



Immunohistochemistry Features of BK Virus-Associated Nephropathy in Chinese Kidney Transplant Recipients

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ABSTRACT

Objective Polyomavirus BK infection has emerged as an important cause of BK virus associated nephropathy (BKVAN) leading to allograft dysfunction and loss. The aim of this study was to investigate immunohistochemistry characteristics of BK virus-associated nephropathy.

Methods We prospectively investigated 36 renal allografts performed in Jinling Hospital. BKVAN was diagnosed by light microscopic examination and a positive immunohistochemistry staining of anti-SV40 large T antibody in a biopsy specimen. The change of CD4+, CD8+, CD20+, CD68+ and CD138+ cells and C4d, IL-2R, HLA-DR expression in renal allografts was observed in the BKVAN group, the acute rejection group and the normal control group. Changes of allograft infiltrating cells was observed in patients with different serum SCR in BKVAN groups (group A, B, C).

Results 36 patients were implemented with SV-40 large T antigen staining of renal tissues, positive scattered BKV tubules can be seen in renal cortex and medulla, that is the nuclear of tubular epithelial cells was brown, the middle was transparent, and the peripheral was burr-like, and exfoliated cell polyomavirus expression can be seen in lumen of the renal tubules. A large aggregation of CD4+, CD8+, CD20+, CD68+, CD138+ cells can be seen in renal interstitial from all renal pathology in BKVAN group. C4d, HLA-DR and IL-2R expression were mostly negative. There was no significant difference in the CD4+, CD8+, CD20+, CD138+ cells, expression of IL-2R and HLA-DR antigen in allograft of BKVAN patients with different SCR levels (group A, group B and group C). However, the CD68+ cells increased with the increase of serum creatinine, and the number of CD68+ cell was significantly in the group C higher than in the group A.

Conclusion BKVAN diagnosis mainly depends on the allograft biopsy pathology, and using SV-40 large T antigen staining can improve the diagnostic rate of BKVAN. Immunohistochemistry changes in the BKVAN group were different from the acute rejection group and the control group, the detections of C4d, IL-2R and HLA-DR antigen of renal allograft are extremely important for identification.

KEYWORDS

Kidney transplantation, BK virus associated nephropathy, Diagnosis, Biopsy, Immunohistochemistry

INTRODUCTION

The steady advances in immunosuppression and immune monitoring of kidney transplant recipients over the past several years has resulted in a remarkable decrease in the risk of acute rejection, but long-term graft survival remains unchanged⁽¹⁾. Although several factors are implicated in long-term graft loss, progressive interstitial inflammatory cell infiltration, interstitial fibrosis and tubular atrophy continues to be a significant factor⁽²⁾. The etiology of interstitial inflammatory cell infiltration is related to a myriad of immunologic and nonimmunologic factors^(3,4). Studies of kidney transplant recipients have shown that acute rejection and BK virus-associated nephropathy (BKVAN) is associated with an increased risk of graft interstitial inflammatory cell infiltration^(5,6) and graft loss^(7,8). The acute rejection and BKVAN diagnosis mainly de-

pends on the renal allograft biopsy. Particularly, BK virus infection can cause acute tubular necrosis, and early diagnosis of BKVAN and use of effective treatment can prevent irreversible allograft damage caused by virus and improve prognosis of BKVAN⁽⁹⁾. Although some transplant centers have been studied on BKV infection after kidney transplantation, the changes of allograft infiltrating cells in patients with BKVAN are rarely reported⁽¹⁰⁾. This study was to investigate immunohistochemistry features of BK virus-associated nephropathy in Chinese kidney transplant recipients.

MATERIALS AND METHODS

Objects Among 351 cases of patients diagnosed with decreased graft functions by renal allograft biopsy in our unit, from August 2005 to January 2015, 36 patients met the inclu-

sion criteria.

Inclusion criteria I. More than 3 months after kidney transplantation, increased serum creatinine (SCR ≥ 1.5 mg/dl or greater than 30% in increase of serum creatinine values above the baseline), and slow clinical progression (increase in serum creatinine < 1 mg/week), accompanied with or without hematuria or proteinuria; II. Vascular and post-renal factors in renal allograft were excluded by color doppler ultrasound. All patients were carried out with blood routine, urine routine, liver and renal function, blood coagulation function and concentration measurements of cyclosporine A (CsA) or FK506 (tacrolimus, Tac) and mycophenolate mofetil (MMF) in blood before biopsy. All subjects have signed informed consent.

Study Design Thirty-six cases of patients with histologically confirmed BKVAN as research object (BKVAN group), and select the same renal transplantation with acute rejection in 42 cases (acute rejection group) and 81 cases of routine biopsy patients as control group, which group of acute rejection were selected renal transplantation after 2 weeks, allograft biopsies with acute rejection patients. Basis of serum creatinine levels(SCR) with BKVAN were divided into three groups: group A(n=16): $1.24 < SCR \leq 2.5$ mg/dl; group B(n=12): $2.5 < SCR \leq 3.5$ mg/dl) and group C(n=8): $SCR > 3.5$ mg/dl.

Pathological study Renal graft biopsy was carried out under the guidance of B ultrasound. The specimens were qualified if the renal tissues contained 2 vessels and more than 10 renal glomeruluses, and were confirmed with conventional light microscopy, immunofluorescence and immunohistochemistry examination. Light-microscopic staining included HE, PAS, PASM and Masson trichrome staining. The pathological diagnosis of allograft can accord with Banff 97 criteria ^[11].

Immunofluorescence Direct fluorescent assay was used to observe the deposition intensities and sites of IgG, IgA, IgM, C3, C4, C1q, and C4d. All frozen tissue sections were confirmed with C4d immunofluorescence staining, and the staining intensities were in accordance with Banff 2001 criteria, in which C4d negative was defined as $< 25\%$ of PTC C4d deposition, reversible focal positive was defined as 25% to 50% of PTC C4d deposition and C4d asystematic positive was defined as $> 50\%$ of PTC C4d deposition ^[12].

Immunohistochemistry Enrolled patients were carried out with CD4+, CD8+, CD20+,CD68+, CD138+ cells, IL-2R, HLA-DR and BK virus immunohistochemical staining. Paraffin immunohistochemistry and Envision two-step method were applied: the paraffin sections were deparaffinized by normal method, treated by 3% hydrogen peroxide to inhibit the endogenous peroxidase, placed into a pressure cooker with 1ml EDTA solution for antigen retrieval, blocked in normal 10% bull serum solution, washed and added with monoclonal mouse anti-human CD3 (1:100), CD68 (1:100), CD20 antibody (1:100) and HLA-DR antibody (1:50), respectively, incubated at room temperature for 2h, added with Envision and incubated at room temperature for 40min, colored with DAB for 5min, counterstained with hematoxylin for 2min, dried and sealed with neutral gum. Read under a Nikon 8100 microscope. SV240 large T antigen was used for positioning (anti-polyomavirus antibody staining).

The infiltration degrees of CD4+, CD8+, CD20+,CD68+,CD138+ cells were classified according to the following criteria: 16 high power fields with a total area of 1mm^2 were randomly selected for counting, the sum of counting > 300 was defined as 3 points, $200 \sim 300$ was defined as 2 points, $100 \sim 200$ was defined as 1 point, and < 100 was defined as 0 point.

HLA-DR expression was judged on the basis of 50 tubular cross-sections, HLA-DR positive tubular accounted for a percentage of the whole tubular. $> 50\%$ was defined as 3 points, $25 \sim 50\%$ was defined as 2 points, $10 \sim 25\%$ was defined as 1 point, and $< 10\%$ was defined as 0 point.

Diagnosed criteria of BKVAN ^[9,13,14] SV240 large T antigen was located in the nuclear of renal tubular epithelial cell. Positive expression was brown and negative cells were not detected. BKVAN was diagnosed through their positive expression in combination with HE staining.

Statistical Analysis The measurement data is represented by mean \pm standard deviation ($\bar{x} \pm s$), $P < 0.05$ has significant difference, $P < 0.01$ has obviously significant difference.

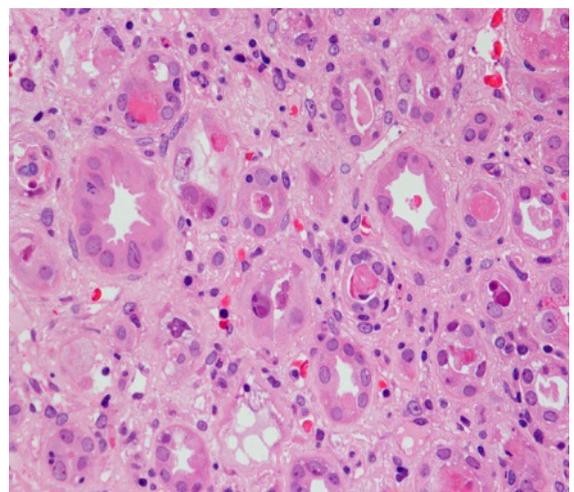
RESULTS

Clinical data BKVAN patients aged 19-56 years old, the morbidity of BKVAN in the shortest time to 3 months after kidney transplantation, the longest 56 months. The lowest serum creatinine 1.58mg/dl, the highest 4.12mg/dl. Acute rejection group aged 20-57 years old, acute rejection occurred in the shortest time 2 months after kidney transplantation, the longest time 49 months. The lowest serum creatinine 1.98mg/dl, the highest 4.53mg/dl. In the control group, the age of 20-51 years old, the time of renal biopsy was 3 months and the longest time was 19 months. The lowest serum creatinine 0.89 mg/dl, the highest 1.24mg/dl(Table 1.).

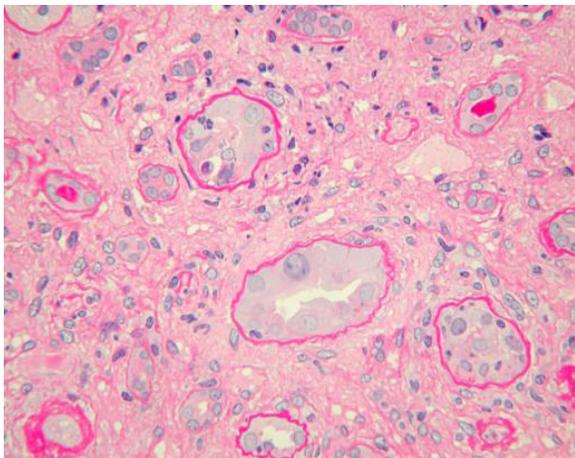
Table 1. Clinical data

	BKVAN group	Acute rejection group	Control group
Gender (Male/Female)□	16/20□	19/23□	28/53
Age (yr)□	37.39±10.01 □	36.49±12.03 □	36.55±10.57
Postoperative onset time (mon)	20.16±2.58 □	16.65±2.67	16.09±2.71
Serum creatinine level (mg/dl)	2.61±1.16	3.13±1.60□	1.05±0.31

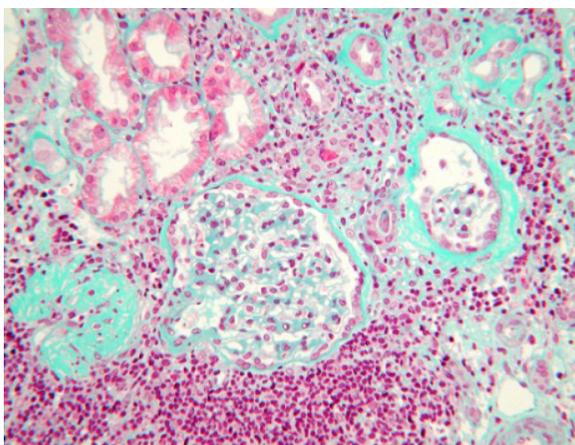
Pathological features of BKVAN The typical pathological features of BKVAN are as follows: different phenotypes of basophilic BK virus inclusion bodies appeared renal tubular epithelial cells, infected renal tubular epithelial cells drop out towards renal tubular lumen, resulting in bare spot on the base membrane (Figure 1.A, B). Interstitial prominent presented as multifocal gathered infiltrating cells, with mononuclear cells and plasma cells as main, and their surrounding tubulars often had tubulitis, multifocal thickening and atrophy of tubular basement membrane (Figure 1.C, D). Later pathological changes mainly include fibrosis, with a few of BKV-infected cells (Figure 1.D).



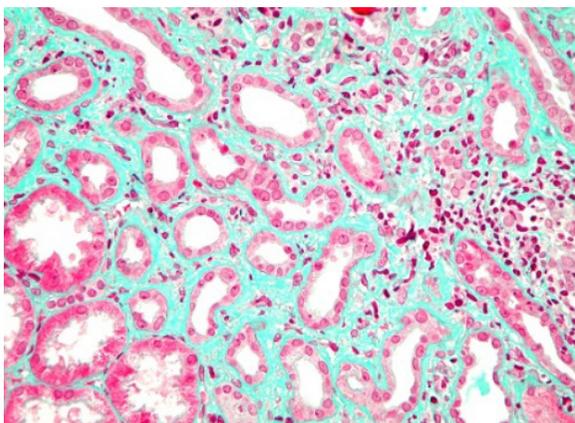
A:Multiple viral inclusions could be seen in the B:Multifocal tubular atrophy and basement membrane renal tubular epithelial cells, and the tubular thickening, focal tubular epithelial cells growth epithelial cells were took off to growth in flat the lumen in flat (x200) (x200)



B:Multifocal tubular atrophy and basement membrane thickening, focal tubular epithelial cells growth epithelial in flat (x200)



C:More mononuclear cells and plasma cells were D:Middle and severe fibrosis of interstitial(x400) infiltrated, small focal neutrophil distribution and a small amount of peritubular capillary inflammation could be seen(x400)

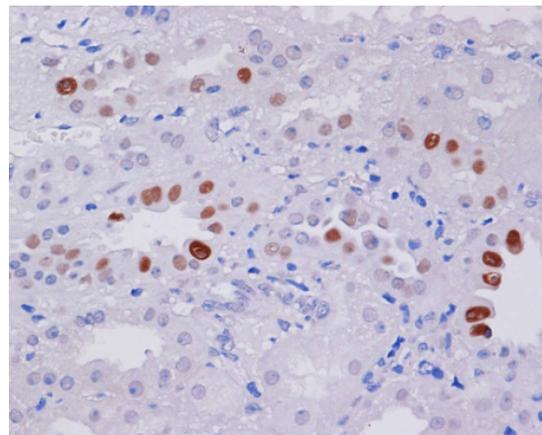


D:Middle and severe fibrosis of interstitial(x400)

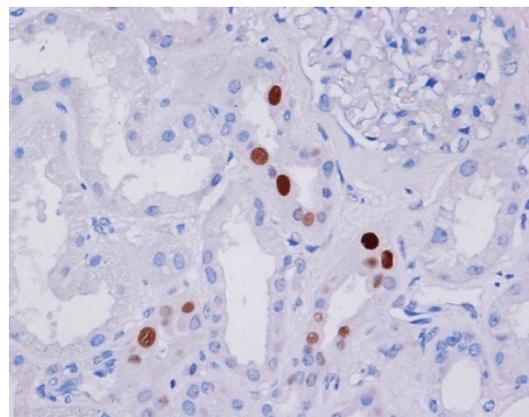
Figure 1. Pathological features of BK virus associated nephropathy

Immunohistochemistry in the BKVAN group 36 patients were implemented with SV-40 large T antigen staining of renal tissues, positive scattered BKV tubules can be seen in renal cortex and medulla, that is the nuclear of tubular epithelial cells was brown, the middle was transparent, and the peripheral was burr-like, and exfoliated cell polyomavirus expression

can be seen in lumen of the renal tubules (Figure 2.A, B). A large aggregation of CD4+, CD8+, CD20+,CD68+,CD138+ cells can be seen in renal interstitial from all renal pathology in BKVAN group. HLA-DR and IL-2R expression were mostly less than 5% and 5/mm², respectively. In one patient combined with rejection, HLA-DR and IL-2R expression were 43.6% and 18/mm², respectively;



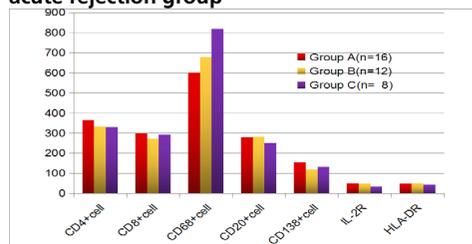
A:BK virus-positive staining cells could be seen in renal cortex and medulla tubular



B:The tubular epithelial perinuclear was in brown, intermediate was the lucent zone and the peripheral was burr-like

Characteristics of renal allograft infiltrating cells in BKVAN group and in the acute rejection group Compared with the control group, a large aggregation of CD4, CD8, CD20, CD68 and CD138 positive cells in BKVAN group and in the acute rejection group (Figure 3.) . CD20+ cells showed no difference between the groups. CD 8+ cells and positive expression of IL 2R and HLA-DR in renal tubular epithelial cells was significantly increased in the acute rejection group. In patients with BKVAN and control group, HLA-DR and IL-2R expression were mostly less than 5% and 5/mm², respectively.

Figure 3. Allograft infiltrating cells in BKVAN group and acute rejection group



C4d detection results in renal allograft It is interesting to find that all renal allograft immunofluorescence IgG, IgM, IgA, C3, C4, C1q were negative in the BKVAN group and in the control group. Allograft were detected by C4d among three groups, only 1 cases of BKVAN group were weak positive, 41 cases were positive in the acute rejection group, C4d test results were negative in the control group.

Changes of allograft infiltrating cells in patients with different serum SCR in BKVAN groups There was no significant difference in the CD4+, CD8+, CD20+,CD138+cells, expression of IL-2R and HLA-DR antigen in allograft of BKVAN patients with different SCR levels(group A,group B and group C) . However, the CD68+ cells increased with the increase of serum creatinine, and the number of CD68+cell was significantly in the group C higher than in the group A (Figure 4).

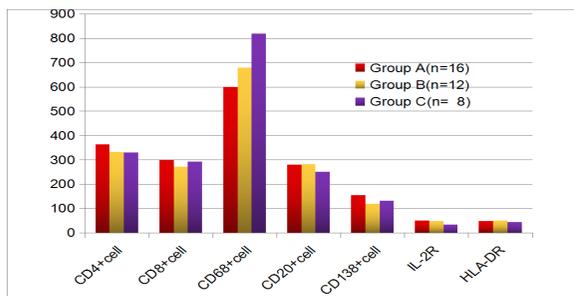


Figure 4.Changes of allograft infiltration in patients with different serum SCR in BKVAN groups

DISCUSSION

The diagnosis of BKVAN needed to be established on the basis of renal allograft biopsy [15,16]. The typical pathology mainly presented as different phenotypes of basophilic BK virus inclusion bodies occurred in the nuclear of renal tubular epithelial cells, infected renal tubular epithelial cells drop out towards renal tubular lumen, resulting in bare spot on the base membrane. Interstitial prominent presented as multifocal aggregation of massive infiltrating cells, in renal interstitium which are mainly mononuclear cells and plasma cells. Tubulitis, multifocal tubule basement membrane thickening and atrophy can be observed as the most common cases with surrounding tubules. Some pathological changes are mainly fibrosis which is only seen in a small number of cells infected with polyomavirus. According to morphological changes, inflammatory cell infiltration, tubular atrophy and interstitial fibrosis, Hirsch et al [17] divided the pathological process of BKVAN into three phases through semi-quantitative evaluation: Phase I: mild pathological changes, such as positive stained large T antigen of focal tubular epithelial cells and virus inclusion bodies, no extensive necrosis and inflammatory infiltration can be seen. Phase II: extensive multifocal diffuse pathological changes of cells, necrosis associated with inflammatory response and initial signs of interstitial fibrosis can be seen. Infiltrated inflammatory cells included polymorphonuclear cells, monocytes and plasma cells. Phase III: renal interstitial fibrosis, scarring and even calcification can be seen. BKVAN observed in this study was mainly Phase II. Therefore, it has extremely important clinical significance for patients with renal allograft dysfunction to carry out renal allograft biopsy for detecting BKVAN in early phase and staging.

Thirty-six patients with BKVAN had obviously renal dysfunction by biopsy. As the early pathological features of BKVAN often similar to the performance of interstitial nephritis, it was difficult to distinguish from acute rejection only through routine staining and light microscopy, especially for mild rejection. Therefore, it becomes necessary for using immune staining on the basis of light microscopy. In this study, SV-40 large T antigen staining was used, which has advantage of specificity. Immunohistochemical staining of renal allograft SV-40 large T antigen was widely recognized "gold standard" for the diagnosis of BKVAN [18].

It is interesting to find that all renal allograft immunofluorescence IgG, IgM, IgA, C3, C4, C1q were negative in the BKVAN group and in the control group. Allograft were detected by C4d among three groups, only 1 cases of BKVAN group were weak positive, 41 cases were positive in the acute rejection group, C4d test results were negative in the control group. Compared with the control group, a large aggregation of CD4, CD8, CD20, CD68 and CD138 positive cells in the BKVAN group and in the acute rejection group. CD20+ cells showed no difference between the groups. Positive expression of IL 2R and HLA-DR in renal tubular epithelial cells was significantly increased in the acute rejection group. In patients with BKVAN, HLA-DR and IL-2R expression were mostly less than 5% and 5/mm². Therefore, HLA-DR, IL-2R expression in the allograft and C4d staining were important indicators for identifying BKVAN and acute rejection [19].

CD20+,CD138 + cells in allograft were significantly increased in BKVAN group, it is possible BKV induced T and B lymphocyte activation and cause renal damage in allograft [20]. This study, number of infiltrating B lymphocyte in graft increased in the BKVAN group than in the acute rejection group. BKV after the invasion by B lymphocyte immune pathway plays an important role in the pathogenesis of BKVAN. Some study showed patients with BKVAN may induce acute rejection.

We found the renal allograft CD4+,CD8+,CD20+,CD68+and CD138+positive cells increased significantly and with serum creatinine elevation was increased in patients with BKVAN, which may with the direct action of the BKV on renal tubule, continuous renal tubular epithelial cell necrosis caused. Of course, allograft injure is more serious in the BKV induced host antiviral immune response than in the acute rejection reaction [21]. These reasons may aggravate transplanted renal interstitial fibrosis, which leads to more CD 68 + cell infiltration, accelerated the deterioration of allograft. We found that BKV can induce immune response to inflammatory cell infiltration in patients with BKVAN.

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