BK polyoma viral infection in renal allograft recipients

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ABSTRACT

BACKGROUND

BK polyoma viral nephropathy (BKVN) has emerged as a significant cause of renal allograft loss. The literature on BK viral infection from India is scarce. The study was therefore undertaken to evaluate impact of BK polyoma viral (BKV) infection on renal allograft recipients in Indian scenario from a service renal transplantation centre.

METHODS

Renal allograft recipients who underwent graft biopsy formed the part of this descriptive cross-sectional study group. The clinicopathological profile of the patients was analysed. The diagnostic modalities employed were histopathology, immunohistochemistry using antibody for Simian virus 40 large T antigen along with real time quantification of the BK viral DNA load in the urine and the serum.

RESULTS

One hundred forty seven renal allograft recipients were evaluated. 73.47 percent (108/147) patients presented with graft dysfunction and rest were protocol biopsies. There were 53 cases of rejection related diagnosis, 8 cases of graft pyelonephritis, 64 cases showed normal histology and rest exhibited miscellaneous causes. Nineteen percent (28/147) cases were positive for BKV DNA (viruria 26/147, 17.6% and viraemia 8/147, 5.44%). 3.4 percent (5/147) exhibited histological and immunohistochemical evidence of BKVN. Nuclear enlargement, smudging and intranuclear inclusions along with plasma cell rich interstitial nephritis were important features observed on histopathology. Concomitant acute rejection was seen in 4/5 cases of BKVN. All cases of BKVN exhibited viraemia (>2500 copies/mL), though cut-off values could not be defined statistically due to small sample size. Positive statistical correlation was observed between use of anti-thymocyte globulin (induction therapy and/or treatment of steroid resistant rejection, Pearson χ^2 value 6.9, $P=0.008$) and rejection episodes (Pearson χ^2 value 9.8, $P=0.007$) with BKV infection.

CONCLUSION

BK polyoma nephropathy should be added to the list of differential diagnosis considered for a renal allograft dysfunction. Renal biopsy remains the

gold standard for diagnosis supplemented by non-invasive molecular techniques for screening and monitoring of BKV infection.

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Key Words: acute interstitial nephritis; acute rejection; BK polyoma nephropathy; immunosuppression

INTRODUCTION

Renal transplantation is technologically advanced form of renal replacement therapy and has become the most acceptable mode of management for the patients with end stage renal disease (ESRD). Advances in immunosuppressive drug therapy have significantly reduced rejection related complications in renal allograft recipients. However, introduction of newer, more potent immunosuppressive drug regimens to combat rejection has come with a price. Viral infections are common after transplantation and may be the biomarkers of excessive immunosuppression. BK polyoma viral allograft nephropathy (BKVN) is an increasingly recognised complication after renal transplantation and has become a significant cause of renal allograft loss.¹ BK polyoma virus (BKV) infects 10–45% of renal allograft recipients and presently is the focus of intense investigation. Human polyoma virus belong to papova virus group, which are small, non-enveloped, 45–55 nm in size having double stranded DNA genome. The most known species of this kind are BK-virus, JC virus and Simian virus 40 (SV40).²

Primary infection usually occurs in childhood and remains latent in the urogenital tract.³ The clinical phenotypes of infection in renal allograft recipients include latent asymptomatic infection with or without serological evidence, asymptomatic re-activation of latent BKV with viraemia-viruria and BKVN with morphological evidence of viral induced cell injury in 1–5% of renal allograft recipients.^{3,4} Prompt diagnosis is crucial as these infections are associated with persistent graft dysfunction and graft loss.

Diagnosis of BKVN relies on renal biopsy, exhibiting viral cytopathic effects and interstitial nephritis supplemented by confirmation by immunohistochemistry, electron microscopy and molecular diagnosis. The management of BKVN includes reducing immunosuppression, treating concomitant inflammation or rejection and antiviral agents like cidofovir.⁴

A descriptive cross-sectional study was conducted for evaluating prevalence of BKV infection and its impact on renal allograft recipients. The diagnosis was established using combination of

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histopathology and immunohistochemistry in graft biopsies along with quantification of viral DNA by real time polymerase chain reaction (PCR) in the plasma and urine. This will enable screening protocols, early diagnosis and appropriate management modalities to be offered, significantly improving the graft survival rates.

MATERIALS AND METHODS

Patients

The study conducted at Army Hospital Research & Referral (R & R) is a cross sectional analysis whereby renal allograft recipients presenting with acute/chronic graft dysfunction and normal healthy allograft recipients were evaluated. Cadaveric and living-related/unrelated HLA matched renal allograft recipients receiving post-transplantation care and attending follow-up clinic were involved in this study. The clinical variables tabulated were age, gender, donor type, type of transplant, use of antithymocyte globulin (ATG) for treatment of steroid resistant rejection and as induction therapy, maintenance immunosuppression, rejection episodes and serum creatinine levels.

Histopathology

Minimum of two cores of renal biopsies were obtained after taking informed consent. Formalin fixed and paraffin embedded sections were stained with haematoxylin and eosin (H & E), periodic acid Schiff and silver methenamine were evaluated. The biopsies were graded as per Banff classification for rejection related diagnosis.⁵

Immunohistochemistry

Biopsies showing histological features suggestive of viral aetiology (tubular epithelial cells exhibiting nuclear enlargement, smudged nuclear chromatin and intranuclear inclusions along with interstitial nephritis rich in plasma cells) were then stained for SV-40 large T-cell antigen (BD Biosciences Catalogue No. 511454) using avidin-biotin peroxidase technique. The renal biopsy of the patients with other rejection and non-rejection related diagnosis not responding to treatment were also processed for immunohistochemical staining.

Molecular Studies

Blood and urine samples from the renal allograft recipients were obtained after informed consent. Since cases of ESRD on haemodialysis are prospective candidates for renal transplants, their blood and urine samples were tested to determine the possibility of reactivation of BKV prior to the renal transplantation. Blood samples were collected in EDTA tubes. Urine was collected as midstream samples in sterile containers and used without centrifugation. DNA extraction was carried out using QIA amp blood DNA extraction kit (Catalogue no. 51104 Qiagen) according to the manufacturer's instructions.

Qualitative PCR Assay

The BK forward and reverse primers were specifically designed to amplify a portion of gene encoding the large T antigen of BK

virus without amplifying DNA derived from JC or SV 40 virus. Each patient's sample was prepared with a reaction mixture consisting of 5 microlitre of 10× Taq DNA polymerase, 5 microlitre of PCR nucleotide mix, 2 microlitre of 5 µM BK forward primer and 2 microlitre of 5 µM BK reverse primer, 33 microlitre of sterile water, 2 microlitre of template DNA, and 1 microlitre of Taq DNA polymerase (5 U/microlitre). The PCR conditions included initial denaturation at 94°C for 5 min, 40 amplification cycles (94°C for 1 min, 45°C for 1 min, 72°C for 1 min) and extension 72°C for 10 min, 20 µL of PCR product was electrophoresed on 1.5% agarose gel. Gels were stained with ethidium bromide (0.5 µg/mL) and examined for band of appropriate size (80 bp).

Quantitative real-time PCR assay were performed using JC/BK Geno-Sen's real time PCR kit (Genome Diagnostics). The PCR amplifications were run in Rotor gene 2000/3000 (Corbett Research). The primers incorporated in the Geno-Sen's real time PCR kit amplify DNA fragment from large T antigen of BKV virus. The cycling profile includes: denaturation at 90°C for 50 sec, annealing at 50°C for 20 sec and extension at 72°C for 15 sec (45 cycles). The results of the run were detected as signals/amplifications in fluorescence channel. Data analysis was performed with Rotor gene 3000 operator manual. The number of the BKV copies was calculated from the standard curve. The data was expressed as copies of viral DNA/mL of urine or plasma.

The results were tabulated and statistical analysis carried out. The Pearsons χ^2 test was used to identify association of BKV infection with clinical variables i.e., age, gender, donor type (related or unrelated), maintenance immunosuppression, rejection episodes and use of ATG.

RESULTS

Study Population

One hundred forty seven renal allograft recipients were evaluated at follow-up clinic of AH (R & R) (November 6–January 8). Mean age of patients 33 years $\pm$ SD 8.9 range 31.79–34.79 and median age 36 years. Males outnumbered females (males 78.91% and females 21%). Ninety-seven percent (144/147) were living donor transplants (related 117 and spousal 27) and three were cadaveric. Ninety-eight percent (145/147) were recipients of first allograft. In three cases the cause of first allograft failure was chronic allograft nephropathy. Eighty five percent (125/147) patients received tacrolimus/mycophenolate mofetil/prednisolone, (tacro/mmfpred); 13% (19/147) received cyclosporine/mycophenolate mofetil/prednisolone (cyclo/mmfpred); and 1.3% (2/147) cyclosporine/azathioprine/prednisolone (cyclo/aza/pred) as maintenance immunosuppression. Twelve percent (19/147) patients were induced by ATG. Thirty-five percent (52/147) had clinical history of acute rejection in the past. Twenty-one percent (31/147) were treated with ATG and 14% (21/147) by pulses of methyl prednisolone. In total 23% (35/147), cases received ATG as rejection treatment and/or as induction therapy.

Molecular Diagnosis

BKV infection was considered when BKV DNA was detected in serum and/or urine of renal allograft recipients. Nineteen percent (28/147) cases were positive by molecular analysis. Viruria in 26/28 cases (viral load 39 copies/mL–110000 copies/mL with median of 2564 copies/mL). Viraemia in 8/28 cases (viral load 11 copies/mL–442660 copies/mL with median of 2550 copies/mL) (Figure 1). In 2/8 cases positive for viraemia, urine samples were not available. The ESRD group was negative except for one positive case (viral load 34 copies/mL).

Histopathological Studies

Renal biopsies of 147 renal allograft recipients were studied. Clinically these patients presented with acute graft dysfunction 53% (83/147), chronic 5.4% (8/147) and acute-on chronic graft dysfunction 5.4% (8/147). Protocol renal biopsies constituted 26% (39/147) of cases. Category of 'others' included 6.12% (9/147) cases presenting with graft dysfunction but clinically suspected for non-rejection related diagnosis i.e., recurrence, de-novo pathology, pneumonia, urinary tract infections, tuberculosis, hepatitis B and hepatitis C viral infection. The renal biopsies in these cases were carried out to exclude associated acute rejection.

The histopathological evaluation of 147 renal biopsies using guidelines by Banff scheme of renal allograft pathology revealed acute rejection (23%, 35/147), chronic allograft nephropathy (6.8%, 10/147), acute-on chronic rejection (5.4%, 8/147), graft pyelonephritis (5.4%, 8/147), interstitial nephritis (7.4%, 11/147), surgical causes (2%, 3/147), drug toxicity (1.3%, 2/147), acute tubular necrosis (2%, 3/147) and recurrence (2%, 3/147). Forty-three percent (64/147) cases showed normal histology. In 20 cases, suspicion of a concomitant infective pathology was entertained in addition to the rejection diagnosis. These cases showed dense interstitial infiltrates rich in plasma cells, tubulorrhexis, sloughing of epithelial cells, neutrophil casts, nuclear enlargement, smudged chromatin and intranuclear inclusions along with persistent graft dysfunction.

Immunohistochemistry

3.4 percent (5/147) cases exhibited intranuclear staining for the virus localised to tubular epithelial cells and were labelled

as cases of BKVAN. The clinical profiles, histological and molecular findings of the patients are listed in Tables 1–3.

Correlation of Histopathological Features and Molecular Studies

BKV infection was detected in 19% cases with viruria in 17.6% and viraemia in 5.44%. Biopsy proven BKVAN was seen in 3.4% cases. These cases were divided into clinicopathological categories as listed in Table 4.

Five of eight cases of viraemia exhibited biopsy proven BKVAN (positive predictive value 65%). All cases of histologically proven BKVAN had viraemia more than 2500 copies/mL. Three cases of viraemia did not show immunohistochemical staining by SV-40. Of these, two patients had very low levels of viraemia (<500 copies/mL). One case had value more than 2500 copies/mL but did not show histological evidence of BKVAN. This case was opined as acute rejection on histopathology but continued to have graft dysfunction despite adequate anti-rejection treatment. Five of 26 viruria cases displayed histological evidence of BKVAN on renal biopsy (positive predictive value 18%). Eight of 19 cases of viruria opined as acute rejection on biopsy, were managed with anti-rejection therapy and 2/19 with re-adjustment of cyclosporine dosage. Nine out of 19 of viruria cases exhibited normal renal histology on biopsy though they responded clinically to anti-rejection treatment Table 4.

Serum BK Viral Infection and Graft Dysfunction

One hundred eight of 147 (73.47%) patients presented with graft dysfunction and 39/147 (26%) were protocol biopsies. Twenty-seven of 108 (25%) patients with graft dysfunction exhibited BKV infection and 5/108 (4.63%) exhibited BKVAN. One of 39 cases of protocol biopsies (2.5%) exhibited asymptomatic viruria.

Serum Creatinine Levels and BK Polyoma Viral Nephropathy

Serum creatinine in BKV infection group ranged from 1.0 mg% to 6.4 mg% (mean value 2.9 mg% SD $\pm$ 1.41 and median value 3.1 mg%) and in BKVAN group values ranged between 2.9 mg and 6.4 mg% (mean value 4.68 mg SD $\pm$ 1.61 and median of 4.7 mg%). BKV negative group displayed mean value 2.4 mg% $\pm$ SD 1.81 and median value of 2.0 mg%.

Figure 1 Real time quantification of BK polyoma virus—graphs of positive cases.

Table 1 Clinical profile of BK polyoma viral nephropathy patients.

Case	Age/ Sex	Time since transplant (mo)	Donor	Clinical features	Creatinine mg%	Immunosuppression	Rejection episodes	ATG induction
1	24 M	21	Living, related	Acute graft dysfunction	3.1	Csa/mmf/pred	1 treated with ATG	Nil
2	20 F	33	Living, related	Fever, dysuria, acute graft dysfunction	2.9	Tacro/mmf/pred	–	Nil
3	32 M	4	Cadaver	Acute graft dysfunction	6.4	Tacro/mmf/pred	3 episodes treated with ATG and rituximab	Yes
4	35 M	10	Living, related	Acute graft dysfunction with fever	5.0	Csa/mmf/pred	2 ATG	Nil
5	41 M	8	Living, related	Acute graft dysfunction hydronephrosis	6.0	Tacro/mmf/pred	2 episodes treated with ATG	Yes

ATG: antithymocyte globulin.

Table 2 Histopathological features of five cases of BK polyoma viral nephropathy.

Case No.	Time of biopsy after transplant (mo)	Tubulitis	Endothelitis	Interstitial inflammation rich in plasma cells	Histopathology Neutrophil casts	Tubulorrhexis	Viral cytopathic effects
1	21	+	–	Dense	+	–	Nucleomegaly smudged chromatin
2	33	–	–	Mild	–	+	Intranuclear inclusions
3	4	+	–	–	–	–	–
	5	+	+	+	–	+	–
	9	+	–	+	–	+	Nucleomegaly smudged chromatin
4	10	+	–	+	–	+	–
	14	+	–	Dense	–	–	–
	18	+	–	Dense	–	+	Nucleomegaly
5	8	+	–	+	+	+	–
	10	+	–	+	+	+	Nucleomegaly smudged chromatin
	11	+	–	+	+	+	Nucleomegaly smudged chromatin

Table 3 Molecular features of five cases of BK polyoma viral nephropathy.

Case	Time of biopsy after transplant (mo)	Histopathological diagnosis	CMV PCR copies/mL	IHC CMV copies/mL	IHC BKV copies/mL	BKV PCR copies/mL
1	21	Acute rejection with concomitant viral infection	–	–	+	2554
2	33	CMV infection	243	–	+	442660
3	4	Acute rejection Banff grade IA				
	5	Acute rejection Banff grade IIA				
	9	Acute rejection Banff grade 1B	–	–	+	11000
4	10	Acute rejection Banff grade 1A				
	14	Acute rejection acute interstitial nephritis	15489	–	+	6246
	18	Acute interstitial nephritis			+	6246
5	8	Graft pyelonephritis				
	10	Acute rejection graft pyelonephritis				
	11	Acute interstitial nephritis	712	–	+	12072

CMV: cytomegalo virus.

Table 4 Clinicopathological groups of BKV polyoma viral infection.

S. No.	Clinicopathological groups	No. of cases	Histopathological features (No. of cases)
1	Asymptomatic viruria	1	Protocol biopsy (1) Within normal histological limits
2	Graft dysfunction with viruria	19	Acute cellular rejection Grade 1A with resolving graft pyelonephritis (1) Acute cellular rejection Grade 1A with chronic allograft nephropathy (1) Acute rejection (4) Borderline rejection (2) Isometric vacuolisation (2) Within normal histological limits (9)
3	Graft dysfunction with viruria + viraemia without immunohistochemical evidence of BKVAN	3	Acute rejection grade IIA (1) Acute rejection grade IB (1) Acute rejection grade IB with interstitial nephritis (1)
4	Graft dysfunction with viruria + viraemia with immunohistochemical evidence of BKVAN	5	Detailed histopathological features as per Table 1

BKVAN: BK polyoma viral nephropathy.

Table 5 Statistical analysis of twenty-eight cases of BK viral infection.

Characteristics	BKV Number (%)	No BKV Number (%)	Total Number (%)	χ^2 DF P value
Total	28 (19.05)	119 (80.95)	147 (100.0)	
Gender				2.23 (1) P=0.135
Male	25 (21.5)	91 (78.4)	116 (78.1)	
Female	3 (9.6)	28 (90.2)	31 (21.09)	
Age (yr)				1.21 (2) P=0.547
0–20	2 (7.14)	8 (6.72)	10 (6.8)	
21–40	20 (71.43)	73 (78.49)	93 (63.27)	
41–60	6 (21.42)	38 (31.09)	44 (29.93)	
Donor				0.02 (1) P=0.882
Unrelated	6 (21.43)	24 (20.17)	30 (20.41)	
Related	22 (78.57)	95 (79.83)	117 (79.59)	

Statistical Analysis

Tables 5 and 6 show analysis of 28 cases of BKV infection in relation to clinical variables studied. No statistically significant association was observed between BKV infection and age, gender, donor type and maintenance immunosuppression (Table 5). Rejection episodes and use of ATG for induction therapy and/or treatment of steroid resistant rejection were found to have statistically significant association with BKV infection (Table 6).

DISCUSSION

BK viral infection has emerged as an important cause of progressive renal allograft dysfunction but the prevalence, diagnostic modalities and potential risk factors have not been completely elucidated especially in Indian scenario. This cross-sectional study involved analysis of renal allograft recipients for BKV infection including those with stable graft functions, where protocol biopsies were performed.

BK viral infection as quantified by the molecular studies was evident in 19% of renal allograft recipients. The rate of biopsy proven BKVAN was 3.4%. Prevalence rates of BKV infection as per the international data range from 10% to 45% whereas the reported prevalence rates of BKVAN are 2.5%, 4.5%, 4.6%, 5.3% and 7.1%.^{6–8}

Indian data is miniscule with only one published study indicating a high incidence of BKV infection (9.3%) where histopathological studies were combined with immunohistochemistry and electron microscopy.⁹

Our study had employed BKV real time PCR that is non-invasive, provides rapid adjunctive diagnosis to the renal biopsy and is convenient for routine management.

Viruria, Viraemia and BK Viral Infection

Viral replication begins early after transplantation and progresses through detectable stages—viruria, then viraemia followed by nephropathy. Urine cytology for decoy cells (viral cytopathic changes in shed epithelial cells) and BKV PCR assay for viruria and viraemia are non-invasive methods for screening, monitoring and clearance of BKV infection.

Randhawa et al in their series reported rates of 30% and 8% for viruria and viraemia.^{10,11} Viruria was observed in 17.6% of graft recipients in our study with low positive predictive value

Table 6 Statistical analysis of twenty-eight cases of BKV infection.

Characteristics	BKV Number (%)	No BKV Number (%)	Total Number (%)	χ^2 DF P value
Total	28 (19.05)	119 (80.95)	147 (100.0)	
Maintenance immunosuppression				(Fisher exact) $P=0.359$
Csa/mmf/pred	5 (17.86)	14 (11.86)	19 (13.86)	
Tacro/mmf/pred	22 (78.57)	103 (87.27)	125 (85.62)	
Csa/Aza/pred	1 (3.57)	2 (1.6)	3 (2.04)	
Rejection episodes				9.8 (2) $P=0.007$
No rejection	13 (46.43)	82 (68.91)	95 (64.63)	
Rejection treated with prednisolone	3 (10.71)	18 (15.13)	21 (14.29)	
Rejection episodes treated with ATG	12 (42.86)	19 (15.97)	31 (21.09)	
Patients receiving ATG (induction + treatment for rejection episodes)				6.91 (1) $P=0.006$
No ATG	16 (10)	96 (65)	112 (76)	
ATG	12 (8.16)	23 (15)	35 (23)	

ATG: antithymocyte globulin.

for the diagnosis of BKVAN. Viruria is sensitive for detecting active BKV infection but lacks specificity for nephropathy and hence is poor predictor of tissue invasive disease as virus can originate anywhere along the urinary tract.¹²

BK viraemia has been reported to have 100% sensitivity, 88% specificity, 50% positive predictive value and 100% negative predictive value. It is speculated that viral DNA gains access to the bloodstream once the activated virus spreads to renal tubules from the urothelium in the highly vascularised renal medulla.^{10,13} Viraemia exhibited overall prevalence of 5.4% with positive predictive value of 65% in our series. Three cases of viraemia were negative for SV-40 staining. The viral load in cases that have viraemia without detectable nephropathy in allograft biopsies is low, as seen in two of our cases. BKVAN cannot be ruled out in third case with high viraemia but without detectable SV-40 immunostaining, as interstitial nephritis and tubular cytopathic changes of BKV nephropathy can be focal or isolated to the medulla and missed on one third of biopsies.^{14–16} All cases of ESRD except one case were negative for BKV viral marker, provides an indirect evidence that the BKV infection was acquired/reactivated after transplantation.

Quantification of Viraemia and Cut-off Levels

Considerable overlap exists regarding threshold cut-off values for viraemia to predict tissue invasive disease (cut-off value of 7700 viral copies/mL in one study).^{12,14} No Indian data is available for defining the cut-off levels. In our study, the sample size was very small as to realistically determine the cut-off levels though all cases of histologically proven BKVAN had viraemia more than 2500 copies/mL.

BK Polyoma Viral Nephropathy

Histological examination of the renal biopsy is a mainstay in the diagnosis and facilitates concomitant diagnosis of rejection.

The morphological hallmarks are viral cytopathic changes (i) type 1 amorphous basophilic ground-glass variant, (ii) type 2, eosinophilic, granular type similar to cytomegalovirus (CMV) inclusions, (iii) type 3, finely granular form lacking a halo, (iv) type 4, vesicular variant with enlarged nuclei and clumped, irregular chromatin.¹⁶ Four cases showed nucleomegaly with clumped irregular chromatin and one case (Case 4) exhibited nuclear inclusions resembling CMV inclusions. Plasma CMV DNA was positive in three cases but the graft staining for CMV antigen was negative. Tubulorrexesis was observed in four cases and cellular casts in two cases (Figures 2 and 3).

However, histopathology has its pitfalls, as at times it becomes difficult to distinguish between rejection and BKVAN especially in cases where there is co-existence of two pathologies. Focal involvement of graft and propensity to involve the medulla also adds to the diagnostic dilemma. Four of 5 cases exhibited dense interstitial inflammation with predominance of plasma cells. As per different international series, approximately 54% of biopsies performed for BKVAN exhibit evidence of cellular rejection with tubulitis and endarteritis.¹⁶ Tubulitis was evident in four of five cases. In three cases, two or more biopsies were opined as acute rejection before viral cytopathic effects were evident. Tissue injury due to acute rejection, graft pyelonephritis or drug toxicity may create a microenvironment favourable for viral activation. Confirmation of diagnosis by immunohistochemistry is essential along with HLA DR and C4d staining accompanied by post-transplant antibody monitoring by ELISA or flow cytometry.

BKV nephropathy is associated with significantly higher levels of serum creatinine.^{1,19} Mean serum creatinine value observed in BKVAN was higher than the viraemia-viruria group without evidence of nephropathy though the data is small for statistical analysis.

Figure 2 Renal biopsy in a case of BK polyoma viral nephropathy exhibiting (A) Interstitial nephritis with focal acute tubular necrosis (H & E $\times 100$), (B) nuclear smudging and enlargement (H & E $\times 400$), (C) showing interstitial infiltrate rich in plasma cells (H & E $\times 400$) and (D) tubular cells positive for SV-40 (Immunoperoxidase staining $200\times$).

Figure 3 Case of BKVAN with renal biopsy showing (A) tubulorrhexis with sloughing of epithelial cells (H & E $\times 100$), (B) intranuclear inclusions (H & E $\times 400$) and (C) tubular cells positive for SV-40 (Immunoperoxidase staining $200\times$).

Graft dysfunction can be attributed unequivocally to BKV infection in 5% of cases (biopsy proven BKVAN). In rest of the cases, other coexistent pathologies were present along with viraemia and viruria. Since reactivation of BKV, infection occurs

as viruria, viraemia and later nephropathy it would be appropriate to follow-up such cases. This will help in generating more meaningful data, for evaluating association between graft dysfunction, acute rejection and BKV infection.

Association with numerous clinical variables have been identified, which includes, immunosuppressive therapy, rejection episodes, male gender, age and HLA mismatch.^{17–19} No specific maintenance immunosuppressive agent has been identified but patients who receive tacro- and mmf-based regimens have been found to be at high risk.¹⁹

In our series, patients with BKV infection receiving tacro/ mmf were high but on analysis were not found to be statistically significant. Statistically significant correlation was found between BKV infection and patients receiving ATG for treatment for steroid resistant rejection and as induction therapy. Many studies have reported association of BKV infection with the use of ATG for management of steroid resistant in patients on tacro/ mmf based maintenance therapy.^{20–22}

Recommendations

In view of high proportion of viral shedding evident in our representative renal allograft recipients, it would be imperative to establish screening protocols. Screening for viruria should be carried out every 3 months during first 2 years post-transplant or when allograft dysfunction is noted by quantitative molecular assay and follow it up with renal biopsy in a scenario of high viraemia or deteriorating graft function. Diagnosis of BKVAN with concomitant acute rejection remains problematic. Ancillary techniques involving SV-40, HLA DR and C4d staining along with post-transplant antibody monitoring may elucidate the diagnosis.^{23–25} The limiting factor of too small a data of BKVAN may be circumvented by generating similar data in other Armed Forces transplantation centres.

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CONFLICTS OF INTEREST

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Journal Scan

Ay C, Dunkler D, Marosi C, Chiriac AL, Vormittag R, Simanek R, et al. Prediction of venous thromboembolism in cancer patients. *Blood* 2010;116:5377–82

The risks of venous thromboembolism (VTE) differ widely in subgroups of cancer patients and depend on the presence of various patient-, tumour- and treatment-related risk factors. This study conducted at Medical University of Vienna included cancer patients, enrolled between 2003 and 2008, with newly diagnosed cancer or progression of disease after complete or partial remission who had not received chemotherapy (in the last three months), radiotherapy and surgery (in the last two weeks). There was no routine VTE screening in the study population. Only when symptoms of VTE developed the patients were subjected to duplex sonography, CT scan for systemic thromboembolism and ventilation perfusion scan for pulmonary thromboembolism.

The calculation of risk score was based on a previously developed Khorana risk model which assigned a score of 'two' for a 'very high risk' site of cancer (stomach, pancreas or brain); one each for 'high risk' site of cancer (lung, kidney, lymphoma or myeloma), platelet count of $350 \times 10^9/L$ or more, haemoglobin $< 10 g/dL$ and leukocyte count $> 11 \times 10^9/L$, BMI $\times 35 Kg/m^2$. This model was further expanded by incorporating two biomarkers and assigning 'one' point to each [soluble P-selectin ($\times 53.1 \mu g/mL$) and d-dimer ($\times 1.44 \mu g/mL$)]. Of the 819 patients included in the study, 61 (7.4%) developed VTE over a median follow-up of 656 days. Pulmonary embolism was fatal in four (6.6% of VTE events) patients. On analysis, the cumulative probability of VTE in the original risk model after six months was 17.7% in patients with highest risk score ($\times 3$, $n=93$),

9.6% in those with score two ($n=221$), 3.8% in those with score one ($n=229$) and 1.5% in those with score zero ($n=276$). The difference in these groups was significant (log-rank test, $P<0.001$). In univariate regression analysis, the hazard ratio of VTE was 2.1 per one point increase in the risk score (95% CI). In the expanded risk model (inclusive of d-dimer and soluble P-selectin), the cumulative VTE probability was 35% in patients with highest score ($\times 5$, $n=30$), 10.3% in those with score three ($n=130$) and 1% in those with score zero ($n=200$). These associations were statistically significant. In patients with highest compared with lowest risk, the probability of VTE was 26-fold higher. For the expanded model, the sensitivity at the cut off point for highest risk was 19.1%, specificity 98.2%, positive predictive value 42.9% and negative predictive value was 94.4%.

The authors concluded by emphasising the usefulness of the scoring system to stratify patients according to VTE risk and the need for interventional trials based on the risk assessment model to demonstrate effectiveness of thromboprophylaxis in high-risk patients. Primary thromboprophylaxis in cancer patients is challenging as the risk of VTE is not equal in all cancer patients, and anticoagulation is associated with increased bleeding complications. Therefore stratification of cancer patients according to their VTE risk is of utmost clinical importance, thus maximising the clinical benefit and cost effectiveness of prophylaxis and additionally minimising risk of bleeding complications.

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