

Prevalence of Viral Infections and Hemorrhagic Cystitis in Hematopoietic Stem Cell Transplant Recipients

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Abstract

Objectives: About 7% to 70% of hemorrhagic cystitis is classified as early and late-onset incidence in hematopoietic stem cell transplant patients. The association between the prevalence of viral infections and hemorrhagic cystitis was evaluated in pretransplant and posttransplant recipients and donors.

Materials and Methods: Ethylenediaminetetra-acetic acid-treated blood and urine samples of 30 recipients and 24 donors were collected before hematopoietic stem cell transplant patients, and monitored for 100 days after transplant. Prevalence of BK virus DNA was investigated by polymerase chain reaction. Prevalence of adenovirus and cytomegalovirus DNA was evaluated by polymerase chain reaction. Type of transplant, conditioning regimens, graft-versus-host disease clinical grading, demographic data, hematologic, and biochemical indexes also were analyzed.

Results: Different grades of hematuria were found in 16 of 30 of the recipients with hemorrhagic cystitis. Severe hematuria and diffuse thickening of the bladder were found in 5 of 30 transplant patients. Multiple infections of BK virus, adenovirus, and cytomegalovirus were seen in 5 patients with severe hemorrhagic cystitis. The viruria of these viruses was decreased in patients with hemorrhagic cystitis as follows: BK virus (5 of 5), adenovirus (2 of 5), and cytomegalovirus (not detected). Also, a significant relation was found between hemorrhagic cystitis and

risk factors including donor-recipient sex mismatches, familial relationships, leukemia as an underlying disease, older age, allogenic type of transplant, prophylactic and therapeutic dose of anti-graft-versus-host-disease regimens.

Conclusions: Detection of single and multiple infections of BK virus, adenovirus, and cytomegalovirus in blood and/or urine samples of hematopoietic stem cell transplant recipients, in combination with 1 or more inducing factors of hemorrhagic cystitis were enforced on the important role these risk factors play in the cause of hemorrhagic cystitis.

Key words: Adenovirus, BK virus, Cytomegalovirus, Bone marrow, Recipients.

Introduction

Hemorrhagic cystitis (HC) is a common and important cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) recipients.¹ Its incidence varies from 7% to 70% in different reports and leads to severe hematuria (grades 3 or 4) in 8% to 27% of HSCT patients.²⁻⁴ Based on the time of appearance, HC can be classified into early or late-onset presentation.² Early-onset of HC has been linked to toxic effects of chemo-irradiative agents, especially drugs used in the HSCT-conditioning regimen such as cyclophosphamide and busulfan.² It also may be encountered by using sodium 2-mercaptoethane sulfonate, which is now included in conditioning regimens with cyclophosphamide. However, despite the use of sodium 2-mercaptoethane sulfonate, HC remains an important clinical problem in HSCT recipients.^{3, 4}

Reported data enforced on several risk factors of HC including type of conditioning regimen, older

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age at transplant, allogeneic source of HSCT cells, graft-versus-host disease, and especially, single or mixed viral infections have been associated with development of HC.⁵⁻⁷ Most of these factors, though, have not been observed consistently and concomitantly in different studies. Also, unknown mechanisms might influence the cause of HC, and identifying an accurate role of HC causative agents' remains unclear.^{8,9}

Based on earlier reports, viral infections have an inducible role in the presentation of HC. From different viral infections, some investigators have found the BK virus in urine samples of HSCT patients with HC.^{5,8,9} Also, the BK virus infection has been found in the blood samples of 75% of patients who developed HC.⁵ A direct relation also has been found between adenovirus excretion, and duration of intensity of hematuria in transplant patients who have developed HC.⁹⁻¹¹ Conversely, widespread morbidity and mortality of *cytomegalovirus* infection in HSCT patients has encouraged us to study the potential responsibility of *cytomegalovirus* infection in developing HC.^{5,12}

For this purpose, the importance of viral infections including polyomavirus (BK virus), adenovirus, and *cytomegalovirus* infections in developing HC as well as determining the incidence, clinical outcomes, and laboratory findings of different risk factors of HC was evaluated in HSCT patients.

Materials and Methods

Patients

A total of 30 HSCT patients including 24 patients (80%) received allogeneic HSCT from 24 HLA-identical sibling-related donors and 6 transplant recipients (20%) underwent autologous HSCT were evaluated in this study. These patients underwent HSCT in bone marrow transplant unit at Namazee Hospital affiliated with Shiraz University of Medical Sciences, Shiraz, Iran, between 2005 and 2007.

Risk factors of HC were collected and evaluated for all patients' pre-HSCT and post-HSCT. Risk factors studied were recipient age and sex, immunosuppressive therapy, underlying disease, kind of transplant, hematologic and biochemical indices, symptoms and grading of graft-versus-host disease, prophylactic and therapeutic doses of anti-graft-versus-host-disease regimen, donor-recipient sex and familial match, remission status, HC

prophylaxis and therapy, ultrasonographic findings, and prevalence of viral infections.

Samples

Two hundred ninety-three EDTA-treated blood and urine samples were collected from HSCT recipients and donors. One sample was collected before the transplant from donors and recipients and also, 1 sample per week for 100 days from HSCT recipients after the transplant. Midstream urine specimens also were collected for microscopic urinalysis and examination of microscopic and macroscopic hematuria. Patients with a grade of 0 of HC were excluded.

Hematopoietic stem cell transplant procedure

All HSCT patients were treated before and after the transplant with regimens including immunosuppressive conditioning regimen, anti-graft-versus-host-disease-prophylactic and therapeutic regimens and prophylactic and therapeutic anti-infective regimens, according to institutional policy defined by diagnosis, disease status, and donor type. On day 0, allogeneic HSCT patients received HLA-compatible marrow from related donors. All recipients received filtered blood products to deplete leukocytes, although all of them had been irradiated with cobalt 3000 rad gamma rays (3000 cGy) to prevent posttransfusion graft-versus-host disease.

Definition and diagnosis of hemorrhagic cystitis

Hemorrhagic cystitis was defined as the presence of *microscopic* or *macroscopic* hematuria, which corresponded to ≥ 100 red blood cells/high-power field in urine microscopy, with or without symptoms of dysuria, frequency of micturition, or suprapubic pain⁶; and in the absence of other clinical conditions such as menstruation, bleeding owing to other gynecologic problems, disseminated intravascular coagulation, multiple organ dysfunction syndrome, or sepsis.⁷ Severity of HC was graded according to the following: grade 0 (no hematuria), grade 1 (≥ 100 red cells per high-power microscopically field), grade 2 (macroscopic hematuria), grade 3 (macroscopic hematuria with clots), and grade 4 (macroscopic hematuria with clots and high creatinine level secondary to obstruction).⁶ Hemorrhagic cystitis was classified into *early onset* (that occurred within ≤ 1 week after HSCT) and *late onset* (occurring > 2 weeks after preparative regimen).^{8,9}

Standard urine analysis was done every day throughout the posttransplant hospitalization and afterward at controlled visits. In all patients with hematuria, genitourinary ultrasonography was performed. Ultrasonographic findings (eg, the thickening of the bladder mucosa and the presence of clots in the bladder) were used in the differential diagnosis of hematuria.

Viral DNA extraction

Based on the types of samples, DNA genomes of adenovirus, BK virus, and *cytomegalovirus* were extracted from collected specimens. Plasma-DNA extraction was done by optimized phenol chloroform method. Also, viral DNA was extracted from Buffy coat samples by optimized boiling and alkaline digestion protocol. DNA was extracted from urine sample by a method organized of polyethylene glycol, Nonidet P40, and boiling extraction.¹²

Polymerase chain reaction protocols

BK virus monoplex-polymerase chain reaction

Detection of BK virus DNA was performed by an in-house qualitative protocol using originally designed primers, forward: 5'-CAA GTG CCA AAA CTA CTA AT-3'; and reverse: 5'-TGC ATG AAG GTT AAG CAT-3', for amplifying a 326 bp fragment of BK virus Vp1 gene.

The BK virus PCR mixture in a total volume of 50 μ L contained 5 μ L of 10 $\times$ PCR buffer, 1.5 μ L MgCl₂ (50 mM), 1 μ L dNTP (10 mM), 1 μ L of each primer (20 pmol), 0.5 μ L Taq (2.5 units), and 5 μ L of the sample DNA. The PCR mixtures were preheated at 94°C for 2 minutes and then subjected to 35 cycles for 1 minute at 93°C, 1 minute at 57°C, and 1 minute at 72°C, and then finally—1 cycle for 5 minutes at 72°C.

Adenovirus and Cytomegalovirus multiplex-polymerase chain reaction

A multiplex-PCR method was optimized for fast, specific, and sensitive diagnosis of adenovirus and *cytomegalovirus* infections in clinical samples. The presence of these viral genomes was preformed simultaneously by 2 primer pairs as follows: An 81-bp fragment of gene 2 of adenovirus, which is highly conserved among different serotypes with newly designed primer sequences, forward: 5'-TTC CTC TAT CTC AGA CAC TGG CTC A-3'; and reverse: 5'-CCA AGC GGC CTC TGA TAA CCA-3'; and a 403-bp fragment of UL123 gene of *cytomegalovirus* by a

new pair of primer sequence: forward: 5'-ACC TAC GAC TAC ATG AAC GGG CGG G-3'; and reverse: 5'-CCA GAG ACC ACC TGG CAC CAA TG-3'.

The mixture of multiplex-PCR reaction in a total volume of 50 μ L containing 5 μ L of 10 $\times$ PCR buffer, 1 μ L MgCl₂ (50 mM), 1.5 μ L dNTP (10 mM), 1 μ L of each primer (20 pmol), 0.5 μ L Taq (2.5 units), and 5 μ L of the sample DNA. Use of 94°C for 3 minutes, and a program that included 35 cycles for 1 minute at 94°C, 1 minute at 62°C, 1 minute at 72°C, and 1 cycle for 5 minutes at 72°C is the thermocycling condition of this PCR method. Each run of Monoplex and multiplex-PCR methods were enrolled with negative and positive controls. The amplified DNA was documented by 1.5% agarose gel electrophoresis and ethidium bromide staining.

Statistical Analyses

A statistical method was used to determine the prevalence of viral infections in HSCT patients with and without HC. Chi-square and Fisher 2-tail exact test methods were used to evaluate correlations between different risk factors and presentation of HC. Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 12.0, SSPS Inc, Chicago, IL, USA). A level of $P \leq .05$ was accepted as statistically significant.

Results

From 30 studied patients, 19 were male (63.3%) and 11 were female (36.7%) (age range, 5 to 53 y; mean $\pm$ SD, 23 $\pm$ 13.73 y). Also, 12 donors (50%) were male and the others were female. The underlying diseases of transplant patients were categorized to thalassemia major (n=10), leukemia (n=10), aplastic anemia (n=3), lymphoma (n=3), multiple myeloma (n=3), and mixed germ cell tumor (n=1; Table 1). Myeloablative and nonmyeloablative conditioning regimen was used in 22 HSCT patients (90%) and 2 HSCT patients (10%). Also for prevention of HC 20 of 30 of HSCT patients (66.7%) received sodium 2-mercaptoethane sulfonate and hyperhydration.

Hemorrhagic cystitis incidence and grading in hematopoietic stem cell transplant patients

Clinical symptoms of HC were diagnosed in sixteen: 11 were male and 5 were female HSCT patients (53.3%). Also, 14 of 30 of these recipients (46.7%)

were asymptomatic for HC symptoms. Early and late-onset HC was detected in 5 of 30 of HSCT patients (16.7%) and 11 of 30 of HSCT patients (36.7%) (Table 1). Clinical signs of early onset of HC were diagnosed in 4 of 30 HSCT patients (13.3) to be continued in the following weeks (Table 1).

The distributing pattern of different grades of HC was as follows in transplant patients: Microscopic or mild hematuria (grade 1) was detected in 12 of 30 recipients with HC (40%) and macroscopic hematuria (grade 2) was diagnosed in 7 of 30 patients with HC (23.3%). Severe hematuria (grades 3 and 4) was seen in 5 of 30 patients with HC (16.7%) (Table 1).

Diffuse thickening of the bladder was confirmed by ultrasonographic protocol in 5 patients (4 male and 1 female) with severe HC (grades 3 and 4). Clinical symptoms of HC were not seen in any autologous HSCT patients.

Table 1. BK virus infection and HC presentation in HSCT patients with HC.

Viral infections patients	BK virus infection n (%) $\pm$ SD			HC Presentation n (%) $\pm$ SD
	Buffy Coat	Plasma	Urine	
1	17 (56.7) $\pm$ 0.5	9 (30) $\pm$ 0.47	20 (66.7) $\pm$ 0.48	7 (23.3) $\pm$ 0.61
2	12 (40) $\pm$ 0.5	11 (36.7) $\pm$ 0.49	7 (23.3) $\pm$ 0.43	7 (23.3) $\pm$ 0.61
3	25 (83.3) $\pm$ 0.37	17 (56.7) $\pm$ 0.5	27 (90) $\pm$ 0.3	8 (26.7) $\pm$ 0.55
4	17 (56.7) $\pm$ 0.48	11 (36.7) $\pm$ 0.5	21 (70) $\pm$ 0.4	7 (23.3) $\pm$ 0.56
5	(63.3) $\pm$ 0.41	13 (43.3) $\pm$ 0.5	21 (70) $\pm$ 0.33	8 (26.7) $\pm$ 0.73
6	14 (46.7) $\pm$ 0.45	5 (16.7) $\pm$ 0.46	16 (53.3) $\pm$ 0.37	10 (33.3) $\pm$ 0.68
7	8 (26.7) $\pm$ 0.51	8 (26.7) $\pm$ 0.52	14 (46.7) $\pm$ 0.39	11 (36.7) $\pm$ 0.82
8	8 (26.7) $\pm$ 0.51	7 (23.3) $\pm$ 0.51	13 (43.3) $\pm$ 0.4	11 (36.7) $\pm$ 0.88
9	10 (33.3) $\pm$ 0.47	5 (16.7) $\pm$ 0.5	11 (36.7) $\pm$ 0.42	9 (30) $\pm$ 1.1
10	9 (30) $\pm$ 0.32	5 (16.7) $\pm$ 0.53	8 (26.7) $\pm$ 0.42	5 (16.7) $\pm$ 0.99
11	8 (26.7) $\pm$ 0.51	5 (16.7) $\pm$ 0.42	7 (23.3) $\pm$ 0.42	5 (16.7) $\pm$ 1.00
12	4 (13.3) $\pm$ 0.44	2 (6.7) $\pm$ 0.42	3 (10) $\pm$ 0.39	3 (10) $\pm$ 1.00
13	(13.3) $\pm$ 0.44	2 (6.7) $\pm$ 0.4	1 (3.3) $\pm$ 0.2	3 (10) $\pm$ 1.00
14	4 (13.3) $\pm$ 0.44	2 (6.7) $\pm$ 0.4	4 (13.3) $\pm$ 0.42	3 (10) $\pm$ 1.00
15	1 (3.3) $\pm$ 0.2	1 (3.3) $\pm$ 0.23	1 (3.3) $\pm$ 0.18	1 (3.3) $\pm$ 1.00

Abbreviations: n, number of patients; ND, not detected; SD, standard deviation; %, percentage of patients

Demographic data and hemorrhagic cystitis

Based on microscopic hematuria and ultrasonographic findings in the bladder, the incidence and severity of some risk factors of HC were presented as follows: Donor-recipient sex mismatch was diagnosed in 9 of 16 of transplant patients with HC (56.5%) and especially detected in 4 of 5 of HSCT recipients with severe HC.

All studied patients received HSC from related donors. Familial relationships between donors and recipients increase the possibility of late onset of HC

and severity in ninth ($P = .014$), 10th ($P = .031$), 11th ($P = .049$), 12th ($P = .046$), 13th ($P = .046$), and 14th ($P = .046$) weeks after HSCT. Also 3 and 2 of five transplanted patients who had shown severe HC symptoms received hematopoietic stem cells from their sisters and brothers.

Leukemia 43.75% and thalassemia 37.5% are the most-prevalent underlying diseases in HSCT patients. Three of 5 patients with severe HC have leukemia, 1 has lymphoma, and the last of them has aplastic anemia. The underlying disease was detected in seventh ($P = .027$), eighth ($P = .05$), and ninth ($P = .019$) week of transplant in patients with HC. The role of old age was redocumented in this study. This criteria was significantly induced HC clinical incidence in sixth week after transplant ($P = .049$) and ninth week after transplant ($P = .038$). In the first ($P = .046$), second ($P = .046$), third ($P = .031$), and fourth ($P = .046$) week after HSCT, allogeneic transplant was significantly induced early and late-onset of HC. The inducing role of sex in HC was reconfirmed when 4 of 5 patients with severe HC were male.

The ultrasonographic findings of HC in the bladder were significantly related to older age ($P = .048$). But this correlation was not detected between other risk factors including donor-recipient relatedness, underlying disease, and type of transplant with HC ultrasonographic findings.

Hemorrhagic cystitis and viral infections

Hemorrhagic cystitis and BK virus infection

In this investigation, significant concordance was detected between BK virus infection and HC in almost all clinical samples of patients at different weeks of HSCT. BK virus was found at the time of HC diagnosis in 15 urine samples, 13 Buffy coat samples, and 13 plasma samples of studied in HSCT patients. Notably, all patients with BK viremia also had BK viruria (Table 2). In 3 HC patients without BK viremia, the BK virus genome was simultaneously not diagnosed in both Buffy coat and plasma samples. BK viremia and viruria were found simultaneously in 5 patients with severe HC. In only 1 patient with grade 1 of HC, both BK viremia and viruria were not documented (Table 2). The therapeutic dosage of prednisolone ($P = .034$), cyclosporine ($P = .034$), and mycophenolate mofetil ($P = .034$) anti-graft-versus-host-disease conditioning drugs are significant inducing factors for BK virus infection in patients with HC.

Hemorrhagic cystitis and adenovirus infection

In contrast to high prevalence of BK viruria, adenovirus viruria was found only in 4 of 30 transplant patients with HC (13.3%). Higher presentation of viremia than viruria of adenovirus was diagnosed in both 11 Buffy coat and plasma samples of HC disordered transplant recipients (Table 2). The genome of adenovirus was not diagnosed simultaneously in all clinical samples of 5 of 16 HC patients. Only 2 of 5 patients with severe HC were infected with adenovirus viruria (Table 2). But Buffy coat and plasma samples of all 5 transplant patients with severe HC had a history of adenovirus infection (Table 2). A prophylactic dosage of cyclosporine level was the only risk factor that significantly correlated with adenovirus infection in patients with HC.

Hemorrhagic cystitis and Cytomegalovirus infection

Cytomegalovirus viruria was not seen in any transplant patients with HC. Also *cytomegalovirus* viremia was found in 5 of 30 (16.7%) of both Buffy coat and plasma samples of HSCT patients (Table 2). *Cytomegalovirus* infection was not diagnosed in both 11 of 16 Buffy coat and plasma samples (68.75%) of transplant recipients with HC (Table 2). Despite other reports that present the less-common role of *cytomegalovirus* infection in severity of HC, the viremia of *cytomegalovirus* infection was detected in all 5 transplant patients with severe HC (Table 2).

Hemorrhagic cystitis and multiple viral infections

The viremia of triple BK virus, cytomegalovirus, and

adenovirus was confirmed in all 5 patients with severe HC. In contrast to viremia, the urinary exertion of these viruses has different patterns in transplant patients with severe HC. The BK viruria that was found in all patients with severe HC, adenovirus viruria was found in only 2 of 5 of these transplant recipients. Also, *cytomegalovirus* viruria was not detected in any of the patients with severe HC.

Hemorrhagic cystitis and graft-versus-host disease clinical grading, anti-graft-versus-host-disease prophylaxis, and therapeutic regimens

Grading of the graft-versus-host disease in transplant patients, with or without HC, is presented in Table 2. Graft-versus-host disease grades 1 to 4 were not significant on HC incidence and severity. Administration of a prophylactic dosage of prednisolone was significantly associated with HC in second ($P = .03$), fourth ($P = .05$), seventh ($P = .023$), and eighth ($P = .046$) weeks after transplant. Hemorrhagic cystitis symptoms were raised significantly after using of cyclosporine as an anti-graft-versus-host-disease prophylactic drug in fourth ($P = .042$), seventh ($P = .023$), and eighth weeks after HSCT ($P = .046$). Use of a prophylactic dosage of mycophenolate mofetil increased hematuria in the seventh week of transplant in recipients ($P = .026$). But administration of anti-graft-versus-host-disease therapeutic regimen—including prednisolone, cyclosporine, and mycophenolate mofetil—did not induce severity of hematuria in transplant recipients.

Table 2. Adenovirus and cytomegalovirus presentation in HSCT patients with HC.

Viral infections patients	Adenovirus infection			Cytomegalovirus infection		
	Buffy coat	n (%) $\pm$ SD Plasma	Urine	Urine	n (%) $\pm$ SD Urine	Urine
1	8 (26.7) $\pm$ 0.45	7 (23.3) $\pm$ 0.43	2 (6.7) $\pm$ 0.25	1 (3.3) $\pm$ 0.18	1 (3.3) $\pm$ 0.18	1 (3.3) $\pm$ 0.18
2	11 (36.7) $\pm$ 0.49	12 (40) $\pm$ 0.49	4 (13.3) $\pm$ 0.35	ND	ND	ND
3	7 (23.3) $\pm$ 0.43	6 (20) $\pm$ 0.41	3 (10) $\pm$ 0.3	1 (3.3) $\pm$ 0.18	1 (3.3) $\pm$ 0.18	1 (3.3) $\pm$ 0.18
4	7 (23.3) $\pm$ 0.45	8 (26.7) $\pm$ 0.47	2 (6.7) $\pm$ 0.27	ND	ND	ND
5	6 (20) $\pm$ 0.44	3 (10) $\pm$ 0.33	3 (10) $\pm$ 0.33	ND	ND	ND
6	7 (23.3) $\pm$ 0.5	6 (20) $\pm$ 0.48	2 (6.7) $\pm$ 0.32	ND	ND	ND
7	4 (13.3) $\pm$ 0.44	1 (3.3) $\pm$ 0.24	ND	ND	ND	ND
8	8 (26.7) $\pm$ 0.52	4 (13.3) $\pm$ 0.45	1 (3.3) $\pm$ 0.25	ND	ND	ND
9	3 (10) $\pm$ 0.45	2 (6.7) $\pm$ 0.37	1 (3.3) $\pm$ 0.48	ND	ND	ND
10	1 (3.3) $\pm$ 0.33	ND	ND	ND	ND	ND
11	2 (6.7) $\pm$ 0.42	ND	ND	ND	ND	ND
12	2 (6.7) $\pm$ 0.4	1 (3.3) $\pm$ 0.2	ND	ND	ND	ND
13	2 (6.7) $\pm$ 0.4	1 (3.3) $\pm$ 0.18	ND	ND	ND	ND
14	1 (3.3) $\pm$ 0.3	2 (6.7) $\pm$ 0.2	1 (3.3) $\pm$ 0.23	ND	ND	ND
15	1 (3.3) $\pm$ 0.23	1 (3.3) $\pm$ 0.25	1 (3.3) $\pm$ 0.22	1 (3.3) $\pm$ 0.3	1 (3.3) $\pm$ 0.3	1 (3.3) $\pm$ 0.3

Abbreviations: n, number of patients; ND, not detected; SD, standard deviation; %, percentage of patients

Discussion

The importance of different risk factors in pathogenesis of HC was evaluated and compared with related studies. Despite the high incidence of HC in HSCT recipients (16 of 30), severe HC was only diagnosed in 5 of 30 studied patients (16.7%), that is comparable to other reports.^{6, 13-15}

Detection of HC in patients only undergoing allogeneic HSCT also has been confirmed by other researchers and diagnosed at a higher incidence of HC in allogeneic recipients compared with autologous transplant patients.^{7, 13-15}

Controlling and treatment of HC symptoms in the early stage promotes prevention of development of HC of lesser grades to severe grades (grades 3 and 4) in transplant patients.²⁻⁵ Microscopic or mild hematuria (grade 1) was detected in 11 of 30 and macroscopic hematuria (grades 2 to 4) was diagnosed in 9 of 30 transplant patients (30%). Also, early and late-onset HC was detected in 5 and 11 transplant patients.

Contrary to some reports, the use of hematopoietic stem cell (HSC) from unrelated donors increases the risk of HC,^{1, 5, 6, 16, 17} and other researches also found similar results in the literature.^{18, 19} Hemorrhagic cystitis clinical symptoms were diagnosed in 16 of 24 allogeneic transplant patients who received HSC from haploidentical-sibling-related donors. Similar to other reports, sex mismatch between donors and recipients increases the risk of severe HC in 4 of 5 HSCT recipients with severe hemorrhage.^{9, 20}

Hematopoietic stem cell transplant patients significantly increased the risk of HC. Myeloma as an underlying disease was mostly found in children who underwent HSCT.²⁰ Hemorrhagic cystitis symptoms occurred more frequently in transplanted patients with leukemia.

Increased age as a risk factor of HC was redocumented in this study, as was older age. Seber and associates and Trotman and associates reported that older age increases the risk of HC.^{13, 14} Sex is one of the risk factors of HC in most studies, also in this report, 4 of 5 patients with severe HC was male.⁵

Other investigators presented acute and chronic graft-versus-host disease as a risk factor of HC.²¹⁻²³ But in this study, graft-versus-host disease did not increase the incidence or the severity of HC. This discrepancy may be due to small studied population,

mismatching of HLA, and the method of T-cell depletion at our institution.^{6, 19}

Use of prophylactic dose of anti-graft-versus-host-disease regimen including prednisolone, cyclosporine, and mycophenolate mofetil significantly increased the severity of HC. These data agree with other investigations that proposed that corticosteroids were a significant risk factor of HC ($P = .0005$).^{21, 22} However, in contrast to other reports, anti-graft-versus-host-disease conditioning regimen was not a risk factor of increasing HC.^{23, 24} Varying the level of hematologic and biochemical indices in prognosis of HC was introduced earlier by this group²⁵ and reconfirmed in this study.

The incidence and severity of HC may depend on the type of viral infection. In this report, high frequency of BK viruria and viremia was found in patients with HC, especially recipients with severe HC. Other investigators, like Cesaro and associates and Gorczynska and associates, have found BK virus infection in 87.5% and 81% of urine samples of HSCT patients with HC.^{5, 24} Also, Cesaro and associates found BK virus infection in blood samples of 75% of patient who developed HC.²⁴ But Gorczynska and associates and Leung and associates found a lower frequency of BK viruria (42% and 18%) in HSCT patients who developed HC.^{5, 26, 27} Similar to other research that announced that the type of conditioning regimen is only strong risk factor for BK viruria in patients with HC,⁵ we found therapeutic dose of anti-graft-versus-host-disease conditioning regimen significantly increase BK virus infection in patients with HC.

Adenoviruria was found in 2 of 5 HSCT recipients who developed severe HC. Adenoviremia has a higher presentation in patients with HC. However, these results propose a strong relation between viruria and especially viremia of adenovirus and severity of HC.

Use of a prophylactic dosage of cyclosporine that significantly correlated with adenovirus infection in patients with HC may result from using an immunosuppressive conditioning regimen and reactivation of latent adenovirus in uroepithelium of HSCT recipients. Londergan and associates reported adenovirus infection as high as 16% in different samples of immunosuppressed patients,²⁸ Raboni and associates, Murphy and associates, and Russel and associates, like Londergan and associates, reconfirmed a direct relation between adenovirus

excretion and duration with intensity of hematuria in transplant patients developed HC.²⁸⁻³¹

Similar to other research, viruria of *cytomegalovirus* was not detected in any transplant patients with HC.^{18, 31-34} Also, *cytomegalovirus* viremia (31.25%) had the lowest incidence in patients with HC. Despite other reports,^{18, 34-36} that present lower influence of *cytomegalovirus* infection in severity of HC and in parallel to other studies,^{2, 37} *cytomegalovirus* viremia was found in all 5 transplant patients with severe HC.

In conclusion, the results of this study reinforce the role of different risk factors of HC, including single and/or multiple viral infections, demographic data of donors and recipients, immunosuppressive, and anti-graft-versus-host-disease conditioning regimens. Therefore, a better understanding of the effect of these risk factors in the pathogenesis of HC requires further prospective investigation in a larger study population.

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