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BKV infection in cancer patients presenting with cystitis

Vian Ibraheem Husain[1], Salah Mahdi Hassen[2], Amal Aziz Kareem[3]
[1]*Health and Medical Technical College\ Kirkuk. Northern Technical University*
[2]*Assistance Professor, College of Health and Medical Technology\Baghdad. Middle Technical University*
[3] *Assistance Professor, College of Health and Medical Technology\Baghdad. Middle Technical University*

Abstract
Objectives: In this study we aimed to determine the incidence of BKV infection in patients undergoing chemotherapy for different types of cancer who present with cystitis. This study provides molecular evidence of a possible association between BKV and cystitis in cancer patient treated with chemotherapy.

Materials and Methods: The study was performed on urine samples from 85 individuals (45 cancer patients during an episode of cystitis and compared with that detected in the urine of 20 cancer patients with no cystitis and 20 healthy individuals) in order to better evaluate the association of BK virus reactivation with cystitis. Detection of BKV in urine by real time PCR assay was done for all of the three groups.

Results: Results on the detection of viral DNA from the urine samples demonstrated that 4 (8.89%) of cancer patients with cystitis, in 1 (5.00%) of cancer patients with no cystitis, while 3 (15.00%) of healthy controls, were positive.

Recommendations:Our findings merit more studies to be done in a larger number of patients including multiple centers to help in establishing a causal association.

Keywords: Cancer BKV, Cystitis, Patients

1. Introduction

Polyomaviruses are a group of evolving pathogens that commonly cause urinary tract infection in human [1]. BK polyomavirus (BKV) is one of presently 13 human polyomavirus (HPV) species identified in humans [2]. The virus's discovery returns to 1971 when Gardner isolated the virus from the urine and the ureteral epithelial cells of a Sudanese renal transplant patient with renal failure and ureteral stenosis. The virus was named after the patients' initials [3]. The new update of the virus taxonomy classified the virus in the Betapolyomavirus genus of the family Polyomaviridae [4]. The Polyomaviridae family includes small, non-enveloped DNA tumor viruses with icosahedral capsids and DNA genomes which is small, circular and double-stranded [4,5]. BKV is holoendemic and has a worldwide distribution in the human population [6]. Primary infection with the virus is typically asymptomatic or linked to insignificant respiratory disease [7,8]. Following primary infection, BKV enters a latent phase with minimal episomal replication in the in renal tubular epithelial cells and the cells of the urogenital tract [9]. However, the virus

can reactivate under conditions of immunosuppression or immunodeficiency, in the kidney or urinary tract and produce lytic infection with tissue destruction [10]. The infection of BKV in immunocompetent individuals, even with the periodic reactivation and shedding of virus in urine, is completely asymptomatic [11,12]. Immunosuppression represents the major risk factor for infection with BKV [13]. therefore, individuals at risk for the development of BKV-associated diseases include those who have altered cellular immunity, such as pregnant women, patients with HIV infection, cancer patients undergoing chemotherapy, renal or other allografts recipients and patients with autoimmune diseases [12,14, 15,16]. In these groups of patients, viral reactivation followed by high-level of replication can result in nephropathy, ureteral stenosis, and cystitis [17]. Cystitis due to BKPyV may be hemorrhagic or non-hemorrhagic [18].

2. Methodology

Study Participants: This study was carried out in Kirkuk Specialist Center for Oncology and Hematology, during the period February 2020 to September 2020 and included 85 individuals. Three groups included in order to better assess the association of BKV reactivation with cystitis: 45 cancer patients who were undergoing chemotherapy for different types of cancer who present with cystitis, 20 cancer patients with no cystitis and 20 healthy individuals with no symptoms of urinary disease.

Specimen Collection: Urine samples (10 ~ 20 ml) have been collected from each individual. Urinalysis using microscopic examination was performed on all urine specimens. Urine specimens were preserved at - 20 °C until processed

Methods:

DNA extraction:

DNA was extracted from the urine manually, using DNA-sorb-AM nucleic acid extraction kit (AmpliSens®, Moscow, Russia. REF K1-11-100-CE). According to manufacturer's instructions and stored at − 20 °C until further analysis.

RT-PCR:

Primers and probes for BKV and internal control were designed using the Beacon designer software (version 8.20; Premier Biosoft, Palo Alto, CA, USA), which were then synthesized by Alpha DNA Company (Montreal, QC, Canada). The specificity of each primer set was assessed by the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) database. 5 µl of extracted DNA from urine sample was used in 25 µL reaction mixture including 10µl of Master Mix, 5 µl of specific primer mix (Primer F: 5'-GTGCAAGTGCCAAAACTAC-3' and primer R 5'-TCTGGGTTTAGGAAGCATTC-3') with hydrolysis probes (ROX-ACCTCTGTAATAGCATCTACCCCAGTT-BHQ) and internal control and 5 µl of nuclease free water. Each PCR assay included a positive control with BKV-DNA and a negative control containing distilled water. PCR for hemoglobin subunit beta (HBB) gene detection was performed to confirm the integrity of DNA extraction.

Realtime PCR was performed on an LM 2012 Real-time PCR Analyzer V8.0.1.8 (SHANGHAI FOSUN LONG MARCH MEDICAL SCIENCE CO., LTD., China). Thermal cycling was initiated with a denaturation step of 95 °C for 15 min. It was followed by 45 cycles of 95 °C for 5 s and 60 °C for 20 s, 72 °C for 15 s.

3. Result:

Results on the detection of viral DNA from the urine samples demonstrated that From a total of 85 (100%) urine samples analyzed, 8 (9.41%) were positive for BKPyV DNA, including all subjects enrolled in the study (Table 1 demonstrate the results). DNA was found in 4 (8.89%) of cancer patients with cystitis, in 1 (5.00%) of cancer patients with no cystitis, while 3 (15.00%) of healthy controls, were positive.

Comparing the occurrence of BK Polyomavirus DNA in the three groups in present study showed no significant difference at P <0.05, the results showed in **Table (1).**

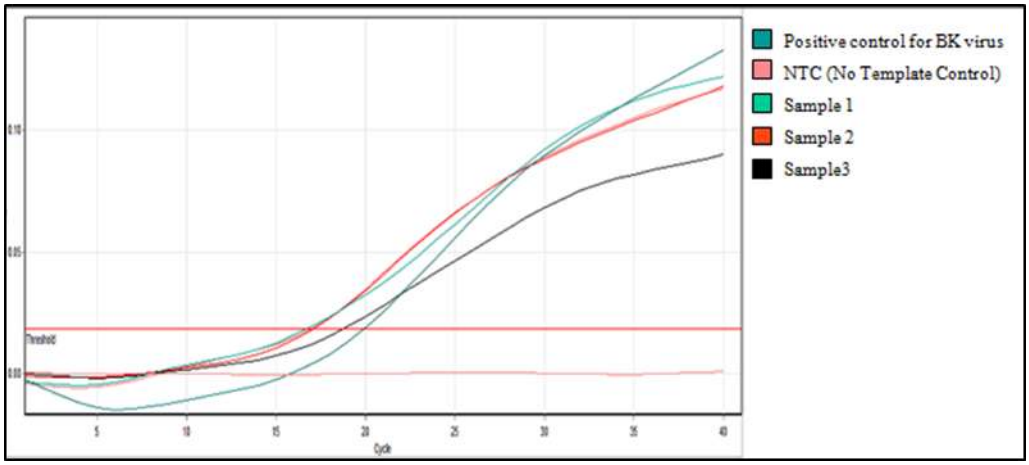

Figure 1: Real Time Polymerase Chain Reaction results using the orange filter- ROX (carboxyrhodamine) for BK virus.

Table 1 Prevalence of BK Polyomavirus Genomes in the Urine by Realtime PCR

BKV realtime PCR -Results	Study Groups			Total
	Cancer patients with cystitis	Cancer patients with no cystitis	Healthy controls	
Positive	4 8.89%	1 5 %	3 15 %	8 9.41 %
Negative	41 91.11%	19 95 %	17 85 %	77 90.59%
Total	45	20	20	85

	100%	**100%**	**100%**	**100%**
Pvalue: 0.5 >0.05 (NS)*				

*** (NS): Non Sig. at P>0.05**

4. Discussion

Lower urinary tract infections (UTIs) are a common worldwide problem and are usually caused by bacteria. Viruses are recognized to be an unusual cause of UTIs in immunocompetent people; yet, viruses are frequently known to cause lower UTIs among patients who are immunocompromised. The predominant pathogens involved include: BK virus, adenovirus, and cytomegalovirus [19].

The majority of studies of BKV infection as a cause of cystitis are in bone marrow transplant recipients. Only a few cases of BK virus associated cystitis in non transplant patients has been reported worldwide. The first of studies was done by Azzi *et al*, 1996 who reported 3 cases of hemorrhagic cystitis and BK viuria out of 55 adults with ALL. All 3 cases were positive for BKV DNA using dot blot hybridization technique[20].. In another study made by Cheerva *et al*, 2007, 3 cases of BK viruria with HC in non-transplant pediatric oncology patients has been reported[21]. Another case of severe hematuria due to BKV reactivation was reported by (Le Calloch *et al*, 2011) in a 15 years old female who was treated with chemotherapy for Hodgkin's Lymphoma. The diagnosis was made by the detection of BKV DNA in the urine[22]. Another case of Haemorrhagic cystitis due to BKV has been reported by Alavi *et al*, 2013 in a five year old boy with ALL on chemotherapy with no stem cell transplant. The urine was checked for the presence of BKV by real-time PCR, which was positive[23]. Moreover, Perram and Estell, 2014 reported another case of symptomatic urological BKV infection in a 38 year old male patient treated for Hodgkin lymphoma[24]. The low prevalence of **BKV** in samples from the cystitis group was possibly due to the small number of samples size, and conclusions about these results are premature. The low rates of BKV infection stated here may signify an epidemiological feature of the BKV in Iraq, which may be linked to the population density and environmental situations, which are of vital importance for the transmission of BKV.

The current study shows high rate of BKV shedding by control group. Asymptomatic urinary shedding of BKV has been reported by many authors [25, 26, 27, 28]. The shedding of BKV in the urine isn't, in of itself, indicative of an active disease. BKV is recognized to be shed by the urine of immunocompetent hosts without causing any hematuria or bladder disease. There is a hypothesis that immunosuppression cause the virus to reactivate. When the replication exceeds a certain level, cytopathic effects of the virus cause the disruption in bladder mucosa and result in hematuria. Another explanation suggests that increased shedding of BKV is secondary to HC or infection with other viruses like adenovirus,. It is possible that disruption in mucosal immunity caused by either adenovirus or HC itself is responsible for BK reactivation [29].

Our findings necessitate further studies in a larger number of patients including multiple centers to help to establish a causal association**.**

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