant recipients are recommended to be under strict surveillance and receive early intervention.

INTRODUCTION

In accordance with the International Committee on Taxonomy of Viruses, polyomaviridae contains 89 recognized virus species that belong to four genera as well as nine species that are not referred to as genera [1]. There are 13 species of polyomaviridae known to infect humans out of all the polyomaviridae [2, 3]. Most of these viruses occur in the human population but are rarely associated with human diseases. Polyomaviruses are omnipresent in nature and species-specific - they cause diseases in humans (JCV, BKV), monkeys (SV40), and mice (mouse polyomavirus) [4]. The Polyomaviridae family of viruses includes only Polyomavirus as a member species, which is a small, non-enveloped virus (45 nm) composed of 72 capsomers with icosahedral symmetry.

A virus was first detected in 1971 in a patient known as BK, whose initials inspired the term. The 'first' patient received a kidney transplant 3 months earlier and presented with anuria and pain around the graft [5]. It was discovered that ureteric obstruction had resulted in ureteric obstruction that, later, was corrected surgically. Biological samples and ureteral segment

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excised during surgery were examined for exposure to an unknown virus. In renal transplant recipients, BKV incidence was associated with morbidity [6-9].

The BK virus (BKV) is a polyoma virus commonly acquired during childhood. There is a seroprevalence rate of 80–90% in adults infected with BKV. There are two main sites where BKV appears, the tubular epithelium of the kidney and the transitional cells of the urinary bladder surface. However, BKV replication can become active in a number of clinical situations resulting in impaired immune competence, including solid organ transplantation, bone marrow transplantation, AIDS, pregnancy, multiple sclerosis, and chemotherapy or biologic therapy [10]. In recent years, BKV has become one of the leading causes of morbidity in renal transplant recipients due to the use of potent immunosuppressive drugs and the enhancement of viral surveillance protocols.

There is an estimated seroprevalence of BK virus in 50% of children under the age of five, and up to 90% of the adult population [11,12]. Primary BKV infections usually occur between the ages of 3 and 4 [13]. A variety of routes can transmit the virus, including faecal-oral, respiratory, blood transfusions, organ transplants, and transplacentally [14]. A BKV infection (with or without trivial symptoms) does not completely eliminate the virus from the host. It may remain latent in renal tubular epithelial cells for life, where the immune system controls its replication [15]. The virus can also be found in the liver, lungs, brain, and lymph nodes. Asymptomatic and clinically insignificant viremia occurs in healthy patients up to 20%, and in immune suppressed individuals and pregnant women, incidences are higher [16,14].

In renal transplant recipients, reactivation of latent viruses occurs within the first three months after immune suppression is implemented. There is no precise mechanism for the reactivation of an infection [17]. The microbiologic characteristics of the virus determine reactivation, the presence of factors triggering its activation in tissues (kidney injury, rejection of grafts, ischemia, drug toxicity), the quantity and nature of viruses present, the severity of the patient's immune deficiencies (such as serological status), the total amount of immune suppression and the host-graft relationship [18,19].

Polyomavirus-associated nephropathy (BKVAN) is one of the main complications of BKV infection after kidney transplantation [20-23]. In 5 to 15% of patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT), polyomavirus-associated hemorrhagic cystitis (BKVHC) occurs [24-26]. Cancer and ureteral stenosis are other complications associated with BKV infection [4,27-30]. Despite being rare, BKV associated pathologies have also been seen in patients undergoing solid organ transplantation (SOT) or those with inherited, acquired, or drug-induced immunodeficiency's [27,31]. There is also pneumonitis, retinitis, liver disease, meningoencephalitis, and BKVAN and BKVHC [32]. The above-mentioned viruses can be detected using various methods, such as antigen-based assays, serology, viral cultures such as the shell vial technique, serology, histopathological examination (HPE), and in-situ hybridization (ISH). Since 90% of adults have seroprevalence of Herpes viruses, serological tests have limited value. It is currently possible to diagnose these viruses using molecular methods because of their rapidity and established sensitivity (97%) and specificity (98%) [33]. In patients suspected of having BKV by RT PCR, quantitative DNA PCR has a sensitivity of 95.9 % [34].

MATERIALS AND METHODS

The present study is conducting an observational study based on laboratory data. A study was conducted at Mahatma Gandhi Medical College & Hospital, Jaipur (Rajasthan) from January 2022 to January 2023. This study processed 88 Blood and urine samples. Demographic data (such as age, sex, in -patient, out-patient status) of the patients were recorded.

Samples were transferred in to multiple storage vials with appropriate labelling and frozen at -80°C before analysis of viral load by quantitative PCR and for detection of viruses by RT PCR In vitro nucleic acid amplification is used to quantify the presence of DNA of the BK virus in human plasma and urine using the artus BK Virus PCR Kit. The polymerase chain reaction (PCR) is used in this diagnostic test kit [35]. Polymerase chain reactions (PCR) are used to detect pathogens by amplifying specific regions of their genomes. Fluorescent dyes are used to detect real-time PCR products. These probes are typically attached to oligonucleotide probes specifically targeting the amplified products.the accumulating product can be detected and quantified without having to reopen the reaction tubes [35]. There are four quantitation standards contained in this package (BK Virus QS-1-4) for analyses. All samples are handled as previously purified samples and are diluted with the

same volume (15 ml). A PCR Instruments standard curve can be generated using the four quantitation standards defined in the "Edit Samples" dialog box.

Cycling Green fluorescence channel detects a signal. DNA from the sample contains BK virus DNA, according to results of the analysis. The detection of a signal in the Cycling Orange channel is not necessary in this case, because a high concentration of BK virus DNA (positive signal in the Cycling Green channel) can result in a reduced or absent internal control signal in the Cycling Orange channel.

In the fluorescence channel Cycling Green, no signal is detected. In the Cycling Orange channel, a signal from the internal control is also displayed. The sample does not contain any DNA from the BK virus. This can be considered a negative. In the case of a negative BK Virus PCR, the detected signal of the internal control rules out the possibility of PCR inhibition. Cycling Green and Cycling Orange channels do not show any signal. The results are not conclusive [35].

RESULTS AND DISCUSSION

The present study is a laboratory-based descriptive observational study. This study was carried out between January 2022 and January 2023 at the Mahatma Gandhi Medical College &Hospital, Jaipur (Rajasthan). 88 whole Blood and Urine sample received in Department of Molecular biology, Mahatma Gandhi Medical College& Hospital, Jaipur (Rajasthan) were included in the study.

The BK virus is a polyomavirus that can cause an infection following a kidney transplant. The BK virus latently infects uroepithelium and renal tubular epithelium. BK virus nephropathy is associated with old age, diabetes, and immunosuppression during organ transplantation, especially renal transplantation. As a result of immunosuppression, it may reactivate and replicate, causing tubule interstitial cell lysis and eventually causing graft invasion.

The present study analyzed 88 samples from suspected BK virus patients from January 2022 to January 2023. There were 88 samples tested, 24 (27.27%) were positive, and 64 (72.72%) were negative. There were also studies conducted in India. Almost the same frequency of positivity was found in the studies.

Table 1: Distribution of BK virus positive & negative samples

Total sample	Positive	Negative
88 (100%)	24 (27.27%)	64 (72.72%)

Table 2: Detection of BK viruses by real time quantitative PCR in whole blood and urine samples

Sample Type	Positive No of sample
Blood sample	18 (75%)
Urine sample	6 (25%)
Total sample	24 (100%)

Table 3: Distribution of BK viral load (copies/ml) and logs

BK viral load (copies/ml) and Log	Total number
1 -1000 copies /ml	14 (58.33%)
>1000 copies /ml	10 (41.66%)
Total	24 (100%)
<3.4 log	14 (58.33%)
>3.4 log	10 (41.66%)
Total	24 (100%)

Table 4: Distribution of IPD and OPD Samples

Total Positive Sample	IPD	OPD
24 (100%)	4 (16.66%)	20 (83.33%)

Table 5: Sex distribution of the study participants

Total Positive Sample	Male	Female
24 (100%)	16 (66.66%)	8 (33.33%)

Table 6: Age distribution of the study participants

Age group	Total no of sample	Positive	Negative
1-20	8 (100%)	4 (50%)	4 (50%)
20-40	26 (100%)	5 (19.23%)	21 (80.76%)
40-60	48 (100%)	14 (29.16%)	34 (70.83%)
60-80	6 (100%)	1 (16.66%)	5 (83.33%)
Total	**88 (100%)**	**24 (27.27%)**	**64 (72.72%)**

Molecular methods detect and distinguish the BKV DNA from other polyomavirus (Randhawa 2005) [36]. The sensitivity of BKV PCR in urine and blood samples of PTRs is similar (100%), though there can be variations in specificity (Hirsch et al., 2002; Kuppachi, 2013) [37,38]. In approximately a third of renal transplant recipients with viruria, viremia will lead to

BKVN, which is estimated at 1-10 percent (Sawinski D 2015). During reactivation BKV is first detectable in urine and several weeks later It detectable in blood. In the present study 6(25%) were reported viruria and 18(75%) were reported viremia. Similar studies have reported that among 17 to 35% of BKV viruria and 21 to 65% developed BKVN (Thakur *et al*., 2011; Sood *et al*., 2012; Hirsch 2013) [39-41]. In our study BK viral load (copies/ml) 1 -1000 copies /ml present in 14(58.33%) and >1000 copies /ml present in 10(41.66%) and <3.4 log present in 14(58.33%) and >3.4 log present in 10(41.66%) This is similar study studies. BKV infection after kidney transplantation may progress gradually from initial viruria through viremia and in a subgroup of 20–40% of viremic patients to histological changes classified as BKV [42]. Currently, BKV diagnosis is based on PCR-based viral load analysis in the plasma and urine. Both quantitative and qualitative tests are being used, with later being much more sensitive. Sustained high urine viral loads of $>7_{log}10$ copies/ml correlate with the onset of viremia [43].

Sustained plasma BKV-DNA load higher than $4_{log}10$ copies/ml is considered as presumptive BKV [44]. Literature data indicate that the urine BKV DNA $>7_{log}10$ copies/ml and/or plasma BKV DNA $>4_{log}10$ copies/ml indicate possibility of BKV. In the present study, among 24 specimens with positive for BK virus. 4 (16.66%) sample were positive from IPD and 20 (83.33%) sample were positive from OPD. Sex distribution in our study 16 (66.66%) were BK positive in male Patients. There were 16 samples BK positive male patients 6 (37.5%) were reported viruria and 16 (62.5%) were reported viremia. 8(33.33%) were BK positive female patients. The age group most commonly affected was 1-20 years (50%) followed by 40-60 years (29.16%) and 20-40 years (19.23 %). 60-80 (16.66%) years. This was similar to study conducted by S. Gonzalez et al. [45]. Immunosuppression is clearly a major risk factor for BKV. This is evidenced by the increase in viral replication observed in immunosuppressed populations and the decrease in viral replication that follows immunosuppression reduction.

CONCLUSION

Polyomaviruses are ubiquitous, and BK virus appear primarily in kidney tubular epithelial cells and the bladder's surface transitional cells. Usually, the virus stays latent; however, in different clinical situations of impaired immunocompetence, it can become active and cause graft failure and life-threatening complications. Since BKV induces both difficult diagnosis and treatment, transplant recipients are recommended to be under strict surveillance and receive early intervention. The study analysed 88 samples from suspected BK virus patients from January 2022 to January 2023.There were 88 samples tested, 24 (27.27%) were positive and 64 (72.72%) were negative.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Vinita Choudhary contributed to the conceptualization, data curation, and formal data analysis. She also contributed in writing the first draft of the manuscript, its review and editing. Chetan Choudhary and Pushpendra Saraswat contributed by supervising the work and investigations. All authors contributed to a critical review of the manuscript for important intellectual content.

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