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RESEARCH

Monitoring of cytomegalovirus, Epstein-Barr virus and adenovirus infections in hematopoietic stem cell transplant recipients

Hematopoetik kök hücre transplant alıcılarında cytomegalovirus, Epstein-Barr virus ve adenovirus enfeksiyonlarının izlenmesi

Buket Şeflek[1], Hatice Hale Gümüş[1], Mehmet Çimentepe[1], Serhan Küpeli[2], Fügen Yarkın[1]

[1]Cukurova University Faculty of Medicine, Division of Virology, Department of Medical Microbiology, [2]Division of Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Adana, Turkey

Abstract

Purpose: Haematopoietic stem cell transplant (HSCT) recipients with iatrogenic immunosuppression are high-risk patients for viral infections. The aim of this study was to investigate the incidence of cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus (ADV) infections in HSCT recipients.

Materials and Methods: We prospectively monitored 35 patients aged 0-17 years who had allogeneic (n=30) and autologous (n=5) HSCT by quantitative real-time polymerase chain reaction tests for CMV, EBV, and ADV. The monitoring was performed one week before HSCT and weekly for the first 100 days, once a month up to one year after HSCT. In addition, seropositivity for viruses was analysed by Enzyme-Linked Immuno Sorbent Assay a week before transplantation.

Results: Before transplantation, all 35 (100%) patients who underwent HSCT were CMV IgG positive, 30 (85.7% - 95% CI: 74.1%-97.3%) HSCT recipients were found to be EBV IgG positive. CMV infection was found in 24 (80% - 95% CI: 65.7%-94.3%), ADV infection in 11 (36.7% - 95% CI: 19.4%-53.9%) and EBV infection in 8 (26.7% - 95% CI: 10.8%-42.5%) allogeneic HSCT patients. In this group, CMV DNA viral load in 8 (26.7%) patients, of which one (3.3%) coinfected with EBV DNA and one (3.3%) with ADV DNA, was higher than 1000 copies/mL which was required for pre-emptive treatment. Among 5 autologous HSCT recipients, CMV DNA was detected in 2 patients, EBV DNA in 5 and ADV DNA in 2. Pre-emptive treatment was given to 11 (%31.4 - 95% CI: 16%-46.8%; 6 CMV, 2 EBV, 1 ADV, 1 CMV-EBV and 1 CMV-ADV infection) of 35 patients. Thus, the development of viral disease was prevented in 7 (63.6% - 95% CI: 35.2%-

Öz

Amaç: İyatrojenik immünsüpresyonu olan hematopoetik kök hücre transplant (HKHT) alıcıları viral enfeksiyonlar için yüksek riskli hastalardır. Bu çalışmanın amacı, HKHT alıcılarında cytomegalovirus (CMV), Epstein-Barr virus (EBV) ve adenovirus (ADV) enfeksiyonlarının insidansını araştırmaktır.

Gereç ve Yöntem: Yaşları 0-17 arasında allojenik (n=30) ve otolog (n=5) HKHT'si olan 35 hasta prospektif olarak CMV, EBV ve ADV için kantitatif gerçek zamanlı polimeraz zincir reaksiyonu testleri ile izlendi. Monitorizasyon, HKHT'den bir hafta önce başladı ve HKHT'den sonra ilk 100 gün için haftalık, bir yıla kadar ayda bir yapıldı. Ek olarak, virüsler için seropozitiflik, transplantasyondan bir hafta önce Enzyme-Linked Immunosorbent Assay ile analiz edildi.

Bulgular: Transplantasyon öncesi HKHT yapılan 35 (%100) hastanın tamamı CMV IgG pozitif, 30 (%85.7-95% CI: 74.1%-97.3%) HKHT alıcısı EBV IgG pozitif bulundu. Allojenik HKHT hastalarının 24'ünde (%80 - 95% CI: 65.7%-94.3%) CMV enfeksiyonu, 11'inde (%36.7 - 95% CI: 19.4%-53.9%) ADV enfeksiyonu ve 8'inde (%26.7) EBV enfeksiyonu bulundu. Bu grupta biri (%3.3) EBV DNA ve biri (%3.3) ADV DNA ile koenfekte olan 8 hastada (%26.7) CMV DNA viral yükü preemptif tedavi için gerekli olan 1000 kopya/mL üzerindeydi. Otolog 5 HKHT alıcısından CMV DNA 2 hastada, EBV DNA 5 hastada ve ADV DNA 2 hastada tespit edildi. Preemptif tedavi 35 hastanın 11'ine (%31.4 - 95% CI: 16%-46.8%; 6 CMV, 2 EBV, 1 ADV, 1 CMV-EBV ve 1 CMV-ADV enfeksiyonu) verildi. Böylece 7 (%63.6 - 95% CI: 35.2%-92.1%) hastada viral hastalık gelişimi engellendi. Toplam 35 hastadan sadece 2'si (%5.7 - 95% CI: 0.0%-13.4%)) viral enfeksiyon nedeniyle kaybedildi.

Address for Correspondence: Hatice Hale Gumus, Cukurova University, Faculty of Medicine, Department of Medical Microbiology, Adana, Turkey E-mail: hhaleag01@hotmail.com
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92.1%). Of the total 35 patients, only 2 (5.7% - 95% CI: 0.0%-13.4%) died due to viral infection.
Conclusion: Early diagnosis of viral infections by prospective monitoring of viral loads in HSCT patients would be effective in preventing morbidity and mortality by ensuring timely initiation of pre-emptive therapy.
Keywords:. CMV, EBV, ADV, hematopoietic stem cell transplantation, quantitative real-time polymerase chain reaction

Sonuç: HKHT hastalarında viral yüklerin prospektif olarak izlenmesi ile viral enfeksiyonların erken tanısı, preemptif tedavinin zamanında başlatılmasını sağlayarak morbidite ve mortaliteyi önlemede etkili olacaktır.

Anahtar kelimeler: CMV, EBV, ADV, hematopoetik kök hücre transplantasyonu, kantitatif gerçek zamanlı polimeraz zincir reaksiyonu

INTRODUCTION

Haematopoietic stem cell transplantation (HSCT) is the infusion of haematopoietic stem cells to reconstitute to produce additional normal blood cells. It is currently used in the treatment of malignancies, replacement of an absent or poorly functioning hematopoietic or immune system, or for the treatment of certain genetic diseases in pediatrics. HSCTs are classified as either allogeneic or autologous according to the source of the transplanted hematopoietic progenitor cells. Cells used in allogeneic HSCTs are collected from a donor that might be a blood relative or an unrelated donor. Allogeneic transplants are quite successful when the donor is a human lymphocyte antigen (HLA)-identical twin or matched sibling. On the other hand, patients who receive allogeneic graft from donors who are not HLA-matched siblings are at a greater risk for graft-versus-host disease (GvHD), suboptimal graft function, and delayed immune system recovery[1,2]. There are three main causes of death to be faced by HSCT patients; GvHD, relapse of primary disease and viral infections. Potent chemotherapeutics in the form of myeloablative therapy or reduced-intensity conditioning (RIC) regimens are used individually to prevent recurrence and treat the primary disease. To reduce GvHD, techniques have been developed to remove T-lymphocytes. Thus, HSCT patients are often immunocompromised[3,4]. Therefore, iatrogenic immunosuppression, T lymphocyte-depleted grafts are associated with a variety of viral infectious complications in HSCT patients, which result in significant morbidity and mortality ranging from 5 to 24%[5,6].

Viral infections develop as reinfection or more commonly as a result of reactivation of persistent latent viruses in donor and/or recipient cells due to the T-cell count and functional decline over several months[2,7]. In the post-transplantation period, the reactivation of viruses, especially cytomegalovirus (CMV), Epstein-Barr virus (EBV) and adenovirus (ADV) predominates. Asymptomatic infection following virus reactivation usually occurs after a viral replication phase that is clinically silent, but in which viral load can be detected[2,8]. To prevent viral infection in HSCT recipients, prophylaxis or pre-emptive therapy is used. Prophylaxis involves the administration of prophylactic doses of an antimicrobial drug to prevent infection in patients at increased risk or recurrent infection. Whereas pre-emptive therapy involves starting antimicrobial therapy based on screening with a sensitive assay (e.g., polymerase chain reaction) in an attempt to detect viremia to avoid progression early infection to invasive disease. Thus, regular monitoring for CMV, EBV, and ADV is essential in HSCT patients to start pre-emptive treatment timely to prevent asymptomatic infection from progressing to disease and mortality[1-2,8].

The vast majority of studies on viral infections in HSCT recipients have focused on adult patients, and very little data exist in the literature regarding CMV, EBV and ADV infections in pediatric individuals. The aim of this study was to investigate the incidence of CMV, EBV and ADV infections in pediatric HSCT patients by quantitative real-time polymerase chain reaction. It was also aimed to demonstrate the clinical outcome of monitoring the viral loads and timely initiation of pre-emptive treatment.

MATERIALS AND METHODS

This prospective, descriptive study was conducted with the approval of Cukurova University Non-Interventional Clinical Research Ethics Committee (Date: 11.10.2013 and Decision No: 25/6). Every blood sample used in this study was taken only under the treating physician's approval. This study was conducted in accordance with the principles of the Declaration of Helsinki, and all patients and their parents provided written informed consent prior to enrollment.

Sample

A total of 35 patients (aged 0-17 years) who underwent HSCT and met eligibility criteria during a year period at the Department of Pediatric Oncology Bone Marrow Transplantation Unit of Cukurova University Hospital were included in the study. The patients who received more than one autologous or allogeneic transplantation during study period, and presented primary disease relapse in the first week post-transplantation were excluded. Of the 35 patients involved in the study, 30 had allogeneic HSCT and 5 had autologous HSCT. Blood samples were collected at the following intervals: a week before transplantation, once a week during the first three months after transplantation, and then once a month up to one year to monitor viral load for CMV, EBV, and ADV using quantitative real-time polymerase chain reaction (PCR). Viral IgG antibody was tested by Enzyme-Linked Immunosorbent Assay (Architect, Abbott, Wiesbaden-Germany) a week before transplantation. Data about patient demographics, clinical characteristics, diagnosis, transplantation type, stem cell source, conditioning regimen, GvHD prophylaxis, complications, and outcomes reported in Table 1 were collected from clinical records, medical charts of children, and used to analyze whether starting timely pre-emptive treatment prevents progression from asymptomatic infection to disease.

Nucleic acid isolation

The blood samples were centrifuged at 5000 rpm for 5 minutes, and plasma samples were stored at -80ºC until further analyses. The nucleic acid was extracted from 400 µl of plasma sample with EZ1 Advanced Automated Nucleic Acid Isolation Instrument (Qiagen GmbH, Hilden, Germany) and EZ1 Virus Mini Kit V2.0 (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendations.

Viral detection

The Artus CMV RG PCR Kit (Qiagen GmbH, Hilden, Germany) for CMV, the Artus EBV RG PCR Kit (Qiagen GmbH, Hilden, Germany) for EBV, and the RealStar Adenovirus PCR Kit (Altona Diagnostics, Germany) for ADV were used for amplification of nucleic acids. For the detection of CMV DNA, 20 µL of DNA extract was added to the mixture consisting of 25 µL of master mix, 5 µL of CMV Mg solution, and 0.2 µL of internal control (IC). The reaction mixture was incubated at 95ºC for 10 min (first denaturation), followed by 45 cycles of 95ºC for 15 s (denaturation), 65ºC for 30 s (annealing), and 72ºC for 20 s (extension). For the detection of EBV DNA, 20 µL of DNA extract was added to the mixture consisting of 30 µL of master mix, and 2 µL of IC. The reactions were incubated using the same protocol as CMV DNA. For the detection of ADV DNA, 10 µL of DNA extract was added to the mixture consisting of 5 µL master mix A, 15 µL master mix B, and 1 µL of IC. The study protocol of ADV DNA amplification was as follows; denaturation at 95ºC for 10 min, amplification by 45 cycles of 95ºC for 15 s and 58ºC for 1 min. Viral DNA quantifications were performed using real-time PCR with Rotor-Gene Q (Qiagen GmbH, Hilden, Germany).

The analytical detection limit of the artus CMV PCR Kit is 0.36 copies/µl ($p = 0.05$) which means that there is a 95% probability that 0.36 copies/µl will be detected. The analytical detection limit of the artus EBV PCR Kit is 1.02 copies/µl ($p = 0.05$) with a 95% probability that 1.02 copies/µl will be detected.

Definitions

The meanings of some specific terms used in this study are as follows:

Seropositivity means having a positive test result for the presence of a specific antibody in the serum.

Viremia refers to having detectable of viral DNA in the blood.

CMV infection means that the virus can be detected in the human body while CMV disease is related to the symptoms caused by the virus.

Co-infection means infection with two or more viruses at the same time.

Myeloablative regimen is high-dose chemotherapy that kills cells in the bone marrow, including cancer cells.

Reduced intensity conditioning kills some cancer cells and somewhat suppress the immune system which decreases the risk of transplant-related complications.

Engraftment is a process in which the body accepts the transplanted bone marrow or stem cells and begins to produce new blood and immune system cells.

Statistical analysis

Variables were expressed as numbers and percentages. For incidences, the 95% confidence intervals were calculated.

RESULTS

Of the 35 pediatric patients (median age 8.2 years; aged 0 to 17 years) who had allogeneic (n=30) or autologous (n=5) HSCT, 74.3% were male and 25.7% were female. Patients' demographics were presented in Table 1.

All patients were prepared with a conditioning regimen for 10 days. Of the 35 patients 68.6% had myeloablative conditioning, and 31.4% had reduced intensity conditioning. Prophylactic acyclovir (10-15 mg/kg) was administered to all of the transplant patients, and the duration of administration was 90 days. All allogeneic HSCT patients received GvHD prophylaxis with cyclosporine beginning one day before transplantation (for 90 days), but autologous HSCT patients did not (Table 1). Donor lymphocyte infusion was not applied to any patient in the study group. Average engraftment time was 14.6 days (at a range of 8-22) in 33 of 35 patients. Before transplantation, all 35 (100%) patients who underwent HSCT were CMV IgG positive, 30 (85.7% - 95% CI: 74.1%-97.3%) HSCT recipients were found to be EBV IgG positive.

Table 1. Patient demographics, clinical characteristics and outcomes.

Characteristics	n	%
Gender		
Male	26	74.3
Female	9	25.7
Age groups (years)		
0-2	6	17.2
3-5	7	20
6-10	11	31.4
11-15	7	20
16-20	4	11.4
Diagnosis		
Fanconi aplastic anemia	8	22.9
Thalassemia major	7	20
Acute myeloid leukemia	4	11.5
Hodgkin lymphoma	3	8.5
Chronic granulomatous disease	3	8.5
Severe combined immunodeficiency	3	8.5
Non-Hodgkin lymphoma	2	5.7
Multiple relapsed neuroblastoma	2	5.7
Wiskott-Aldrich syndrome	1	2.9
Osteopetrosis	1	2.9
Langerhans cell histiocytosis	1	2.9
Transplantation type		
Allogenic	30	85.7
Autologous	5	14.3
Stem cell source		
Bone marrow	30	85.7
Peripheral blood	5	14.3
Donor and HLA matching		
Matched related donor	28	93.3
Haploidentical	2	6.7
Conditioning regimen		
Myeloablative	24	68.6
Reduced intensity	11	31.4
GvHD prophylaxis		
CsA	8	26.7
CsA + MTX	17	56.7
CsA + MMF	4	13.3

CsA + MMF + MTX	1	3.3
Complications		
Fever	27	77.1
Gastroenteritis	15	42.8
Skin rash	12	34.3
Oral mucositis	10	28.5
Kidney dysfunction	3	8.6
Bleeding diathesis	3	8.6
Cystitis	2	5.7
Coagulation disorder	2	5.7
Liver dysfunction	2	5.7
Pneumonia	1	2.8
GvHD		
Yes	31	88.6
No	4	11.4
Outcome		
Healthy	31	88.5
Exitus due to viral disease	2	5.7
Exitus due to non-viral reasons	2	5.7

HLA, human leukocyte antigen; GvHD, graft versus host disease; ATG, antithymocyte globulin; CsA, cyclosporine, MMF, mycophenolate mofetil; MTX, methotrexate.

In the analysis performed prior to HSCT, CMV viremia was positive in 8 of the 35 patients before transplantation, of which one with CMV DNA above 1000 copies/mL. One of them was coinfected with ADV. All of these 8 patients also had viremia (one with ADV viremia) after HSCT. There was only one patient with EBV viremia in pre-transplantation and post-transplantation periods. Thus total 9 (25.7%) patients positive for viremia before and after HSCT, of which 3 (33%) had viral load higher than 1000 copies/mL (2 CMV, 1 CMV-ADV infection) required for pre-emptive treatment in post-transplantation period. However, of 26 patients without pre-transplant viremia, 8 (30.7%) were found to develop significant viremia after transplantation (4 CMV, 1 ADV, 2 EBV, 1 CMV-EBV infection).

Patients were monitored for CMV, EBV and ADV viremia once a week for the first three months and monthly for up to one year. The median observational period was 204.2 days (range 172-359 days). In the allogeneic HSCT group, CMV DNA positivity was 80% (n=24 - 95% CI: 65.7%-94.3%), EBV DNA 26.7% (n=8 - 95% CI: 10.8%-42.5%)), and ADV DNA 36.7% (n=11 - 95% CI: 19.4%-53.9%)). In 5 patients who underwent autologous HSCT, CMV infection was seen in 2 (40%), EBV infection in 5 (100%) and ADV infection in 2 (40%). Thus, in the whole patient group, CMV infection was 74.3% (26/35), EBV infection was 37.1% (13/35), and ADV infection was 37.1% (13/35).

Among 30 patients who underwent allogeneic transplantation, 28 (93.3%) was positive for single infection or coinfection. Of these patients, 16 (53.3%) had a single infection (13 cases of CMV, 1 case of EBV and 2 cases of ADV infection), 12 (40%) multiple infections, of which 5 had CMV-ADV, 3 CMV-EBV, 1 EBV-ADV coinfection and triple infection was observed in 3 patients. Two patients in whom infection did not develop were in the allo-HSCT group. However, viremia was observed in all 5 patients (100%) who underwent autologous transplantation. Single infection was found in 2 (40%) cases (EBV in two cases) and multiple infection in 3 (60%) cases (2 had dual infection, of which CMV-EBV in one case, EBV-ADV in one case and triple infection in one case).

Thus, in our study group, viral infection as a single or co-infection developed in 33 (94.3%) of all 35 patients. Eighteen (18) of them (51.4%) had a single viral agent, of which CMV (13) was the most frequently detected. Multiple viral infections were detected in 15 (42.9%) patients. Triple infections (CMV, EBV and ADV) were found in 4 patients (11.4%), and dual viral infections, mostly CMV-ADV and CMV-EBV, were identified in 11 patients (31.4%).

The most of the patients with CMV, EBV and ADV viremia were between 6-10 years of age (34.6%, 38.5% and 61.5%, respectively) and infected patients in the study group were mostly male (84.6%, 69.2%

and 75%, respectively). Of 26 CMV DNA positive patients, 8 patients with CMV DNA load >1000 copies/mL, which is the threshold for pre-emptive therapy, were allogeneic HSCT patients (26.7%, 8/30). Of 13 children with EBV DNA viremia, 3 patients had EBV DNA loads >1000 copies/mL, one of them was in allogeneic group (3.3%, 1/30), and two were in the autologous HSCT group. Among 13 patients with ADV DNA viremia, 2 patients had ADV DNA > 1000 copies/mL, one in allogeneic group (3.3%, 1/30) and one in autologous group.

The median time at which viral DNA was detectable for CMV, EBV, and ADV were 28.1 days (range of 2-160 days), 74 days (range of 4-215 days) and 81.9 days (range of 12-304 days), respectively. The median time to reach viral load >1000 copies/mL for CMV, EBV and ADV requiring pre-emptive treatment was 50.4 days (range, 12 to 160 days), 185.3 days (range, 30 to 304 days), and 16.5 days (range, 12 to 21 days), respectively.

As a result, there were 11 patients (31.4%) in the study group with a viral load above 1000 copies/mL (6 CMV, 2 EBV, 1 ADV, 1 CMV-EBV, and 1 CMV-ADV infection). Pre-emptive treatment was applied to all of them, and the development of viral disease was prevented in 7 (63.6% - 95% CI: 35.2%-92.1%). However, two patients whose viremia was cleared, died due to nonviral infections (one from acute myeloid leukaemia relapse, and the other from cardiovascular collapse). The other two patients (one with CMV and the other with CMV-ADV coinfection) died as a result of multiorgan failure secondary to viral infection despite the pre-emptive treatment. Thus, only 2 (5.7% - 95% CI: 0.0%-13.4%) died due to viral infection among 35 patients.

DISCUSSION

Viral infections with CMV, EBV and ADV are common in pediatric recipients of allogeneic HSCT. There is lack of data on viral infections after HSCT in pediatric recipients in the literature[8]. In this study, we prospectively monitored 35 pediatric HSCT patients to investigate the incidences of CMV, EBV and ADV infections using quantitative real-time PCR.

Serological tests can also be used in the diagnosis of viral infections. Acute infection is defined by IgM response, IgG avidity test or seroconversion. Unfortunately, an adequate antibody response is generally not detectable in transplant recipients due to immunosuppression. Therefore, serological tests are less helpful for diagnosis in allo-HSCT recipients. In addition, antigen detection tests such as immunofluorescence tests and enzyme immunoassays are rapid diagnostic tests, but their sensitivity is low compared to molecular diagnostic tests[2,9].

The incidence of viral infections in our cohort was 94.3% after HSCT. This ratio was higher than the incidence reported (68.2%) in a single center study that was conducted in 107 pediatric patients following allogeneic HSCT[11]. Current data on the incidence of viral infections in children after HSCT vary widely. CMV infection, defined as the development of CMV viremia, remains one of the most important viral infections, occurring in 15-20% of children after allo-HSCT. It is usually the result of reactivation of endogenous virus, occurring in up to 80% of seropositive patients[8]. In this study, CMV viremia in the allogeneic group in post-transplant period was 80%, while it ranged from 19.9% to 46.1% in similar studies conducted in pediatric allogeneic HSCT[10-15]. The higher incidence of CMV infection (80%) might be due to high CMV IgG seropositivity (100%) in our study population.

About 40-50% of recipients with ADV viremia develop disease, ranging from asymptomatic viral syndrome (fever, elevated liver enzymes, and pancytopenia) to invasive localized and disseminated disease (pneumonitis, nephropathy, haemorrhagic cystitis, colitis, myocarditis, central nervous system disease). Mortality rate due to ADV disease is estimated to be approximately 22%; moreover, disseminated disease has a mortality rate up to 80%. It is crucial to monitor ADV DNA in high risk populations[8,16]. The incidence of ADV viremia, which we found to be 36.7% in the allogeneic group, was consistent with 25-50.4% reported in other studies[10,17-18]. Additionally, the incidence of EBV viremia in the allogeneic group, was 26.7%, which was also within the range previously reported (22.6-34.5%)[10-11,15].

Our findings also showed that significant viremia rates, which were 33% and 30.7% in the post-transplant period were similar in patients with and without pre-transplant viremia, respectively. CMV infection usually occurs by reactivation of endogenous virus in up to 80% of seropositive individuals, and 20-35% progress to CMV disease[8]. Duver et al. reported that transplant from an unrelated donor and in vivo T-cell depletion had a

significant effect on infection rates, whereas for CMV, the strongest effect was seen by recipient seropositivity before transplantation[10]. All patients were seropositive for CMV IgG in our study. And, all HSCT recipients with significant CMV viremia above 1000 copies/mL were observed in the allogeneic HSCT patient group. Similarly, it was previously reported that viral infections were more frequent in allogeneic versus autologous transplants[15].

We found that the median time of developing viremia in the patients affected by CMV (28 days), EBV (74 days) and ADV (81 days) infections was within the first 100 days after transplantation. That is the time when the highest immunosuppression was expected. It is strongly recommended that allogeneic recipients at risk for CMV infection should be screened once a week from 10 days to 100 days after HSCT[19]. Notably, in this study, the mean time for viral DNA load to reach >1000 copies/mL was 50.4 days (12-160 days; higher viral load was developed in only one patient after 100 days) for CMV DNA, and 16.5 days (range, 12 to 21 days) for ADV, but 185.3 days (30-304 days) for EBV DNA (for 3 patients on day 30, 222 and 304 post HSCT).

It was reported that the risk of infection increases due to the duration and severity of neutropenia in the pre-engraftment period, which depend on the conditioning regimen used[8]. In this study, engraftment did not occur in 2 patients, except for 33 patients whose average engraftment time was 14.6 days. One of those two patients, who received myeloablative regimen, had a dual infection of CMV and ADV (with viremia over 1000 copies/mL on the 12th day post-HSCT) and died of multiorgan failure on the 14th day. The other patient, who had a reduced intensity regimen, had no significant CMV viremia, and survived. Duver et al. also reported two cases of failed engraftment; in one patient, engraftment was preceded by a CMV infection, and in another patient, engraftment was preceded by an HHV-6 infection, but they reported that these infections were concomitant and not the cause of rejection[10].

Of 35 patients in our group, 24 received myeloablative therapy, while 11 received low-intensity therapy. While CMV DNA, EBV DNA and ADV DNA positivity were 61.5%, 76.9% and 53.8%, respectively, in patients who received myeloablative conditioning regimen, these rates were found to be lower in patients receiving reduced-intensity conditioning regimen; 38.5%, 23.1%, and 46.2%, respectively. This could be explained by the fact that reduced-intensity conditioning regimens have a higher risk of rejection than myeloablative regimens, but a lower risk of engraftment delay or failure and faster immune recovery, which potentially lead to the lower risk of infection[20-21].

As allo-HSCT recipients have insufficient host immunity to control viral replication, pre-emptive therapy is used in patients with asymptomatic viral infections to prevent viral diseases[2,8,22]. In this study, the development of viral disease was prevented in 7 of the 11 patients who had a viral load of >1000 copies/mL (CMV n=6, EBV n=2, ADV n=1, CMV-EBV n=1 and CMV-ADV n=1) by pre-emptive treatment. The monitoring of viral load with specific and sensitive methods such as quantitative real-time PCR allows early initiation of pre-emptive treatment and eradication of viremia. The remaining 4 patients (CMV DNA n=3, CMV-ADV n=1) died. In two of them, the viremia was eradicated with pre-emptive therapy, but they still died of nonvirus-related causes (acute myeloid leukaemia relapse and cardiogenic dysfunction). The other two patients died as a result of multiorgan failure due to CMV and CMV-ADV coinfection. Thus, the mortality rate due to viral infection in 35 pediatric HSCT patients was found to be 5.7%.

The limitation of this study was that it was a hospital-based single center study with a small sample size. We simply focused on the viruses that are commonly observed in this population.

In conclusion, viral infections are relatively common with the highest incidence of 80% for CMV, and 36.7% for ADV, 26.7% for EBV among pediatric HSCT patients in this study. Pre-emptive treatment was given to 11 patients which had viremia above 1000 copy/ml. Thus, the development of viral disease was prevented in 7. Of the total 35 patients, only 5.7% died due to viral infection. Management of viral infections after HSCT is essential to limit virus-related morbidity and mortality. Screening and prospective monitoring of viral infections in the pre-transplantation and post-transplantation periods with sensitive and specific method such as quantitative real-time PCR, will contribute to the early detection of viremia, the early implementation of treatment options, and the reduction of virus-related morbidity and mortality.

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