

DETECTION OF BK POLYOMA VIRUS AMONG SUDANESE RENAL TRANSPLANTATION RECIPIENTS USING CYTOLOGY AND MOLECULAR BIOLOGY TECHNIQUES

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ABSTRACT

Objective: In the developing countries there is lack of recent information about detection of Polyoma virus using urine cytology and PCR, especially in Sudan. Polyoma virus-associated nephropathy is cause of graft dysfunction after kidney transplantation. The aim of this study is to compare between urine cytology and PCR-based assays for detection of BK Polyoma virus among Sudanese patients attending Sudanese Renal Transplantation Society Clinic. **Material &Methods:** one hundred and fifty renal transplant recipients attending Sudanese Renal Transplantation Society Clinic, both gender were included different age groups, patient were subjected to many types of immunosuppressive drugs, and different transplant duration. Urine specimen was collected, centrifuged and prepared for cytology according to Papanicolaou method. The remained deposit was prepared for PCR technique, extraction was done using guanidine technique.

Results: The 150 patients 97 (64.67%) were males and 53(35.33%) were females. Most of the study groups were transplanted for duration less than 25 years. Urine cytology showed 5

(3.33%) nuclear inclusions associated with BK polyoma virus infection. whereas PCR showed 11 (7.33%) positive for BK polyoma virus DNA.) **Conclusion:** PCR was more sensitive than urine cytology for the detection of BK Polyoma virus in renal transplant patients. However Urine cytology is a simple, rapid, and cost-effective method.

KEYWORD: BK Polyoma virus, renal transplantation, PCR, Cytology.

INTRODUCTION

The transplantation of kidney allograft has become a standard therapy for end stage renal disease. Transplant rejection occurs when a transplanted organ or tissue is not accepted by the body of the transplant recipient.^[1] In the developing countries there is lack of recent information about detection of Polyoma virus using urine cytology and PCR, especially in Sudan. The first kidney transplantation in Sudan was in 1973.^[2] Polyoma virus-associated nephropathy is a major cause of graft dysfunction after kidney transplantation.^[3] With prevalence rates of 1-10 %.^[4]

Polyoma virus infection in kidney transplant recipients is of increasing interest and research. Although the two human polyoma viruses, BK virus (BKV) and JC virus (JCV), were reported in 1971.^[5] In most immunocompetent individuals, the infection with BK Polyoma virus is completely asymptomatic.^[6] Despite frequent episodes of viral reactivation with shedding into the urine. Reactivation can lead to severe disease.^[7] Diagnosis of Polyoma virus is mainly achieved by quantitative PCR of urine and plasma, but also by cytology.^[8] The current study investigates screening for BK polyoma virus (BKV) infection with urine cytology and PCR among patients attending Sudanese renal transplant society clinic. Studies comparing the performance of both methods are scarce internationally. According to literature, this study is the first addressing this topic in Sudan. The aim of this study is to compare between urine cytology and PCR-based assays for detection of BK Polyoma virus among Sudanese patients attending Sudanese Renal Transplantation Society Clinic.

MATERIALS AND METHODS

one hundred and fifty urine samples from both sexes, adult and asymptomatic renal post transplanted recipients. The urine samples were collected and centrifuged. Part of the deposits was smeared in clean labeled microscopic slides, fixed wet and stained with Papanicolaou stain and examined microscopically for presence of BK pyloma virus inclusion.

The remained deposit was prepared for PCR extraction using guanidine technique and the following oligonucleotide sequences, derived from the BKV (Dunlop strain; GenBank accession no. (NC001538) capsid protein-1 (VP-1) gene.^[9] Was used for detection of viral DNA.

Statistical analysis

The obtained results were written in tables, the statistical analysis was done using statistical package for social science (SPSS-version 17), using chi-square test for the categorized data.

RESULTS

Out of the 150 patients 97 (64.67%) were males and 53(35.33%) were females. the study group was transplanted for duration less than 25 years. Cytological results among the study group was as follow, five (3.33%) showed the nuclear inclusions associated with BK polyoma virus infection. According to the gender and detected infection, cytology detected three (2%) in males and two (1.33%) in females. In PCR result males were eight (5.33%) and females were three (2%) as shown in table (1).

Table (2) shows the relation between the BKV infection and duration of transplantation. All the positive cases in cytology the five (3.33%) were within the age group 1-10 years. on the other hand, the PCR result detect, eight (5.33%) positive, it was found with the same age group 1-10 years. Three (2%) positive cases were within in recipients who have been transplanted for duration between 2-10 months. Most of study groups were tacroleomous user 100(66.67%) among those, three (2%) were positive in cytology while seven (4.67%) were positive in PCR result. Cyclosporine users were 43(28.67%) recipients, two (1.33%) were positive in cytology and four (2.67%) were positive by PCR as shown in table (3).

Table (1): Relation between gender and BKV infections using cytological findings and PCR results.

Gender	Cytology (BKV)		PCR (BKV)		Total
	Negative	Positive	Negative	Positive	
Male	94 (62.67%)	3 (2%)	89 (59.33%)	8 (5.33%)	97 (64.67%)
Female	51 (34%)	2 (1.33%)	50 (33.33%)	3 (2%)	53 (35.33%)
Total	145 (97.67%)	5 (3.33%)	139 (92.67%)	11 (7.33%)	150 (100%)

Table (2): Relation between duration of transplantation and BKV infection using cytological findings and PCR results

Transplantation duration	Cytology (BKV)		PCR (BKV)		Total
	Negative	Positive	Negative	Positive	
less than 1 year	29 (19.33%)	0	26 (17.33%)	3 (2%)	29 (19.33%)
1 -10 Year	108 (72%)	5 (3.33%)	105 (70%)	8 (5.33%)	113 (75.33%)
11 -23 Years	8 (5.33%)	0	8 (5.33%)	0	8 (5.33%)
Total	145 (96.67%)	5 (3.33%)	139 (92.67%)	11 (7.33%)	150 (100%)

Table (3): Relation between the BK polyoma virus infection and immunosuppressive drugs.

Immunosuppressant drug	Cytology (BKV)		PCR (BKV)		Total
	Negative	Positive	Negative	Positive	
Tacrolimus	97 (64.67%)	3 (2%)	93 (62%)	7 (4.67%)	100 (66.67%)
Cyclosporine	41 (37.33%)	2 (1.33%)	39 (26%)	4 (2.67%)	43 (28.67%)
Prednisolone	1 (0.67%)	0	1 (0.67%)	0	1 (0.67%)
Tac + Cycl	1 (0.67%)	0	1 (0.67%)	0	1 (0.67%)
Cellcept	1 (0.67%)	0	1 (0.67%)	0	1 (0.67%)
Myfortic	1 (0.67%)	0	1 (0.67%)	0	1 (0.67%)
Imuran	3 (2%)	0	3 (2%)	0	3 (2%)
Total	145 (96.67%)	5 (3.33%)	139 (92.67%)	11 (7.33%)	150 (100%)

DISCUSSION

The results of this study found no association between BK polyoma virus infection and sex, duration of transplantation and types of immunosuppressive drugs, this finding is agreement with previous literature. with regard to cytology finding in this study five (3.33%) decoy cells were found. this result agreed with Sudanese study done by El Howeris, five (2.7%) BK polyoma virus infection was examined.^[10] And other study Koukoulaki et al positive decoy cells were identified in 14 (7.3%) urine samples.^[11]

In higher percentage Selvaggi reported positive Pyloma (BK) viral inclusions in 19.7% of urine samples from renal transplant patients. However the high percentage in the later study may explained by the large sample size $n= 7116$.^[12]

According to the result 7.33% were found to have BK polyoma virus in their urine specimen using PCR method. While Merlino in Italy in 2003 and his group reported 53% of the patients with BK polyoma virus in urine specimen.^[13] Further more Bergallo in Italy in 2002 and his colleagues, found 43.1% urine samples positive for BK virus.^[14] There is huge difference between the finding of this study and the other international previous two studies, this difference may be due to difference in the study group as most of study group population was suffering from nephropathy, and on the other hand our study group was asymptomatic recipients.

CONCLUSION

Urine cytology is a simple, rapid, and cost-effective method, but it's not sensitive in the early detection of the BKV infection. The PCR technique is more reliable and highly sensitive in the detection of the BKV infection. Farther studies are needed to study the same object with large sample size. Urine cytology may be used as regular simple screening test among renal transplant recipients. PCR preferably to be use for detection of BKV infection.

Conflicts of interest

Authors have no conflicts of interest.

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