Original Article

BK Polyomavirus Nephropathy leads to rejection in Kidney Transplant Recipients

Sanjukta Goswami[1], Arpita Ghosh Mitra[2], Sudip Roy[3], Dilip Kumar Pahari[4]

Over the past decade, infection of kidney transplants with BK polyomavirus has become increasingly appreciated. The infection is manifested by both an inflammatory response and subsequent fibrotic response, leading to renal dysfunction and eventual irreversible graft loss. The pathogenesis of this disease isdue to over-immunosuppression with concomitant tubular injury. It remains unclear whether the tubular damage is either due to viral effect or virally-directed immune response. Renal biopsy is the gold standard of diagnosis of BKV nephropathy. Post-transplant BKV quantification has been performed by Real Time PCR. Pre transplant HLA typing has been done by SSOP method using Luminex. Pre transplant Patient Donor crossmatch has been performed by serological method with DTT and AHG augmentation. Post-Transplant protocol biopsies are done for all the patients. Statistical analysis has been done with Origin Pro 9.0. In our study we have seen 64 biopsies from kidney transplant recipients. 15 patients among 64 have been diagnosed with active BKV infection by quantitative PCR and 2 of them experienced graft loss. The histological hallmarks of this disease are viral cytopathic changes in renal tubular epithelial cells, which occur in medullary and distal tubules in early disease, and proximal tubules in more advanced stages. Other site includes vascular and parietal glomerular epithelium along with interstitial inflammatory cell infiltration.Some clinicians contend that this infiltrative process is an appropriate viral-specific immune response, and for that reduction in immunosuppression is warranted.

[*J Indian Med Assoc* 2018; **116:** 40-3]

Key words **: BK virus Nephropathy, Kidney Transplantation.**

The frequency of BKV nephritis and subsequent nephropathy are observed in kidney transplantation. After so many years of through investigation the key factors associated with increasing incidents remain unclear. There are four variants of inclusion body observed; type IV out of them is related to graft loss. Therefore, post transplantation chronic rejection due to BK Virus infection is not a matter of negligence.

The establishment of immune suppressive agents such as mycophenolate mofetil (MMF) and Tacrolimus (TAC) has been thought to play main causative role in BKVN. Out of 100 approximately 80 individuals observed with antibody of BKV which can cross react with virus of same family like SV40, JC virus and frequency is notable in renal disease, kidney donor and transplant recipients[2,6]. Renal dysfunction is one of the major symptoms[2,5]. Apart from that hallmark characteristics are uretic obstructions, occasionally seen hydronephrosis[14]. Renal failure reported in 30-60%[2]. Viral cytopathic changes in renal tubular epithelial cells occurs in medulla in early stages followed by proximal tubular in advance stages.

Two major hypotheses has been proposed regarding source of infections. First hypothesis conducted the transmission probably through the donor tissue to recipient who has never exposed to BKV in their life span[7,8]. Second, stated that the latent state of BK virus in renal epithelial which enters into lytic cycle become reactive after transplantation due to defective immune Surveillance. Reactivation of virus in urothelial cells of recipient is a potential threat for transplantation. Replication takes place at early stage of transplantation, detectable stages are: viruria, viremia, nephropathy[19]. Ischemic injury is also possible reason for creating a suitable environment for viral replication followed by infection which leads to nephritis that becomes a vital challenge for clinician to prevent graft loss. Non coding control region (NCCR) is a unique sequence present within BKV which shows a greater level of variability. Due to this reason individual diagnosed with same BKV has different sequence in different patients.

Hyper Ig-M immune deficiency is one of the major lethal cases in the BKV infection antibody production against BKV[4]. But, lack of development of BKV specific

HLA & Molecular Laboratory, Medica Superspeciality Hospital, E M Bypass, Kolkata 7000091
[1]MSc Student
[2]PhD, PDF (HMS), Technical Manager
[3]MBBS, MD, Head of the Department of Microbiology
[4]MBBS, MD, DM, DNB, FASN, FISN, Head of the Department of Nephrology

IgG and CD40 ligand function is considerable in disease manifestation. In this case class switching is endangered. So, IgM Class switching is help us to save a recipient from nephritis followed by death.

Another important parameter was noted that recipients and donor with high level of HLA mismatch leads to nephropathy and subsequent rejection but in this case graft loss is less[12]. Viral cytopathic effect specifically urinary decoy cells and virus itself from blood, urine, tissue and immunity to virus specific antibody is the key basis of diagnosis of BKV infection. Urinary decoy cells screening considered as a parameter to detect the infection but give poor positive predictive value around 20% as well as it is not able to detect BKV nephropathy[19].

There are a lot of potential antiviral drugs which are to some extent able to prevent this rejection which includes cidofovir of leflunomide[12-15] but decreasing the level of immunosuppressive drugs immediately caused draft rejection which causes further problem in recipients. Scientists have observed the BKV load in renal transplant recipients as well as in the peripheral blood of those individuals who reported as PCR positive[10]. The frequency of occurrence BKVN lowered after second transplantation.

Material and Method

Patient : 15 post-transplant patients have been included in this study in between October 2012 to May 2015 at Medica Superspecialty Hospital, Kolkata, India.

Lymphocyte crossmatch (CDC) : Pre transplant Lympho Cytotoxicity Test was performed using both direct complement-dependent cytotoxicity (CDC) crossmatch and CDC crossmatch with added anti-human globulin (AHG-CDC).

Real Time PCR : Viral DNA from patient sera has been extracted using QIAmp blood mini kit (QIAGEN). Real Time PCR was performed using Rotor Gene Q QIAGEN using artus BKV RG PCR kit (QIAGEN). To generate the standard curve, positive and negative controls were run in parallel along with the patient's sample.

Kidney Biopsies : Patient underwent protocol biopsies post-transplant to determine allograft dysfunction and rejection.

Statistical analysis : All statistical analysis has been done using Origin 9 and Microsoft Excel 2010.

HLA Typing : HLA Typing has been done using reading software Luminex 1001S v.2.3. The lab type SSOP HLA locus A, B, C, DR, DP, and DQ has been procures from One Lambda USA. All the data has been analyzed using Fusion 3.0 software.

Results

We had received 64 samples for BKV quantitative PCR amongst kidney transplant allograft recipients, where clinically indicated. Among those 15 (23%) patients were detected with active BK virus infection. The mean age of all affected patients (n=15) is 51 years, and male: female ratio is equal to 2:1. All the infected patients had undergone a living unrelated kidney transplants. All selected infected patients had negative Serological Crossmatch for both Classes I and II (Table 1).

Table 1 — *Patients information including number of patients (n=15), mean age, sex, donor's information, transplant information, HLA match, and CDC crossmatch*

Recipient's information	Age (Mean) years	51
	Male	10
	Female	5
Donor's information	Deceased	0
	Living	15
Transplantation	First Transplantation	15
	Second Transplantation	0
HLA-A,B and DR mismatches	Matched	0
	Mismatched	15
CDC crossmatch	Positive	0
	Negative	15

Among the 15 infected kidney allograft recipient the BK virus Quantitative Real-time PCR reveals a mean viral peak of 340394 copies/ml. The highest viral load detected 3029030 copies/ml and the lower range detected 5.0 copies/ml. Among all infected patients, 3 patients were detected with viral load above 500000 copies/ml. Among the infected patients of high viral load (>100000 copies/ml), 2 of the patients are presented with BK virus associated nephropathy and kidney allograft rejection (Fig 3).

The patient presented with BKVN is a 58 years male with a viral load of 2556840 copies\ml. The renal biopsy reveals tubular epithelial cells with clear intracellular viral inclusion and with a positive staining by SV40. The mean creatinine level of this patient is 2.37 mg/dl and the post-transplant creatinine concentration appears a highest peak of 2.80 mg/dl on 14th day of transplantation.

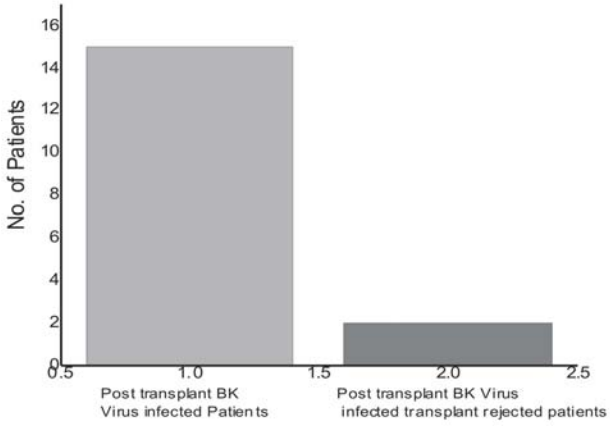

Fig 1 — Two (2) recipients (13%) out of 15 patients had been detected with rejection of allograft

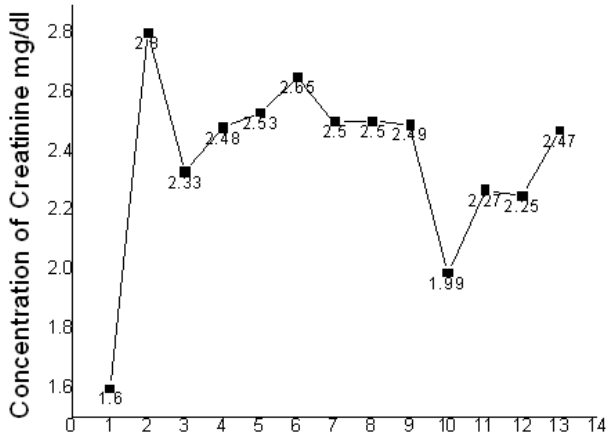

Fig 2 — This graph shows serum creatinine level of the patient at different time point indicating BK virus mediated nephropathy. 13 tests were done which plotted on x axis. All tests are done after kidney transplantation and all the data in this graph are arranged date wise

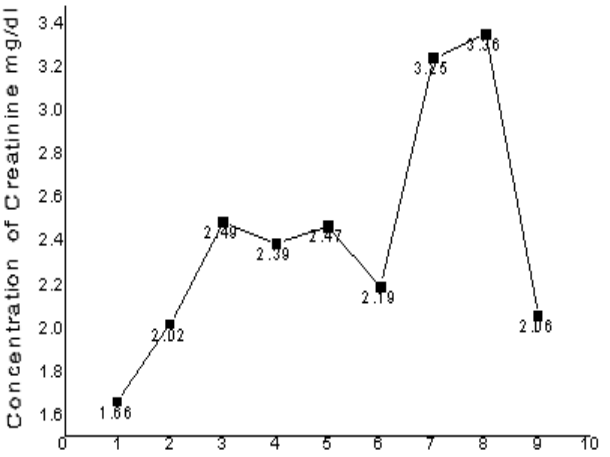

Fig 3 — This graph shows serum creatinine level of the patient at different time point indicating renal allograft rejection. 9 tastes are done after transplantation. All tests are done after kidney transplantation and all the data in this graph are arranged date wise

The 48 years old male patient presented with kidney allograft rejection symptoms exhibiting a viral load of 643395 copies/ml. The renal biopsy reveals a positive C4d staining, that confirm the rejection phenomenon. The mean creatinine value of the patient after transplantation is 2.43 mg/dl and the highest peak of creatinine ie, 3.36 mg/dl reaches on the 64th day of transplantation.

The patient with the highest viral load of 3029030 copies/ml does not exhibit any kind of nephropathy or rejection symptoms possibly because she had more than 50% HLA matched with her donor. Although this 29 year old female patient had the mean creatinine value of 3.908 mg/dl and her peak creatinine value was 8.85 mg/dl.

Discussion

A total number of 64 patients underwent renal transplantation in between October 2012 to June 2014, 15 of those post transplants recipients has viral DNA in blood plasma which is a potential indicator of active BKV infection. Mean age of all allograft patients is 51, 2 of them experienced graft loss. Among 15 infected kidney allograft BK virus Quantitative real-Time PCR reveals a mean viral peak of 340394 copies/ml. highest viral loads detected in PCR 3029030 Copies/ml and lower range 150.0 copies/ml. Among all infected patients, 3 patients were detected with viral load above 500000 copies/ml.

Among these 3 patients 2 patients were male (48 and 58 years.) and 1 female (29 years.). Among the infected patients of high viral load 2 patients were diagnosed with BK virus nephritis and associated nephropathy, experience kidney allograft rejection. There are three stages of viral nephropathy: First, initially, asymptotic, but the presence virus detected by urine decoy cells indicating viruria. Second, high dose of immunosuppressive drugs like tacrolimus. Third, observation of viral load in blood plasma along with tubular injury.

Among 3 patients with high viral load, first patient 58 years male with viral load of 2556840 copies/ml. Renal biopsy reveals the tubular epithelial cells with clear viral inclusion and it was positive stained by SV40 which confirms the presence of virus. Total 13 tests were done to identify the serum creatinine level which shows the mean 2.37 mg/dl and post-transplant creatinine concentration appeared a highest peak of 2.80 mg/dl. This elevated level revealed the renal dysfunction. The symptoms of BKVN started appearing within 2 weeks initiating from presence of urine decoy cells to viremia.

All the patients administered with immunosuppressive drugs of tri combination like tacrolimus, mycophenolate mofetil along with corticosteroids. C4d staining confirms the rejection and patient experienced rejection on 70th day.

Second patient 48 years male with highest viral load of 643395 copies/ml and allograft rejection observed on 64th day, C4d staining confirms the rejection phenomenon. 9 tests were done and the mean creatinine level after transplantation is 2.43 mg/dl with highest peak of serum creatinine 3.36mg/dl, a marker of renal dysfunction. Symptoms started appearing after 3 weeks with inclusion body in urine.

In both cases C4d staining acts as a biomarker for confirming the antibody mediated rejection phenomenon. According to numerous researches, inclusion body bearing cells more likely attached to medulla and infect other cells which looks like rounded. Here vital challenge is to differentiate between interstitial inflammation induced by virus and cellular rejection. In both cases the possible rea-

son of rejection is spreading of viral infection in renal cortex which further enhanced by viremia. When virus enters into blood stream, it started infecting tubular cells along with entire nephron leads to inflammation and associated nephropathy. Some researchers concluded that human leukocyte antigen plays a vital role. HLA DR stimulates lymphatic reaction which in turn induces T cell lysis and eventually cellular rejection took place. And also high dose of administration of immunosuppressive drugs create an environment for virus to gain the access of host system.

Third patient 29 years female had high viral load of 3029030 mg/dl does not exhibit any kind of nephropathy or rejection symptoms possible because she had more than 50% HLA matched with her donor and also taken into consideration her younger age and possibly a comparatively stronger immunological status gave advantage over other patients. Although she had high Creatinine level 8.85mg/dl but did not exhibit any signs and symptoms of nephropathy or graft failure after several months of transplantation.

Recently there is no antiviral therapy is available to treat the BKV infection. So it is wise to reduce the amount of immunosuppression to prevent the viral infection which leads to allograft rejection. Leflunomide and Cedofovir are the drugs used for treatment.

Conclusion

Post-transplant BKV nephropathy is one of the major concerned areas to prevent allograft rejection. Early stage monitoring and subsequent reduction of immune suppression could be the salient steps for the prevention and management of the stage leading to BKV nephropathy associated graft failure in renal transplant.

References

1 Shah KV, Daniel RW, Kelly Jr TJ — Immunological relatedness of Papovaviruses of simian virus 40-polyoma subgroup. *Infect immune* 1977; **18:** 558-60.

2 Hussain S, Bresnahan BA, Cohen EP, Hariharan S — Rapid kidney allograft failure in patients with polyoma virus nephritis with prior treatment with antilymphocyte agents. *Clin transplant* 2002; **16:** 43-7.

3 Gupta M, Miller F, Nord EP, Wadhwa NK — Delayed renal allograft dysfunction and cystitis associated with human polyomavirus (BK) infection in a renal transplant recipient: a case report and review of literature. *Clin Nephrol* 2003; **60:** 405-14.

4 Rosen S, Harmon W, Krensky AM — Tubulo-intertial nephritis associated with polyomavirus (BK type) infection. *N Engl J Med* 1983; **308:** 1192-6.

5 Randhawa PS, Finkelstein S, Scantlebury V — Human polyoma virus associated interstitial nephritis in the allograft kidney. *Transplantation* 1999; **67:** 103-9.

6 Stolt A, Sasnauskas K, Koskel P — Seroepidermology of the human polyomaviruses. *J Gen Virol* 2003; **84:** 1499-1504

7 Rubino MJ, Walker D — Immunosuppression and murine polyomavirus infection. *Virus Res* 1998; **9:** 1-10.

8 Andrews CA, Shah KV, Daniel RW — A serological investigation of BK virus and JC virus infection in recipients of renal allograft. *J infect dis* 1988; **158:** 176-81.

9 Greenlee JE, Clawson SH, Pheleps RC, Stroop WG — Distribution of K-papovavirus in infected newborn mice. *J Camp Pathol* 1994; **111:** 259-68.

10 Hussain S, Orentas R, Walczak J — Prevention of nephritis by monitoring BK viremia in renal transplant recipients: a prospective study. *Graft* 2004; **7:** 28-30.

11 Major EO — Field's virology, 4th edn. vol. 2. Lippincot Williams and Wilkins: Philadelphia 2001; 2175-96 (chapter 64).

12 Vats A, Shapiro R, Singh Randhawa P — Quantitative viral load monitoring and Cidofovir therapy for the management of BK virus-associated nephropathy in children and adults. *Transplantation* 2003; **75:** 105-12.

13 Kadambi PV, Joshephson MA, Williams J — Treatment of refractory BK virus- associated nephropathy with cidofovir. *Am J Transplant* 2003; **3:** 186-91.

14 William JW, Javaid B, Kadami PV —- Leflunoamide for polyomavirus type BK nephropathy. *N Engl J Med* 2005; **352:** 1157-8.

15 Al-Jedai AH, Honakar MR, Trofe J — Renal allograft loss as the result of polyomavirus interstitial nephritis simultaneous kidney pancrease transplantation: results with kidney transplantation. *Transplantation* 2003; **75:** 490-4.

16 Ramos E, Vinceti F, LU WX — Renal transplantation in patients with graft loss caused by polyoma virus nephropathy. *Transplantation* 2004; **77:** 131-3.

17 Drachenberg CB, Papadimitriou JC, Mann D, Hirsch HH, Wali R, Ramos E — Negative impact of human leukocyte antigen matching in the outcome of polyomavirus nephropathy. *Transplantation* 2005; **80:** 276-8.

18 Awadalla Y, Randhawa P, Ruppert K, Zeevi A, Duquesnoy RJ — HLA mismatching increases the risk of BK virus nephropathy in renal transplant recipients. *Am J Transplant* 2004; **4:** 1691-6.

19 Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J — Prospective study of polyomavirus type BK replication and nephropathy in renaltransplant recipients. *N Engl J Med* 2002; **347:** 488-96.

20 Chen Y, Trofe J, Gordon J, Du Pasquier RA, Roy-Chaudhury P, Kuroda MJ, Woodle ES, Khalili K, Koralnik IJ — nterplay of cellular and humoral immune responses against BK virus in kidney transplant recipients with polyomavirus nephropathy. *J Virol* 2006; **80:** 3495-505.

21 Smith JM, MacDonald RA, Finn LS — Polyomavirus nephropathy in pediatric kidney transplant recipients. *Am J Transplant* 2004; **4:** 2109-17.

22 Hariharan S — BK virus nephritis after renal transplantation. *Kidney Internation* 2006; **69:** 655-62.