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## Prevalence of cytomegalovirus and BK polyoma virus infection in post-renal transplant patients in a tertiary care centre in South India

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### ABSTRACT

**Background:** Viral infections are a significant cause of graft loss and dysfunction in kidney transplant recipients. Cytomegalovirus and BK polyomavirus have often been explained as the most common viral etiological agents.

**Objectives:** The current study was undertaken to assess the prevalence of cytomegalovirus and BK polyomavirus infection in post-renal transplant individuals in a tertiary care centre in South India and also to study the histopathological changes of such infections in the kidney allograft biopsies.

**Patients and Methods:** We conducted a retrospective investigation of 100 cases using archival renal biopsy specimens which were subjected to immunohistochemical stains to detect cytomegalovirus and BK polyoma virus. These findings were then correlated with the histopathological alterations detected in H&E sections.

**Results:** We detected the prevalence of cytomegalovirus in 7% and BK polyoma virus in 3%. Cytomegalovirus was statistically associated with pre- and post-transplant infections along with diabetic status. We noted that, out of the seven patients who were immunohistochemically cytomegalovirus positive, only five had positive cytomegalovirus IgM status. With BK polyoma virus, we noted a statistical significance with pre- and post-transplant infections. However, we did not find evidence of cytomegalovirus and BK polyoma virus co-infection in any of the renal allograft biopsies.

**Conclusions:** Routine immunohistochemical evaluation of cytomegalovirus and BK polyoma viral infections in kidney allograft recipients must be done, especially in those with pre- and post-transplant infections and diabetes.

### *Implication for health policy/practice/research/medical education:*

Routine immunohistochemical evaluation of cytomegalovirus and BK polyoma viral infections in renal allograft recipients must be done, especially in those with pre- and post-transplant infections and diabetes.

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### 1. Background

Viral infections are an important cause of allograft dysfunction in kidney transplant recipients. These infections have been observed with increasing frequency in the recent years mainly due to the use of more potent

immunosuppressive therapy. Cytomegalovirus (CMV) and BK virus has been described as the most common viral etiological agents in kidney allograft recipients in the West. Detection of such viral infection associated graft dysfunction requires a judicious decrease in

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immunosuppression and monitoring for acute rejection. However histological features of viral infection involving the kidneys are not very distinct and have a morphological overlap with features of rejection. Graft rejections have diminished due to increasing potency of immunosuppressive regimens but the susceptibility to infections continues to rise.

CMV remains the most significant viral pathogen despite availability of effective antiviral drugs and validated strategies for prophylactic, pre-emptive and therapeutic intervention (1). With frequent recurrences and increasing rate of antiviral resistance, CMV replication can affect almost every organ system. Together with secondary long-term effects, CMV significantly decreases graft and patient survival after solid organ transplantation. CMV nephritis can arise from reactivation of recipient's latent strain or from primary infection or super-infection with virus from donor kidney. CMV infection in the allograft kidney has a wide-ranging spectrum of histological appearance varying from scattered inclusion bodies without any inflammatory response to severe interstitial nephritis.

Asymptomatic primary BK polyomavirus infection is seen in most individuals since early life. It remains latent in the urinary tract and gets reactivated due to drug induced immunosuppression in renal transplant recipients. A high prevalence of BK virus nephropathy has been earlier reported from India in post renal transplant patients (2).

## 2. Objectives

This paper highlights our understanding of CMV and BK polyomavirus infections in renal allograft recipients in a tertiary care setting in South India. The prevalence of such infections was assessed by using immunohistochemistry along with clinical and histopathological correlation.

## 3. Patients and Methods

### 3.1. Patients

The present study is a retrospective analysis of 100 archival renal allograft biopsy specimens done for clinically suspected rejection between 2009 and 2012 at Madras Medical Mission, Chennai, India. Histological scoring and immunohistochemistry for CMV and BK virus status was subsequently carried out in the Department of Pathology, Pondicherry Institute of Medical Sciences, Pondicherry. Clinical data was recorded from the patient's medical records which included the age, sex, method and period of pre-transplant dialysis, blood transfusions, diabetic/hypertensive status, pre- and post-transplant infections, transplant indications, post-transplant drug therapy, duration before clinically suspected graft rejection, serum creatinine at time of

biopsy as well as one month post-transplant and other relevant details.

The results of anti-CMV IgM were noted from the records for all the cases. CMV IgM by ELISA test was performed on urine samples. We also recorded the results of BK DNA PCR from the charts for available cases. The PCR assay was also performed on urine samples, amplifying two BKV genomic regions (LT and VP1). The retrospectively selected archival paraffin blocks were serially sectioned for immunohistochemical staining for both CMV and BK polyomavirus.

### 3.2. Immunostaining methods

For CMV and BK polyoma virus immunostaining, we used monoclonal anti-CMV clones CCH2+DDG9 [DAKO] and monoclonal anti-SV40 large T cell antigen antibody Clone PAB 100 (BD Biosciences) respectively. Tissue sections were taken on 0.1% poly-L-lysine coated slides after being cut at 4 µm and kept for overnight incubation at 45°C to 50°C. Subsequently, microwave antigen retrieval was done at 120°C for 15 minutes with sections in TRIS-EDTA buffer (pH 9.0).

### 3.3. Assessment of morphologic lesions

The histological alterations/parameters studied in H&E stained sections were tubulitis, tubular atrophy, acute tubular necrosis, isometric vacuolization, interstitial fibrosis, interstitial inflammation whether acute or subacute or chronic, nuclear changes, vascular changes including any evidence of endothelial cell inclusions, casts, glomerular changes and manifestation of any recurrent renal diseases in the renal allograft biopsy. Tubulitis and interstitial fibrosis were graded according to severity. Tubulitis was assessed in non-atrophic tubules. We correlated immunohistochemical findings with clinical history, CMV IgM status, BK DNA PCR and histological features.

### 3.4. Ethical issues

The research followed the tenets of the Declaration of Helsinki and its later amendments. This study was approved by the Ethics Committee of Pondicherry Institute of Medical Sciences.

### 3.5. Statistical analysis

Statistical analysis was done using SPSS software (version 20). Fisher's exact test and the Mann-Whitney U-test were used for statistical analysis. A *P* value <0.05 was considered statistically significant.

## 4. Results

The demographic and clinical features of the study population are summarized in Table 1. Amongst the study

**Table 1.** Demographic and clinical characteristics of study population

Total number of recipients	100
Mean recipient age (y)	40.6 ± 12.3
Gender	Male (75%) and female (25%)
Method of pre-transplant dialysis	HD (92 %), PD (6 %), HD+PD (2 %)
Mean period of pre-transplant dialysis (months)	37.6 ± 16.4
Pre-transplant infections	Bacterial UTI – 22, HBV – 4, HCV – 2, Tuberculosis - 1
Number of recipients who received pre-transplant packed cell transfusions	18
Diabetes/hypertension	Diabetes – 45, Hypertension – 30, Diabetes and hypertension – 11
Transplant indications	Chronic glomerulonephritis – 34%, Diabetic nephropathy – 23%, Hypertensive nephrosclerosis – 17%, Reflux nephropathy – 5 %, IgA nephropathy – 4%, APKD – 3%, Others – 14%
Post-transplant infections	Bacterial UTI – 25
Mean serum creatinine at 1 month post-transplant (mg/dL)	1.2 ± 0.2
Mean serum creatinine at allograft biopsy (mg/dL)	2.6 ± 1.0
Drug therapy	Triple drug regimen (100%)
Duration of transplant before clinically suspected rejection (months)	18.7 ± 43.5
Post-transplant CMV IgM status (Positivity)	21
Post-transplant BK DNA PCR	3

Abbreviations: HD, hemodialysis; PD, peritoneal dialysis; UTI, urinary tract infection; HBV, hepatitis B virus; HCV, hepatitis C virus; APKD, adult polycystic kidney disease

population, 15 were Africans and 85 were Asians (South-East), predominantly South Indians. The common indications for live donor renal allograft transplantation were chronic glomerulonephritis (34%), diabetic nephropathy (23%) and hypertensive nephrosclerosis (17%). Amongst 29 patients who had pre-transplant infections, the most common was bacterial urinary tract infection (UTI). Post-transplant, 25 patients developed bacterial UTI. The serum creatinine levels at 1 month post-transplant ranged from 0.8 mg/dL to 1.9 mg/dL (Mean  $1.2 \pm 0.2$  mg/dL). The proportion of patients with serum creatinine  $\leq 1.3$  mg/dL at 1 month post-transplant was 82 individuals. However at the time of renal allograft biopsy which was done due to clinically suspected rejection, the serum creatinine levels ranged from 1.5 mg/dL to 7.4 mg/dL (Mean;  $2.6 \pm 1.0$  mg/dL). Most of the patients (85%) had serum creatinine in the range of 1.4 to 3.3 mg/dL at the time of clinically suspected rejection. All the patients in our study were on triple drug regimen (cyclosporine, prednisolone and MMF). Immunohistochemistry was done in all 100 allograft biopsies. We detected heterogeneous nuclear viral staining in tubular epithelial cells for CMV in seven cases (Figures 1A and 1B) and BK polyoma virus in 3 cases (Figures 2A and 2B). None of the cases showed positivity for both these infections.

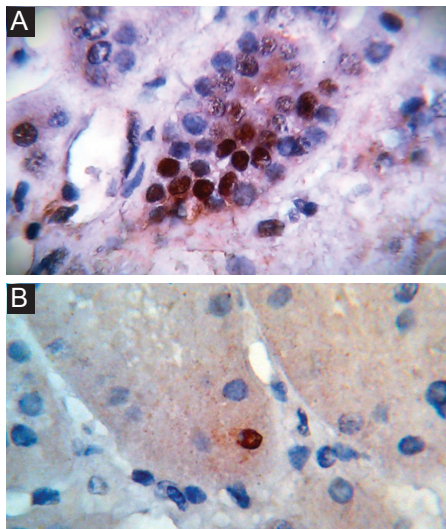
Table 2 shows a comparison of various clinical and laboratory parameters of cases immunopositive and negative for CMV and BKV.

#### 4.1. CMV nephropathy

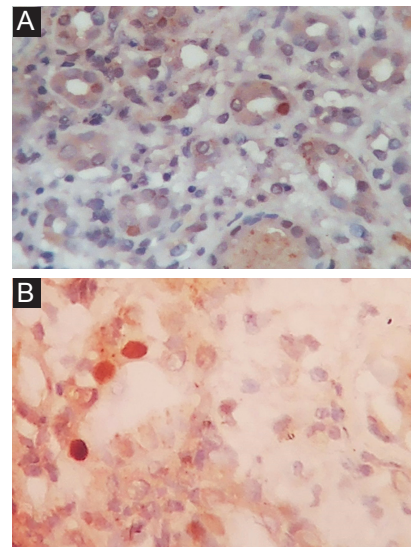
CMV was statistically associated with pre- and post-transplant UTIs, and diabetic status. No significant association was seen with respect to age, sex or ethnicity. Though the prevalence of CMV immunopositivity was slightly higher in Africans (2/15 cases, 13%) in comparison to South-East Asians (5/85 cases, 6%), the difference was not statistically significant. Liver dysfunction was also more common in CMV positive cases. Histologically, the allograft biopsies of CMV positive cases showed mild to moderate tubulitis along with mononuclear interstitial inflammatory infiltrate composed of plasma cells and lymphocytes. Nucleomegaly were seen in only four patients, one of whom had evidence of cytopathic effect (type 4 viral inclusions) (Figure 3). No changes were noted in renal glomeruli. We also noted that out of the seven patients who were immunohistochemically CMV positive, only five were CMV IgM positive. Histological features of acute rejection were observed only in 28.6% (2/7 cases) of CMV positive cases in contrast to 43.0% (40/93 cases) of all CMV negative cases. No significant association was evident with acute rejection ( $P$  value = 0.695).

#### 4.2. Polyomavirus nephropathy

With BK polyoma virus, a statistical significance with pre- and post-transplant UTIs was noted (Table 2). The histological alteration of mild tubulitis and chronic inflammation composed of plasma cells was seen in all the three cases while tubular atrophy and acute tubular



**Figure 1.** (A,B) Immunohistochemistry for CMV by using Monoclonal Anti- CMV antibody (Clones CCH2+DDG9 - Dako) – Nuclear positivity in the tubular epithelial cells. (×400)



**Figure 2.** (A,B) Immunohistochemistry for BK polyoma virus by using SV40 Large T cell Antigen antibody (Clone PAB 100, BD Biosciences) – Nuclear positivity in the tubular epithelial cells (×400).

necrosis were evident in two patients. We did not find any histological evidence of fibrosis, glomerular changes, cytomegaly, cellular casts, endothelial cell inclusions or any viral inclusions. The pattern of immunostaining was heterogenous in the tubular epithelial cells. Post-transplant PCR for BKV DNA revealed viruria in all the three patients whose renal biopsies were immunopositive for BKV. Histological features of acute rejection were evident in two cases.

**5. Discussion**

Graft survival is still a major concern in the field of renal

transplantation. The reason for this is not only rejection, but also graft dysfunction due to infectious diseases out of which CMV and BK virus nephropathy are the two main important causes that have been implicated. CMV infection is a cause of increased morbidity and mortality in transplant recipients (3).

BK virus has also been recently recognized as a cause of renal allograft dysfunction. It is thought that most of the BK virus infections in post-transplant patients stem from reactivation of latent virus in the renal allograft (4,5). It was also found that, such infections occurred in

**Table 2.** A comparison of cases whose renal allograft biopsies were immunopositive and negative for CMV and BK virus

	CMV positive (7 cases)	CMV negative (93 cases)	P value	BKV positive (3 cases)	BKV negative (97 cases)	P value
Mean (median) recipient age	43.9±12.6 (40)	40.4±12.3 (42)	0.552	47.7±10.3 (45)	40.4±12.3 (42)	0.368
Male	5 (71.4%)	70(75.3%)	1.000	2 (66.7%)	73 (75.3%)	1.000
Female	2 (28.6%)	23(24.7%)	1.000	1 (33.3%)	24 (24.7%)	1.000
Race African	2 (28.6%)	13(14.0%)		0 (0.0 %)	15 (15.5%)	1.000
Asian (South-East)	5 (71.4%)	80(86.0%)	0.282	3 (100.0%)	82 (84.5%)	1.000
Mean (median) months of dialysis before transplant	40.6±16.5 (44)	37.4±16.4 (36)	0.534	46.3±23.1 (33)	37.31±16.2 (36)	0.498
Mean (median) serum creatinine at biopsy (mg/dL)	2.4±0.4 (2.5)	2.6±1.0 (2.3)	0.850	2.3±0.7 (1.9)	2.6±1.0 (2.4)	0.620
Mean (median) duration of transplant in months before clinically suspected rejection	6.6±3.2 (6)	19.6±45.0 (5)	0.730	4.3±3.2 (3)	19.2±44.1 (5)	0.447
Pre-transplant UTI	4 (57.1%)	18 (19.4%)	0.04	3 (100.0%)	19 (19.6%)	0.01
Pre-transplant hepatitis B and C positivity	2 (28.6%)	4 (4.3 %)	0.055	0	6 (6.2%)	0.629
Post-transplant UTI	6 (85.7%)	19 (20.4%)	0.001	3(100.0%)	22 (22.7%)	0.014
Diabetes	7 (100.0%)	38 (40.9%)	0.003	2 (66.7%)	43 (44.3%)	0.587
Liver dysfunction	6 (85.7%)	32 (34.4%)	0.011	2 (66.7%)	36 (37.1%)	0.556
Post-transplant CMV IgM positivity	5 (71.4%)	16 (17.2%)	0.004	0	21 (21.6%)	1.000



**Table 3.** Comparison of CMV IHC positive cases in the present study with similar studies published in literature

Author, (no. of cases)	Mean age in years (range), M:F ratio	Mean serum creatinine in mg/dL (range)	Patients with rise in serum creatinine	Tubulitis	Interstitial inflammation (chronic), plasma cells	Cytomegaly	Viral inclusions	Cellular casts	CMV prevalence and immunostaining pattern
Sachdeva et al <sup>2</sup> (6 cases, 8 bx)	NA	NA	NA	6/8 (75%)	8/8 (100%), Plasma cells - NA	NA	NA	2/8 (25%)	1.9% Nuclear, Focal. In TEC (7), VEC (2), IC (1), G (1)
Agarwal et al <sup>12</sup> , (4)	50 (37-56), 4:0	3.85 (1.3-7.5)	3/4 (75%)	0/4	4/4 (100%), Plasma cells-4/4 (100%)	4/4 (100%)	Type II and IV	NA	1.2% Nuclear, HGN. In TEC& VEC
Present study (7)	43.9 (24-63), 2.5:1	2.4 (1.8-3.1)	7/7 (100%)	7/7 (100%)	7/7 (100%), Plasma cells-6/7(85.7%)	1/7 (14.3%)	1/7, Type IV	1/7 (14.3%)	7% Nuclear, HGN. In TEC (7).

Abbreviations: TEC, tubular epithelial cell; VEC, vascular endothelial cell; IC, interstitial cell; G, glomerular staining; HGN, heterogeneous; NA, not available; bx, biopsies.

3 to 4 months post-transplant when immunosuppression is the highest (6). These infectious processes are often identified late or continue to remain undetected because they tend to impersonate non-infectious pathology such as allograft rejection and drug induced toxicities. The cause is probably due to compromised inflammatory responses brought about by the immunosuppressive therapy in these transplant recipients.

All of our patients were on triple drug regimen receiving prednisolone, cyclosporine, tacrolimus or MMF. Increased risk of viral infections is seen in those patients who have received immunosuppressive drugs such as calcineurin inhibitors and MMF (7).

As mentioned earlier, the common indications for renal allograft transplant was chronic glomerulonephritis (34%), diabetic nephropathy (23%) and hypertensive nephrosclerosis (17%). Our data was closely similar to a study in a tertiary care centre in North India which found chronic glomerulonephritis (34.5%) to be the most common cause of end stage renal disease amongst all age groups, followed by diabetic nephropathy (20.5%) (8). A recent cross-sectional study of the Indian chronic kidney disease (CKD) registry revealed that the most common cause of end stage renal disease was diabetic nephropathy (31%), followed by CKD of unknown etiology (16%), chronic glomerulonephritis (14%) and hypertensive nephrosclerosis (13%) (9).

Out of the 100 renal allograft biopsies which were evaluated immunohistochemically, 7% of patients were found to harbour CMV infection while 3% were found to be infected with BK polyomavirus. None of these patients were found to have both CMV and BK polyomavirus infections. The prevalence of CMV and

BKV varies with countries. This variation can be due to the appropriateness of various diagnostic tests used for the detection of these viral infections. The threshold values used for diagnosis can also be a potential source of variation (10). One study in Korea showed that 10 (5.2%) out of 191 patients with renal transplant had BK virus associated nephropathy. Four out of the 10 patients had concurrent CMV infection (11). An earlier study conducted in North India showed a prevalence of BKV and CMV to be 9.3% and 1.9% respectively in renal transplant patients having graft dysfunction (Tables 3 and 4) (2). According to a recent study conducted in North India, a prevalence of 4% for BK virus infection and 1.2% for CMV associated nephropathy was found. Cortical necrosis was seen in one of these cases (12). During the period of intense immunosuppression in renal allograft recipients, viral infections can cause allograft dysfunction during the period of 1 to 6 months. Beyond 6 to 12 months, renal allograft recipients can also develop such infections due to community acquired exposure which also depends on the duration of maintenance immunosuppression (7).

### 5.1. Cytomegalovirus

The mean time taken for CMV infection to manifest was  $6.6 \pm 3.2$  (range 1 to 11) months. The mean serum creatinine at the time of biopsy which was done for clinically suspected rejection for this group was  $2.4 \pm 0.4$  (range 1.8 to 3.1) mg/dL. These findings were closely similar to a study where 14.2% live related allograft recipients developed CMV disease after a median interval of  $7.18 \pm 4.35$  months from transplantation with a mean serum creatinine of  $1.9 \pm 0.6$  (1.3 to 3.6) mg/dL at the

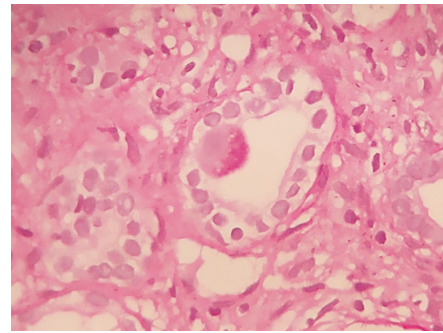
time of the diagnosis (13).

In our study, we found a statistical significance between pre-transplant infections and CMV nephritis. UTIs were seen in 57.1% (4/7) of CMV immunopositive cases. Hepatitis B virus and hepatitis C virus positivity was seen in one patient each (2/7 cases, 28.6%). A recent study from Turkey revealed positive hepatitis B and C serology in 17.6% of CMV positive cases (14). Another study found HCV positivity in 28.6% of CMV positive recipients (15).

We noted a statistical significance of post-transplant bacterial UTIs which developed in 6 out of 7 patients (85.7%) who immunohistochemically tested positive for CMV. Factors that might contribute to the development of UTIs include increased immunosuppressive therapy and prolonged instrumentation of the urinary tract such as urethral catheters and ureteric stents. A recent study revealed that CMV can be a risk factor for developing UTI during the first year after renal transplantation. CMV can weaken host immune responses thereby predisposing to infections. Alternatively UTI can reactivate latent CMV virus by releasing pro-inflammatory cytokines which trigger CMV replication (16). Urine leucocyte counts were also found to be higher in CMV positive recipients (14). Further research is essential to understand the link between UTIs and CMV infection. All the patients in our study have received ganciclovir prophylaxis for CMV for a period of three months. Prophylaxis has been shown to be effective in reducing the incidence of CMV disease (17). There was a statistical significance between pre-transplant diabetes and CMV infection in the allograft recipients. Diabetes was the commonest comorbid condition for post-renal transplant CMV infection in another Indian study also (15).

Our study did not identify any significant association of CMV with acute rejection. A recent Chinese study also did not identify significant differences in age, gender or acute rejection in CMV infected and uninfected patients (18). Another study from North India also did not reveal any morphological evidence of acute rejection in the immunohistochemical CMV positive cases but showed varying degrees of tubulointerstitial inflammation (12), similar to our study. In contrast the study from Turkey showed that acute rejection episodes are more frequent in the CMV-positive group (14). CMV infection is associated with chronic allograft nephropathy that causes myointimal thickening leading to graft failure (3). An earlier study revealed that recipients who had both acute rejection and CMV developed chronic rejection sooner along with a higher incidence when compared to those with acute rejection but no CMV (19).

The pattern of immunostaining was nuclear and



**Figure 3.** CMV – Cytopathic effect in a renal tubular epithelial cell showing markedly enlarged nuclei and prominent nucleoli ( $\times 400$ , H&E).

heterogeneous in the tubular epithelial cells in all the seven cases. On H&E sections, four of the seven cases showed nucleomegaly, one with cytopathic effect in the tubular epithelial cells (Figure 3). Chronic interstitial inflammation was present in all the seven biopsies with plasma cells present in six cases. All the seven patients had tubulitis irrespective of the severity. Cellular casts were present in only one of the seven cases. There was no evidence of endothelial cell inclusions in our study. In comparison with another study, none of the CMV positive cases had tubulitis, but mixed interstitial inflammation and plasma cells were present in all the cases (Table 3). The pattern of immunostaining was nuclear and heterogeneous seen in the tubular epithelial and vascular endothelial cells. Nucleomegaly and cytomegaly was seen in all the CMV positive cases (12). The findings in another study were also similar to our study with presence of tubulitis in six out of the eight immunohistochemical CMV positive cases while interstitial inflammation was present in all the cases. The pattern of immunostaining in their study was predominantly seen in the tubular cells. Immunostaining of endothelial cells was seen in two cases, interstitial cells and glomerular staining in one case each (Table 3) (2).

### 5.2. BK polyoma virus

The mean period of diagnosis was  $4.3 \pm 3.2$  months (range 2 to 8) amongst the three immunohistochemical BK positive patients who also had rising serum creatinine levels with the mean being  $2.3 \pm 0.7$  mg/dL (range 1.9 to 3.1) at the time of biopsy. In a North Indian study, the mean period of diagnosis of BK polyoma viral infection was 12.4 months (7 days to 3.5 years) (12). We found a statistical association of allograft BK polyomavirus positivity with pre- and post-transplant bacterial UTI. In one study, three patients had bacterial UTI out of a total of seven patients who had renal allograft BK positivity (20).

Pre-transplant diabetes was present in 2/3 of

**Table 4.** Comparison of BKV IHC positive cases in the present study with similar studies published in the literature

Author, (no. of cases)	Mean age in years (range), M:F ratio	Mean serum creatinine in mg/dL (range)	Patients with rise in serum creatinine	Tubulitis	Interstitial inflammation (chronic), Plasma cells	Cytomegaly	Viral inclusions	Cellular casts	BK Prevalence and Immunostaining pattern
Sachdeva et al <sup>2</sup> , (30 cases, 31 bx)	NA	NA	NA	28/31 (90.3%)	31/31 (100%), Plasma cells - NA	NA	NA	4/31 (12.9%)	9.3% Nuclear. In TEC(31), VEC(5), IC(1), G(8)
Agarwal et al <sup>2</sup> (13)	36 (22–52), 12:1	3.76 (1.2-11.5)	12/13 (92.3%)	9/13 (69.2%)	13/13 (100%), Plasma cells–13/13 (100%)	3/13 (23%)	Type I and III	NA	4% Nuclear, HGN. In TEC.
Present study (3)	47.7 (39–59), 2:1	2.3 (1.9-3.1)	3/3 (100%)	3/3 (100%)	3/3 (100%), Plasma cells-3/3 (100%)	0/3	0/3	0/3	3% Nuclear, HGN. In TEC (3).

Abbreviations: TEC, tubular epithelial cell; VEC, vascular endothelial cell; IC, interstitial cell; G, glomerular staining; HGN, heterogeneous; NA, not available; bx, biopsies.

immunohistochemically BK polyoma positive cases. A study on the prevalence and risk factors of BK polyomavirus replication in patients who had undergone simultaneous pancreas/kidney transplantation, found that the duration of pre-transplant diabetes and graft function delay were individually associated with BK polyomavirus positivity (21).

It is interesting to note that many studies have been undertaken to estimate the prevalence of BK virus nephropathy. Two North Indian studies have reported an incidence of 4.0% (12) and 9.3% (2), while an Iranian study found it to be 13.1% (22). However another Iranian centre detected BK virus in only 0.93% of all allograft biopsies (23). A Japanese study found 6.9% of renal allograft biopsies to be positive for BKV (24). A recent study from the United Kingdom found the incidence to be low (2.1%) (25). Thus there is a wide variation among transplant centres worldwide.

Histological alteration of tubulitis was seen in all the BKV positive cases. Chronic inflammation along with plasma cells was seen in all the cases while there was no evidence of fibrosis, cytomegaly or viral inclusions in any of the positive cases. The pattern of immunostaining of tubular epithelial cells was nuclear and heterogeneous. One study showed that 9/13 cases had tubulitis (69.2%). Mixed interstitial inflammation and plasma cells, however were seen in all 13 patients. The immunostaining pattern was heterogeneous and nuclear in the tubular epithelial cells (Table 4) (12). Another study showed that 28 of 31 allograft renal biopsies which were immunohistochemically positive for BK polyomavirus showed tubulitis. Varying degree of interstitial inflammation was seen in all 31 biopsies

(Table 4) (2). Tubulitis in polyomavirus nephropathy (PVAN) probably represents anti-viral host immunity. It may also be non-specific which occurs secondary to tubular injury. Excessive tissue destruction might occur once the patient's immunity improves (26).

Histopathology has difficulties in distinguishing between rejection and PVAN especially where there is co-existence of both the disease process (27). Focal involvement of graft and tendency to involve the medulla also adds to the diagnostic problem. BK virus nephropathy can be underdiagnosed if immunohistochemistry is not used routinely.

Though the present retrospective study is limited by a relatively small sample size, our results are comparable to various studies conducted in transplant centres worldwide. It is essential to monitor CMV and BKV infection in the early post-transplantation stage in renal allograft recipients and immunohistochemistry is required for diagnostic confirmation. Larger studies are essential in developing nations like India to understand the prevalence and risk factors of CMV nephritis and BK virus nephropathy in renal allograft recipients.

#### Limitations of the study

This retrospective study is limited by a relatively small sample size.

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#### Author's contribution

RGV and GA designed the study. KM, MMA and IV

drafted the manuscript. KM and MMA collected the data, conducted statistical analysis along with interpretation of the data. RGV, GA and IV provided technical support. All the authors have reviewed and approved the final manuscript.

### Conflicts of interest

The authors declare no conflict of interest.

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