Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq

BK Polyomavirus-infected Decoy Cells in Urine Cytology Specimens of Renal Transplant Recipients

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Abstract

Background BK polyomavirus is one of the common post-transplant viral infections, affecting ~15% of renal transplantation recipients (RTR), leading to graft loss in more than half of cases.

Objectives Study the rate of detection of BK virus (BKV) in RTRs in Pap-stained urine cytology specimens.

Methods A single center study, urine samples were collected from 99 RTR patients, with 15 Living Donors (LD) and 15 patients with chronic kidney disease (CKD) were taken as controls. And urine cytology smears were Pap stained for detection of decoy cells (DCs).

Results Out of the 99 RTRs, 27 (27.3%) patients were decoy positive, 8 out of these 27 patients had uncommon DCs, and 5 out of these 27 cytology positive patients (18.5%) had biopsy proven BKV nephropathy (BKVN).

Conclusion This study suggests that the finding of BKVN in 18.5% of the DC positive patients stresses the importance of screening for BK polyomavirus with Pap-stained urinary cytology in RTR.

Key wards BK polyomavirus, renal transplantation, decoy cells

List of abbreviations: BKV = BK virus, BKVN = BK virus nephropathy, RTR = renal transplant recipient, LD = living donor, CKD = chronic kidney disease, DC = decoy cell.

Introduction

Opportunistic polyomaviruses infections mainly BK virus (BKV) and JC virus have become increasingly common problem among renal transplant recipients (RTR). Polyomaviruses are circular, double-stranded DNA viruses. The most important and commonest among these viruses is BKV infection, which was reported in ~15% of RTRs in the first post-transplant year in the absence of an effective prophylaxis strategy [1-3].

BKV presents with an asymptomatic gradual rise in serum creatinine with a tubulo-interstitial nephritis mimicking rejection, making a treatment dilemma. The decrease in immune-suppression that is needed to treat BKV infection is opposite to the increases in immune-suppressive drugs that are needed to treat rejection [4].

Once the virus has reactivated, there will be an ascending infection via cell-to-cell spread [5]. In the absence of an appropriate immunologic control, a progressive lytic infection could take place [6]. This results in large nuclear virus-containing inclusions in the tubular cells. Lysis of these urothelial infected cells leads to spread of the virus into the tubule lumen and then urine, as well as to the tubular interstitium and then spread to the surrounding cells. Subsequently, there will be tubular cell necrosis and cast formation [4,7].

Urine cytology screening for viral inclusion-bearing, so called decoy cells (DCs) allows for the early identification of BKV infection, and it has a relatively high sensitivity and a negative predictive value above 95%, besides being a cost-effective non-invasive assay [8-10]. Detection of DCs in the urine is one of the earliest assays, in this assay urine is Papanicolaou-stained and examined under light microscope to look for virus infected cells "decoy cells", which are epithelial cells with enlarged nuclei, and large basophilic ground-glass intranuclear viral inclusions [8-12].

Thus, the objective of the present study was to evaluate the prevalence of BKV infection in RTRs based on the detection of urinary DCs in Pap-stained urine cytology specimens.

Methods

A total of 99 RTR patients who attended the (Center of Kidney Diseases and Transplantation) in the Medical City of Baghdad, were enrolled in the study. A consent letter was signed by each patient, and the study was approved by the Ethical Committee of Al-Nahrain University.

Urine samples were collected from the patients, 33 of them had normal renal function, and the remaining 66 had impaired renal function. Two control groups were included in the study, 15 living donors (LD), and 15 non-transplanted patients with chronic kidney disease (CKD). Living Donors (who are apparently healthy individuals, not diabetic, not hypertensive, not receiving any medications, and their serum creatinine and creatinine clearance tests are normal).

Urine (10-ml aliquots) was centrifuged in Falcon tubes at 1500 rpm for 5 min for DCs screening. The supernatant was discarded and the sediment was re-suspended in the remaining urine. For each patient; two slides were prepared; one was immediately stained with the Papanicolaou method and examined under light microscope at 40 and 100X; the other was stored unstained at -20 ^{o}C for confirmation of diagnosis if required (slides preparation and staining were conducted in the Teaching Laboratories in the Medical City of Baghdad).

Identification and Quantification of Decoy Cells

Activation and replication of polyomaviruses was detected by identification of DCs, which are viral inclusion-bearing epithelial cells characterized by a ground-glass appearance with an enlarged nucleus, occupied by a basophilic inclusion surrounded by chromatin [10,11]. Some of the DCs appear resembling the tail of a comet [13]. For DCs quantification; a cut-off level of ≥ 10 DCs / (removed) slide, is defined as decoy positive [14]. In addition to the quantification of common ground-glass DCs, the uncommon (clumped) variants were also looked for; as their presence reflects the pathological stages of BKVN, if the uncommon (clumped) variants are more than 25% of the total decoy cell count; then BKVN can be predicted with more than 75% probability [15].

Statistical analysis

Statistical analysis was performed with the software SPSS version 21.0, and Microsoft Excel 2013. Categorical data formulated as count and percentage. Fisher exact test was used to describe the association of these data. Numerical data were described as mean, standard deviation of mean. ANOVA was used for comparison among more than two groups. $P \leq 0.05$ was considered statistically significant.

Results

This prospective study involved 99 RTR, 33 of them had normal renal function, and the remaining 66 had impaired renal function, 78/99 (78.79%) were males. Their mean age was 37±13years ranging between 18 and 67 years.

The mean serum creatinine value in the RTRs was 2.33±1.7 mg/dl, and their mean post-transplantation period was 17.5±9.7 months ranging from 2-30 months

Among these 99 RTRs, 19.2% had renal allograft rejection (biopsy-proven), five of them (5.1%) were receiving antithymocyte globulin (ATG) as anti-rejection therapy.

In addition, 5.1% had biopsy proven BK virus nephropathy (BKVN) (biopsy was studied in a separate laboratory), and 4.0% had ureteric stenosis (diagnosed by ultrasonography).

Papanicolaou-stained urine cytology smears revealed high rate of DCs shedding among RTR as compared with both control groups; LD and CKD that were all DCs negative, table 1.

On the other hand, uncommon DCs variants were present in 8 out of 99 RTR as shown in table 1 and fig. 1.

The most frequent variant of DCs was the amorphous, basophilic, ground-glass–like nuclear appearance. While in the other variants (uncommon type), the nucleus appeared eosinophilic and granular, and could be surrounded by a halo, or with a finely granular without a halo (Fig. 1).

Table 1. A; Decoy cells shedding, and B; Uncommon decoy cells shedding in renal transplant recipients

Feature		Study groups		
		LD	RTR	CKD
Decoy cells	Negative (%)	15 (100.0)	72 (72.73)	15 (100.0)
	Positive (%)	0 (0.00)	27 (27.27)	0 (0.00)
	Total	15	99	15
Uncommon Decoy cells	Negative (%)	15 (100.0)	91 (91.92)	15 (100.0)
	Positive (%)	0 (0.00)	8 (8.08)	0 (0.00)
	Total	15	99	15

LD = living donor, RTR = renal transplant recipient, CKD = chronic kidney disease

In addition, the results of this study revealed that 19 out of these 27 cases (70.4%) were males, their mean age was 34±7 years with no significant correlation with decoy cell positivity, and their mean post-transplant period was 18.2±8 months which also not significantly correlatedwith decoy cell positivity.

On the other hand, 21/27 (77.8%) of these DC positive patients had impaired renal function with a mean serum creatinine value 2.3±0.9 mg/dl, which is significantly correlated with DC positivity (p=0.01).

Table 2 demonstrates sensitivity and specificity of urine cytology in which all of the 5 patients who had biopsy-proven BKVN had positive urine cytology for DCs, i.e. 18.5% of them.

Two main standard immunosuppressive regimes are mainly followed in our transplantation center in Baghdad; the old regimen which includes cyclosporine A (CSA), mycophenolate (MMF), and prednisolone, the second regimen includes tacrolimus (TAC) instead of CSA, in addition to MMF and prednisolone.

Table 2. Sensitivity and specificity of Urine cytology as compared with renal biopsy in the diagnosis of BKVN

	cytology+	cytology-	Total
Biopsy +	5	0	5
Biopsy -	22	72	94
Total	27	72	
Sensitivity	100%		
Specificity	76.6%		

On comparing with the type of immunosuppression used, 55.6% of DC positive patients were on tacrolimus regimen, and 44.4% were on cyclosporine A regimen, which is not significantly correlated with DCs positivity and 4/5 (80%) of patients who were on ATG (anti-thymocyte globulin) were decoy positive, among the 26.3% (5/19) patients who had rejection. Finally, 3/4(75%) of ureteric stenosis patients (diagnosed by ultrasonography), were DC positive.

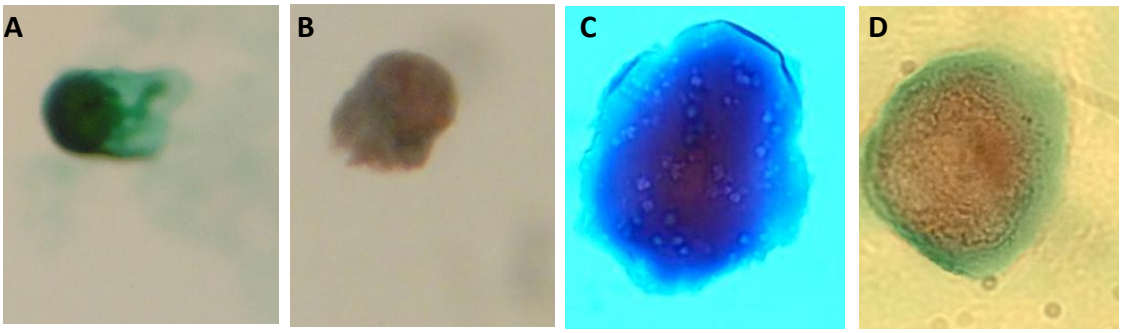

Fig. 1. Urine cytology: The activation and replication of polyomaviruses can be monitored by searching for viral inclusion-bearing epithelial cells, i.e., decoy cells (DC), in routine urine cytology specimens, (A,B) typical DC phenotype resembling the tail of a comet. And (C) uncommon (atypical) eosinophilic DC, (D) uncommon finely granular DC. Papanicolaou stain, (A&B) X400, (C&D) X1000.

Discussion

BKV shedding into the urine occurs in 10-30% of renal transplant recipients, and prospective monitoring of RTRs may identify patients with active infection before deterioration of the renal function. BKV cytopathic effect is a well-recognized entity in urine cytology specimens. Virus-infected cells termed (decoy cells) can be found in urine samples, and may mimic the nuclear changes that occur in urothelial cancer however, experienced cytopathologist could easily differentiate between them [8,9,16].

Decoy cells were found in the urine of 27.3% of the patients, mostly within 1-2 years following renal transplantation, and more than 50% were above 40 years age, matching findings from international studies reporting urinary decoy cells in 20-30% of patients from the 16th week of transplantation onwards [17-20].

Based on the morphologic features alone, one cannot always distinguish between BKV excretion and other viral infections. DCs might result from infection with BKV, JCV, and less commonly, adenoviruses [18,21]. However JCV and adenoviruses rarely cause nephropathy in RTRs [22,23]. The detection of uncommon DCs in about 30% of positive cases raises the possibility of BKV reactivation with more than 75% probabilityof BKVN [15]. This could support the specificity of this assay.

Detection of DCs in all of the five biopsy-proven BKVN cases indicates a high sensitivity of this screening method, a result that is in agreement with the majority of studies on DCs [8-10]. According to Drachenberg et al [24], the absence of DCs in urine rules out BK-associated nephropathy in up to 99.4% of instances. In addition, because urinary cytology is noninvasive, inexpensive, fast, and simple to perform, it remains a feasible alternative to immunohistochemistry and molecular biology for monitoring BKV infection in transplantation centers with limited resources [8].

Clinical manifestations associated with post-transplantation BKV infection include interstitial nephritis or BKV-associated nephropathy, ureteral stenosis, systemic infection, and bladder cancer [25,26]. In this study, 21/27 (77.8%) of positive cytology patients had impaired renal function with high serum creatinine, among which 5 patients had BKVN, and 3 patients had ureteric stenosis (diagnosed by ultrasonography).

Patients developing BK nephropathy oftenturn and remain 'DC positive' months before the initial diagnosis of viral nephropathy, repeating urine cytology is useful for proper risk assessment. Decoy cell positive renal allograft recipients fall into risk level 1; they have to be closely monitoredat 4-week intervals using repeat cytology examinations and additional quantitative (plasma) polymerase chain reaction tests [17]. DC can bedetected in urine when more

than 10^6 viral gene copies/ ml are excreted in urine [14].

Finally, 5 out of these 27 patients had rejection, and 4 of them were on ATG antirejection therapy, this could be explained either due to a concurrent BKV reactivation with rejection [14,27,28], though it is rare. Or more commonly, it is usually difficult to differentiate BKVN from the reaction of an interstitial cellular rejection (Banff 1 A/B) [29].

According to Pillai et al [30], in view of the increasing number of RTRs in South Indian states, Papanicolaou screening of urine cytology specimens for DCs is now a simple and efficient routine procedure for identifying patients at risk of developing BKVN and, of course, for ruling out disease. The test is now mandatory for all organ transplant recipients [31].

In conclusion, the finding of BKVN in 18.5% of patients with urinary decoy cells, stresses the importance of screening for BKV with urinary cytology. The test is sensitive, noninvasive, inexpensive, fast, and simple to perform, and is therefore highly indicated for transplantation services lacking immunohistochemistry and molecular biology testing facilities.

Acknowledgment

Authors would like to acknowledge the Center of Kidney Diseases and Transplantation in the Medical City of Baghdad, Iraq for there cooperation in accomplishing this research.

Author contribution

Al-Obaidi and Kadhim collect the specimens, Abd refer the patients, Habib do the sample preparation and processing, Qasim primarily read the cytolopathology slides, and Hussain and Abdlqader revise the cytolopathology slides.

Conflict of Interest

Authors declare no conflict of interest.

Funding

This research is funded by the Research and Development Department “RDD” of the Iraqi Ministry of Higher Education and Scientific Research, project of supporting post-graduate students (www.rddiraq.com).

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Received 3rd Dec. 2014: Accepted 17th Mar. 2015