New Microbiologica, 46, 2, 141-145, 2023, ISSN 1121-7138

FULL PAPER

BK Virus Infections and Hemorrhagic Cystitis in Allogeneic Hematopoietic Stem Cell Transplant Recipients

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SUMMARY

BK virus (BKV) associated with hemorrhagic cystitis (HC) is the most important complication that develops after hematopoietic stem cell transplantation (HSCT) in patients with hematological malignancies. This study aims to investigate BKV infections and HC in pediatric patients after allogeneic hematopoietic stem cell transplantation. Between November 2018 and November 2019, a total of 51 patients between the ages of 11 months and 17 years were included in the study. BKV Bosphore® v1 quantification kit (Geneworks Anatolia, Turkey) was used for the detection of BKV DNA in urine and blood samples. Among the total of 51 patients, the incidence of BKV infection was found to be 86.3%. Allogeneic HSCT was performed in 40 patients and autologous HSCT in 11 patients. BK viruria and/or viremia were detected in 85% (44) of patients who underwent allogeneic HSCT and in 90% in the autologous group. High-level BK viruria (>10^7 copies/mL) was found in 41% (9) of 22 patients who were BKV positive before transplantation, while in 27.5% (8) of 29 patients who were BKV negative before transplantation; thus, BKV positivity before transplantation was considered a risk factor for high-level BK viruria. Acute GVHD developed in 6 of 40 patients in the allogeneic group. HC was prevented in 12 (67%) of 18 patients who received preemptive treatment, while HC developed in 6 (33%). HC occurred at a median of 35 days (17-49 days) post-transplant. Despite preemptive treatment, 6 (15%) patients who developed HC associated with BKV were in the allogeneic group but not in the autologous group. Of these patients with HC, 5 received a myeloablative treatment regimen, and 1 patient was given a reduced-intensity treatment regimen. The viral load in urine was found to be 10^{7-9} copies/mL within 2 weeks before the development of HC and has been identified as a prognostic indicator. In conclusion, early diagnosis of viral infections by monitoring BKV viral load in HSCT patients will be effective in preventing the progression of complications such as BKV-associated HC by providing timely initiation of preemptive treatment.

Received September 11, 2022 *Accepted* February 18, 2023

INTRODUCTION

BKV belonging to the Polyomaviridae family is a non-enveloped DNA virus that is quite common in healthy adults with seropositivity of up to 90% (De Padua Silva *et al.,* 2010). BKV was first isolated in 1971 from an immunosuppressive kidney transplant recipient with ureteral stenosis and was named BKV, using the initials of the patient's name and surname (Cesaro *et al.,* 2018; Ambalathingal *et al.,* 2017). Transmission occurs through the respiratory tract, especially in early childhood. The infection is usually asymptomatic or associated with mild upper respiratory tract symptoms with fever. After primary infection, BKV remains latent in the kidneys, urothelium, and other organs and is reactivated during immunosuppression (De Padua Silva *et al.,* 2010). Clinically significant reactivation of latent BKV occurs in certain immunocompromised conditions, such as HIV infection or transplantation (Ambalathingal *et al.,* 2017). However, urinary excretion of BKV is detected in 7-14% of immunocompetent individuals (Han *et al.,* 2014). Immunodeficiency associated with allogeneic HSCT results in high susceptibility to infection. BKV reactivation after allogeneic HSCT is associated with diseases ranging from asymptomatic viruria to severe HC. HC is often a serious complication of renal and hematopoietic stem cell transplantation and has the potential to cause serious morbidity. HC is char-

Key words:
BKV, PCR, HSCT, hemorrhagic cystitis.

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acterized by hematuria, dysuria, bladder spasm, and increased urinary frequency and may cause renal failure (Rorije *et al.,* 2014). Early-onset or pre-transplant HS occurs over time from 48 hours to two weeks and is caused by chemotherapeutic agents such as cyclophosphamide, ifosfamide, busulfan, and etoposide (Han *et al.,* 2014). Late-onset HC occurs after neutrophil engraftment as a result of viral infections or acute graft versus host disease (GVHD), occurring 2-4 weeks after transplantation. Late-onset HC is commonly caused by BKV reactivation, but also by ADV infection and rarely by HSV, CMV, or can also develop due to JC virus infections (Cesaro *et al.,* 2019; Dalianis *et al.,* 2019). HC is seen in 9-31% of HSCT recipients and may lead to longer hospitalization and mortality (Han *et al.,* 2014). Determination of BKV load in urine and/or blood by quantitative PCR has been shown to increase the specificity and positive predictive value of BKV detection in HSCT patients with HS. A BKV viral load $>10^{6-7}$ copies/mL in urine and $>10^{3-4}$ copies/mL in plasma is an important finding for the development of HC (Cesaro *et al.,* 2008). While BKV infection is a common cause of morbidity and mortality in patients after allogeneic HSCT, it is rarely seen in patients with autologous HSCT (Berber *et al.,* 2014). In this study, BKV infections and HC in pediatric patients who underwent allogeneic HSCT was investigated.

MATERIALS AND METHODS

A total of 51 pediatric patients, age 11 months to 17 years (mean age 7.8 years) who underwent HSCT between November 2018 and November 2019 in the Department of Hematology and Oncology, Faculty of Medicine, Cukurova University, were prospectively investigated. The study protocol was approved by Cukurova University Institutional Ethics Committee (01.02.2019 16/85).

Demographic information and clinical characteristics of the patients were noted, such as gender, age, primary disease, donor type, donor gender, transplantation type, stem cell source, immunosuppressive therapy received, and complications (*Table 1*). A 10-day conditioning regimen was applied to transplant patients before allogeneic (40) and autologous (11) transplantation. In our study, 41 (80%) of 51 patients received myeloablative therapy and 10 (20%) received low-intensity therapy (*Table 1*). ATG, MTX, and prebrol were given to all allogeneic patients as GVHD prophylaxis. Acyclovir (10-15 mg/kg) treatment given for preventive purposes was administered to all trans-

Table 1 - *Demographic and Clinical Characteristics of Patients With HSCT.*

Patient gender	*n (%)*	*Donor gender*	*n (%)*
Male	31 (61%)	Female	18 (45%)
Female	20 (39%)	Male	22 (55%)
Age groups		*Transplant type*	
0-2 age	7 (14%)	Bone marrow	24 (47%)
3-5 age	8 (16%)	Peripheral blood stem cells	25 (49%)
6-10 age	22 (43%)	Cord blood and bone marrow	2 (4%)
11-15 age	12 (23%)		
16-17 age	2 (4%)		
Donor type		*Stem Cell Source*	
HLA- matched unrelated	19 (47%)	Otolog	11 (22%)
HLA matched related	21 (53%)	Allogenic	40 (78%)
GVHD		*Preparation mode*	
Non	28 (70%)	Myeloablative regimen	41(80%)
Acut GVHD	6 (15%)	Low-intensity regimen	10 (20%)
Chronic GVHD	6 (15%)		
Diagnosis		*Diagnosis*	
Thalassemia majör	18 (34%)	Immunodeficiency	1 (2%)
Neuroblastoma	7 (14%)	Hemophagocytic	1 (2%)
Fanconi Aplastic anemia (FAA)	4 (8%)	Myelodysplastic syndrome (MDS)	1 (2%)
Acute lymphoblastic leukemia (ALL)	4 (8%)	MHC class2	1 (2%)
Acute myeloid leukemia (AML)	4 (8%)	MPS1	1 (2%)
Hodgkin lymphoma (HL)	4 (8%)	Lack of Dock8	1 (2%)
Sickle cell anemia	2 (4%)	Genetic cause unknown	1 (2%)
Osteopetrosis	1 (2%)		
Complications		*Complications*	
Fire	31 (61%)	Hematuria	7 (14%)
Gastroenteritis	23 (45%)	Bleeding diathesis	4 (8%)
Skin rash	15 (30%)	Multiorgan failure	3 (6%)
Oral mucositis	12 (24%)	Pneumonia	1 (2%)
Liver dysfunction	12 (24%)		

plant patients for 90 days. HSCT patients were followed up once a week before transplantation, once a week for the first three months after transplantation, and once a month for up to one year for BKV viruria and/or viremia.

Urine in a sterile container and blood samples in a tube with EDTA were taken from the patients. Urine and blood samples were delivered to the laboratory in a short time. Urine samples were placed into a centrifuge device (Hettich EBA 8S) at 2000 rpm for 5 minutes and the supernatant portions were placed into sterile tubes. The supernatant part of the blood samples was taken and centrifuged again at 2000 rpm for 5 minutes and the plasma part was separated. Samples were stored at -20ºC until analysis. The kit (Bosphore BKV quantification kitV1, Anatolia Geneworks, Turkey) was used for extraction of viral DNA from plasma and urine samples. DNA extraction was performed according to the manufacturer's recommendations. Automated nucleic acid isolation device Magnesia 16 (Anatolia Geneworks, Turkey) was used for viral DNA extraction from plasma and urine samples. BKV DNA amplification was performed with the RT-PCR kit Bosphore® BKV quantification kit v1 (Anatolia Geneworks, Turkey). The Montania 4896 RT-PCR device was used for this process. Preemptive treatment was applied to patients with BKV DNA viral load above $>10^7$ copies/mL and $>10^4$ copies/mL, respectively, in urine and plasma samples of the patients. First of all, for preemptive treatment the dose of immunosuppressive treatment was reduced. Treatments such as cidofovir, oral levofloxacin, intravenous immunoglobulin use, and bladder irrigation were applied to patients who developed HC, depending on the clinical situation.

RESULTS

In our study, 40 (78%) of 51 patients underwent allogeneic and 11 (22%) autologous HSCT. BKV DNA positivity in the urine and/or blood of 44 (86.3%) of 51 transplanted patients, including 34 (85%) of 40 patients who underwent the allogeneic transplant and 10 (90%) of 11 patients who underwent autologous transplant (28 boys and 16 girls) were identified. Of the 44 patients with the BK virus, 28 (64%) were male and 16 (36%) were female, and it was observed that 50% of these patients were in the 6-10 age group. Of the 18 (35%) patients with a BKV DNA urinary viral load $>10^7$ copies/mL required for preemptive therapy, 17 had a high viral load post-transplant and 1 had a pre-transplantation viral load. Of these 18 patients, 14 (78%) were in the allogeneic HSCT group (1 positive before transplantation and 13 after transplantation), and 4 (22%) in the autologous group. Of the 26 patients with viral DNA load below 10^7 copies/mL and positive for BKV DNA, 20 (77%) were allogeneic and 6 (23%) were autologous, and it was determined that the virus was cleared in these patients or did not rise above 10^7 copies/mL. The incidence of BKV infection was 86.3% (44/51) in a total of 51 patients. The median time of onset of virus in 44 patients with BK virus was found to be 3.6 weeks (1-20 weeks). In 17 patients with post-transplant BKV DNA viral load $>10^7$ copies/mL, the median onset time of high BK virus was 2.8 weeks (1-5 weeks). In our study, the presence of BKV DNA in the urine and/or blood of 51 patients before transplantation was investigated. BKV positivity was detected in 22 (43%) of the patients before transplantation, and BKV viruria was found in 18 (35%) and BKV viremia in 4 (8%) patients. Of these patients, 18 had BKV viruria $<10^7$ copies/mL, 3 had BKV viremia $>10^4$ copies/mL, and 1 had both high viruria ($>10^7$) and viremia ($>10^4$). While 9 (41%) of 22 patients with BK virus positive before transplantation developed high BK virus ($>10^7$copies/mL) after transplantation, 8 (27.5%) of 29 patients with negative BK virus before transplantation developed high BK virus. HC developed in 2 patients in the allogeneic group with pre-transplant BK viremia. At the time of engraftment, 47 patients had a mean of 13.2 days (6-19 days), while 4 patients did not develop engraftment. When the post-transplant BK viral load was high ($>10^7$ copies/mL) and those with low ($<10^7$ copies/mL) post-transplant BK virus positive (20 patients) were compared, it was observed that the engraftment times were similar at 13.3 days and 12.3 days, respectively. Acute GVHD developed in 6 of 40 patients in the allogeneic group. A high viral load ($>10^7$ copies/mL) was found in 3 (50%) of 6 patients who developed acute GVHD, while a low viral load ($<10^7$ copies/mL) was detected in 11 (32.3%) of 34 patients who did not develop acute GVHD. The development of HC was prevented in 12 (67%) of 18 patients who received preemptive treatment. BK-related HC was seen in only 6 (33%) patients. HC occurred at a median of 35 days (17-49 days) post-transplant. Despite preemptive treatment, 6 (15%) patients who developed HC associated with BKV were in the allogeneic group but not in the autologous group. Of these patients with HC, 5 received a myeloablative treatment regimen, and 1 patient was given a reduced-intensity treatment regimen.

DISCUSSION

BKV is found in approximately 50% of bone marrow transplant recipients, and high levels of the virus are associated with a higher risk of developing HC. While BKV is excreted in urine asymptomatically in healthy individuals, and in bone marrow with immunosuppression in transplant recipients, detection of viral load in the urine $>10^{6-7}$ copies/mL and BK viremia $>10^4$ copies/mL is associated with a high risk of HC. BKV-related HC is seen in 18% (8-25%) of children who underwent allogeneic HSCT and typically

occurs 2-8 weeks after transplantation (Cesaro *et al.,* 2018). BKV DNA positivity was detected in urine and/or blood in 44 (86.3%) of 51 patients who underwent HSCT; 34 (85%) of them were allogeneic and 10 (90%) were in the autologous group. In 18 (35%) patients, urinary BKV DNA viral load was $>10^7$ copies/mL and preemptive treatment was applied. BKV DNA urinary viral load $>10^7$ required for preemptive therapy 14 of the 18 patients with copies/mL above were included in the 14 (35%) allogeneic and 4 (36.4%) in the autologous HSCT group. Pre-transplant BK viremia was found to be $>10^4$ in 4 (8%) of 51 patients who underwent HSCT, and 3 were in the allogeneic and 1 in the autologous group. The rates of urinary BKV DNA viral load $>10^7$ copies/mL after transplantation in patients with urine BK virus positivity before transplantation (41%) and in patients who were negative (27.5%), respectively 41% and 27.5%, and BKV DNA positivity before transplantation were accepted as a risk factor for the high virus. While the viral load of 50% in urine was $>10^7$ copies/mL in 3 of 6 patients who developed acute GVHD, 11 (32.3%) of 34 patients who did not develop acute GVHD had BKV positivity with a lower rate in allogeneic HSCT patients ($>10^7$ copies/mL) and the presence of acute GVHD was determined as a risk factor for an increase in BKV DNA viral load to a level that requires preemptive treatment. While the development of HC was prevented in 12 (67%) of 18 patients who received preemptive treatment, HC developed in 6 (33%). Thus, HC developed in 6 (15%) of 40 patients who underwent allogeneic HSCT. HC occurred a median of 35 days (17-49 days) after transplantation. In our study, the rate of BKV DNA in 51 patients who underwent HSCT was 86.3%, and Robin et al. had a rate of 79%. The incidence of BKV infection in allogeneic HSCT patients was 85%. Oshrine *et al.* found 69.1% and Laskin et al. found 47% (Robin *et al.,* 2013; Oshrine *et al.,* 2013; Laskin *et al.,* 2013). As a risk indicator for HC, the BK virus level was $10^{7\text{-}9}$ copies/mL within 2 weeks before the development of HC, and similar to the findings of Hayden et al., they found the BK virus level to be $>10^9$ copies/mL 13 days before HC. In other studies, Kwon et al. $>10^{10}$ copies/mL and Umeda *et al.* detected BK virus at a level of $>10^8$ copies/mL (Hayden *et al.,* 2015; Kwon *et al.,* 2013; Katsutsugu *et al.,* 2018). In our study group, BKV viremia was found to be $>10^4$ copies/mL 2 weeks before the development of cystitis in only 1 of 6 HC cases, which is similar to Oshrine *et al.*, finding of $>10^4$ copies/mL. On the other hand, Gilis *et al.* reported $>10^{2\text{-}5}$ copies/mL (Oshrine *et al.,* 2013; Gilis *et al.,* 2014). In our study, the urinary viral load value was $>10^7$ copies/mL in 50% of the patients who developed acute GVHD, and 32.3% in those who did not. Kwon *et al.* 58%, Hayden *et al.* 50%, and Gilis *et al.* reported acute GVHD with a rate of 14% as a risk factor for BKV infection (Hayden *et al.,* 2015; Kwon *et al.,* 2013; Gilis *et al.,* 2014). In our study, the rate of HC in allogeneic HSCT patients was 15%, while Park *et al.* 26.1%, Gilis *et al.* 13.3%, and Kesherwani et al. 8.3% (Gilis *et al.,* 2014; Park *et al.,* 2016; Kesherwania *et al.,* 2019). In this study, HC occurred a median of 35 days after HSCT. During this period Robin *et al.* reported that HC developed at 27 days, and Megged *et al.* at 57 days (Robin *et al.*, 2013; Megged *et al.,* 2011). In conclusion, a urinary viral load of $10^{7\text{-}9}$ copies/mL was found in the 2 weeks before the development of HC in 6 cases with BKV-associated HC, which was determined as a prognostic indicator for the development of HC. Early diagnosis of viral infections by monitoring BKV viral load in HSCT patients will be effective in preventing the progression of complications such as BKV-associated HC by providing timely initiation of preemptive treatment.

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