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RESEARCH ARTICLE

A study on some factors affecting CMV reactivation in allogeneic hematopoietic stem cells transplantation

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ABSTRACT

Background: In developing countries, cytomegalovirus (CMV) seropositivity has been widely propagated. Following hematopoeitic stem cell transplantation, the apparent risk of CMV reactivation increases. With effective surveillance and study of the effect of some factors on CMV reactivation, timely treatment with anti-viral treatment may decrease morbidity and mortality associated with CMV reactivation. **Aim:** This study aimed to evaluate the effect of some factors on CMV reactivation in allogeneic hematopoietic stem cell transplantation (HSCT). **Materials & Methods:** We retrospectively analyzed 25 patients with Serostatus positive of CMV IgG antibodies who underwent allogeneic hematopoietic stem cell transplantation at two Bone Marrow Transplantation centers in Egypt from November 2016 to June 2017. **Results:** In our study, the patient male percentage was 57.1% in CMV reactivation group with the most common pre-transplantation diagnosis was FA, CML and βTM. All donors in the reactivation group (100 %) were positive for CMV IgG ab. This group had 42.9% matching donors in sex and blood groups. While the stem cell source was 92.9% peripheral blood source with a median dose of 7.0 cells $\times 10^6$ / kg. **Conclusion:** The incidence of CMV infection following hematopoietic stem cell transplantation is comparable to that recorded in Western literature because of a higher seroprevalence rate in developing countries. More research is needed to study factors affecting cytomegalovirus reactivation in allogeneic hematopoietic stem cells transplantation (HSCT).

Keywords: Bone marrow transplantation; CMV reactivation; Stem cells

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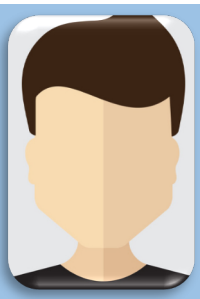

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INTRODUCTION

Bone marrow transplantation (BMT) is increasingly successful in the treatment of a variety of hematologic and immunologic disorders. As bone marrow transplantation and solid organ transplantation have progressed to become the preferred therapeutic methods for a variety of malignancies and dysfunctions of the end-stage of the organ, these types of therapy are now more constrained by the availability of organs than by technological capacity or lack of sufficient immunosuppression. Despite the progress of transplantation medicine, the transplant recipient tends to be afflicted by infection, which remains the leading cause of death in this population (Michael and Peter, 2019**;** Sarah et al., 2019).

The principle behind BMT for malignant and nonmalignant hematologic diseases is the ablation of abnormal marrow followed by the rescue of marrow function by replacement of the ablated marrow elements with normal donor marrow (Alberto et al., 2020). With bone marrow transplantation as a stem cell regenerative treatment, significant progress has been achieved over the last 50 years. However, insufficient numbers of HSCs are still a major constraint in clinical applications.HSCs, the pivotal cells in this important tissue, are the subject of extensive studies to provide insights into new methods for enhanced stem cell therapies unique to patients.

Nowadays, new and/or improved sources of transplantable HSCs were found. These-now-

include-the-CD34+-CD38---fraction-of-adult-bone-marrow,-mobilized-peripheral-blood-HSCs and-the-CD34+-CD38---fraction-of-umbilical-cord-blood. Three-sources-of-haematopoietic stem cells-have-generally-been-utilized;-syngeneic-bone-marrow-from-a-genetically identical twin, allogenic-bone-marrow-from-histocompatible-sibling-donor-and-autologous cryopreserved bone-marrow-cells-(Hatizimichael-and-Tuthill,-2010).

The use of peripheral blood stem cells (PBSCs) as a source of stem cells is associated with faster grafting than is seen in autologous transplants with harvested bone marrow. After autologous SCT, the minimum number of hematopoietic progenitors needed for engraftment is 2×10^6 $CD34^+$cells⁄kg. Although raising the number of transplanted $CD34^+$cells from more than $5\times10^6CD34^+$cells kg, it is helpful and concerns remain that could be associated with an increased risk of tumor contamination (Vose et al., 2009).

For patients undergoing a sibling allograft, PBSCs are the most widely used stem-cell source and are increasingly used in unrelated recipients of donor transplants (Weisdorf et al., 2009). While, in pediatric transplantation, bone marrow remains the preferred stem cell source. In recipients of a PBSC allograft, the lowest appropriate stem cell dose for healthy engraftment is considered to be 2×10^6 $CD34^+$cells/kg (Zhang et al., 2012).

UCB contains a high proportion of hemopoietic progenitors and HSCs are an increasingly important stem cell source in pediatric and adult transplantation. Importantly, HLA disparity appears better tolerated in the recipient of UCB and as a consequence, the incidence of severe GVHD is lower with mismatched UCB than would be expected using a comparably mismatched unrelated donor. A major factor limiting the uptake of UCB transplantation has been delayed or failed engraftment, which used to be a common problem, particularly in the adult recipient. The two most important factors determining the likelihood of neutrophil and platelet engraftment after UCB transplantation are nucleated cell dose and HLA disparity (Batten et. al.,2013). Conflicting data were documented with respect to the relative risk of bone marrow harvest (BM) as a stem cell source compared to peripheral blood stem cells (PBSC) (Pinana et al., 2010; Guerrero et al., 2012).

In the peripheral blood, only a few hematopoietic stem and progenitor cells are present. Recent studies have shown that administration of hemopoietic growth factors such as granulocyte colony-stimulating factor (G CSF) interferes with the adhesion of progenitors to the stroma of the bone marrow, resulting in large numbers of them mobilizing into the peripheral blood. This influences the practice of transplantation, resulting in the substitution of bone marrow by peripheral blood as the most common hemopoietic source (Pelus, 2017).

Bone marrow transplantation starts with conditioning treatment in which high-dose toxic marrow radiation and/or chemotherapy is administered to kill malignant cells and suppress host immunity to prevent donor cell rejection. The intravenous infusion of donor marrow is administered. It is the preparative regimen given before HSCT to eradicate the malignancy exploiting the dose response phenomena that most cancer cells exhibit, suppress the host immune system, in the setting of allogeneic HSCT, giving better chance for engraftment and creating space for homing of new graft, in the setting of allogeneic HSCT (Gyurkocza and Brenda, 2014).

Early HSCT complications occur within the first 100 days, while late complications occur after the 100^{th} day of transplantation (Trajkovska et al., 2017). The early complications of HSCT include infections bacterial, fungal, and viral infections, acute GVHD (skin, liver, gut), interstitial pneumonitis, hemorrhagic cystitis, in addition to venoocclusive disease and cardiac failure**.** However, the late complications of HSCT include varicella-zoster and capsulate bacterial infections, chronic GVHD (arthritis, malabsorption, hepatitis, scleroderma, pulmonary disease), chronic pulmonary disease, autoimmune disorders, cataract, infertility, and second malignancies (Miano et al., 2008).

Human cytomegalovirus (HCMV) is a member of the viral family known as Herpesviridae or herpesviruses. HCMV belongs to the subfamily Betaherpesvirinae, which also includes another mammalian cytomegalovirus (Koichi et al.,

2007). HCMV infections are frequently associated with the salivary glands. HCMV stays latent during life within the body and can be reactivated at any moment. It may eventually cause mucoepidermoid carcinoma and other malignancies, such as prostate cancer (Michael et al., 2016). HCMV infection can appear as primary infection, reinfection or reactivation. CMV infection is a major problem in allogeneic bone marrow transplant (BMT) cases, 30%-50% of cases show clinically significant infection (Sissons and Carmichael, 2002).

Transplantation of solid organs clearly can transmit CMV, so other cells than those mentioned can harbor and transmit the virus. Whether the infected cell type in these organs is blood cells, macrophages, or other cell types, however, has not been clarified. T-cell mediated cellular immunity is the most important factor in controlling CMV replication (Sissons and Carmichael, 2002; Bhat et al., 2015).

Other studies also showed that the serology status of the recipient remains a predominant risk factor for BMT rejection and associated mortality. The host immune system recognizes virion after infection and leads to the activation of the host immune system. Several studies have reported that after bone marrow transplantation CD4-T cells regenerate relatively at a slow rate, which subsequently provides limited help to cytotoxic T cells for control of CMV replication. Patients undergoing Haplo-SCT have a higher incidence of CMV antigenemia than HLA matched transplantation. Other risk factors for CMV infections in hematopoietic stem cell transplantation (HSCT) cases are advancing age, immunosuppression because of whole-body irradiation, antithymocyte globulins, chemotherapeutic regimens, and transplantation of umbilical cord blood. Recipients of non-myeloablative (HSCT) are more prone to have late CMV infection, mostly due to chemotherapy containing alemtuzumab or antilymphocyte globulins (Ozdemir et al., 2007).

CMV infection is defined as the detection of CMV, typically by DNA PCR, pp65 antigenemia, or mRNA nucleic acid sequence-based amplification, from plasma or whole blood in a CMV-seronegative patient (primary infection) or a CMV-seropositive patient (reactivation of latent or persistent virus or superinfection with another strain of CMV) (Manuel et al., 2009). This research aims to study the effect of some factors on CMV reactivation in allogeneic hematopoietic stem cell transplantation (HSCT).

PATIENTS AND METHOD

This study was conducted on 25 patients with Serostatus positive of CMV IgG antibodies who underwent allogeneic hematopoietic stem cell transplantation. They were of Bone Marrow Transplantation Unit, Tanta University International Teaching Hospital and Bone Marrow Transplantation Unit, Nasser Institute for Research and Treatment. The duration of the study was from November 2016 to June 2017. Informed consent had been taken from all participants in the research and the privacy of the data had been greatly considered. Peripheral collection of hematopoietic stem cells and HLAmatched identical sibling donor are considered criteria for the incorporation of allogeneic transplantation.

All patients included in the study were subjected to the following:

Complete clinical examination: General examination for lymphadenopathy, a manifestation of anemia such as polar, palpitation, dyspnea, general weakness, etc.Abdominal examination for liver, spleen, and any mass and chest and heart examination.

Laboratory investigation including Hematology: Complete blood count including differential leucocytic count, peripheral blood smear, reticulocytic count, blood group, coagulation screening as PT, PTT, INR and fibrinogen.Bone marrow aspiration and biopsy to confirm the diagnosis, for cellularity, morphology, immunophenotyping especially for PNH clone (CD55, CD59) – Cytogenetic analysis and exclusion of infiltration.Viral marker screening (HBsAg, HBs Ab, HBcAbIgm, HCV Ab, HIV Ab, CMV Ab(IgM and IgG), EBV Ab. and PCR for HCV RNA both qualitative and quantitative.

Biochemistry: Renal and liver function tests, electrolytes (Sodium, Potassium, Calcium, and magnesium levels), random blood glucose, serum ferritin. Immunology assay: Autoantibody screening (ANA, RF and antids DNA). HLA typing

for both the recipient and donors: HLA class I and II typing was performed by serology and real-time polymerase chain reaction (sequence-specific primers and sequence-specific oligonucleotide probes) (Ena Wang et al., 2018).

System overview: Including echocardiography, Chest X-ray, electrocardiogram (ECG), computerized tomography of chest and sinuses. All donors – after identification of HLA matched sibling–were subjected to pre-transplantation evaluation including the same hematological, biochemical, virology screen and echocardiography, as done for the patients in addition to bone marrow aspiration and cytogenetic study. A vessel assessment was done for the possibility of apheresis through the peripheral wide pored cannula versus the central venous axis.

Donor stem cells that were mobilized using hemopoietic GCSF alone had been given at a dose of 10 mg/kg day for 5 days. One or more apheresis sessions (using Spectra Optia machine for cell separation) were performed on the fifth day of GCSF until the yield was achieved. Some donors were equipped for harvest operations to obtained stem cells on the fifth day of GCSF until the yield was achieved. The yielded evaluation was done through assessment of $CD34^+$ mononuclear cell number expressed per kilogram of recipient weight. The typical target yield was: a collection of $2\text{-}5x10^6$ /kg $CD34^+$ cells.

Analysis of CD 34^+ cells: Fresh PBSC harvest aliquots are immunostained and analyzed for $CD34^+$ cells (using Coulter XL flow cytometer) aiming to collect at least a total of $CD34^+$cells of 3 x 10^6/Kg of Bodyweight (BW) of the recipient after a maximum of three leukapheresis (Shi and DiPersio, 2009).

A whole blood staining method was performed, where 100µL of whole blood was incubated with 10 µL class III $CD34^+$ monoclonal antibody (HPCA2 clone PE, B & D) in one tube and is control in another tube. After 30 minutes of incubation, cells were washed twice once with a haemolysing solution and the other with phosphate buffer saline (PBS). This was followed by suspension of the cells in 500 µL PBS. Cells were then analyzed for forward and side scatters using Coulter XL flow cytometer. The gates were defined on the population with low side scatter and CD34+ve events estimated. Total CD 34 cells= % of CD 34+ve cells (test control) X % of gated cells x TNC x volume of harvest. The value obtained is then divided by the patient's body weight.

CMV reactivation (replication)

CMV reactivation was tested by real-time polymerase chain reaction (PCR) weekly.

Statistical analysis of the data

Data was fed to the machine and analyzed using version 20.0 of the IBM SPSS software package (Armonk, NY: IBM Corp) (Kirkpatrick and Feeney,2013). Qualitative data were represented using numbers and percentages. The Kolmogorov Smirnov test was used to check the normality of distribution The range (minimum and maximum), mean, standard deviation and median data were used to define the quantitative data. At the 5% mark, the importance of the findings obtained was significant.

RESULTS

The two groups (recipients with CMV reactivation and without reactivation) were determined by CMV PCR weekly. The group with CMV reactivation shows a positive PCR result.

In our study, we study some factors affected by CMV reactivation such as:

- Patient gender and age
- Pre-diagnosis
- Donor gender and age
- Microbial screening of donor
- Sex and blood group matching between donor and recipient
- Source and dose of stem cells
- The days of hospital stay
- The degree of kinship between the recipient and the donor
- The conditioning regimen
- Regimen-related toxicity such as oral mucositis and microbial infection.

There was no statistical difference between both groups as regard patient gender and pre-diagnosis, the percentage of males was 63.6% and 57.1% for recipients with CMV reactivation and patients with no CMV reactivation respectively. The most common pre-transplantation diagnosis in recipients with CMV reactivation was AML, βTM and SAA which

represented 36.4%, 27.3 and 27.3% respectively. The most common diagnosis in recipients without CMV reactivation was FA, CML, and βTM which represented 21.4%, 14.3and 14.3% respectively (Table 1).

The donor male percentage was 54.5% (6 cases) and 57.1% (8 cases) for non-reactivation and reactivation groups respectively, while the female percentage was 45.5% (5 cases) and 42.9% (6 cases) for two groups respectively. As regards, viral screening of donors, the non-reactivation group, had 4 donors (36.4%) positive for HBs Ab, 9 donors (72.7%) positive for CMV IgGAb, and 2 donors (18.2%) positive for toxoplasma IgG, in comparison to the reactivation group that exhibited 3 donors (21.4%) positive for HBs Ab, 2 donors (14.3%) positive for toxoplasma IgG and 14 donors (100%) positive for CMV IgG Ab (Table 2).

There were no significant statistical differences between the two groups according to sex and blood group matching, CMV reactivation group had 6 (42.9%) matching donors and 8 (57.1%) non-matching (4 of them had female donor and 4 of them had male donor) in comparison to the non-reactivation group that had 6 (54.5%) matching donors and 5 (45.5%) non-matching (3 of them had female donor and 2 of them had male donor). The percentage of matching blood groups was 63.6% (7 matching donors) and 42.9% (6 matching donors) in non-reactivation and reactivation groups respectively. On the other hand, the reactivation group had 8 non-matching donors (57.1%) (Table 3).

The source of stem cells was from peripheral blood in 81.8% (9 from 11cases) of the non-reactivation group and 92.9% (13 from 14 cases) of the reactivation group, while 18.2% (2 from 11 cases) of the non-reactivation group and 7.1% (1 from 14 cases) of the reactivation group was bone marrow harvest. The median dose of stem cells was 7.0 for both the non-reactivation and the reactivation groups. While the days of hospital stay ranged from 27 to 62 days with a median value of (34.0) in the non-reactivation group and reactivation group, they ranged from 22 to 55 days with a median value of(32.5) (Table 4).

As regards the degree of kinship between the recipient and the donor, in the non-reactivation group the percentage of sister and brother was 54.5% and 45.5% respectively, while in the reactivation group the percentage was 28.6% and 35.7% respectively. Nevertheless, there were no patients categorized as father or mother in the non-reactivation group, while in the reactivation group the percentage of father and mother was 21.4% and 14.3% respectively. There were no significant statistical differences between the two groups as regards the degree of kinship (Table 5).

As a conditioning regimen in the not reactivated group, eight cases (72.7%) were taken BU and CY as a conditioning regimen, while three cases only from 11 (27.3%) took FLU and BU. While, in the reactivated group, four cases (28.6%)were taken BU and CY, two cases (14.3%)were taken FLU and ALK, two cases (14.3%)were taken FLU and CY, one case only was taken FLU and BU, two cases (14.3%) were taken BU, CY, and ATG and three cases were taken FLU, CY and ATG as a conditioning regimen (Table 6). The oral mucositis percentage in the non reactivation group was 27.3% (3 cases), 54.5% (6 cases), 9.1% (1 cases), and 9.1% (1 cases), for no, mild , moderate and severe respectively while in the reactivation group, was 7.1% (1 cases),50% (7 cases), 28.6% (4 cases) and 14.3% (2 cases) respectively (Table 6).

The infections in the non-reactivation group were only three cases (27.3%) with bacterial infection only, while the other eight cases (72.7%) were not infected. In the reactivation group, the infection rate was 50% of the patients (7 cases), four cases of them (28.6%) were infected with bacterial, one case(7.1%) had fungal infections, and two cases (14.3%) had bacterial/fungal infections (Table 6).

DISCUSSION

In patients undergoing hematopoietic stem cell transplantation (HSCT), opportunistic infections such as cytomegalovirus (CMV) are among the main causes of morbidity and mortality. Human cytomegalovirus (CMV) reactivation occasionally occurs following allogeneic hematopoietic stem cell transplantation (HSCT) during the early stages of immune recovery (Valadkhani et al.,2016). However, studies interrogating such effects are limited in the modern transplant era.

Table 1. Demographic data and clinical diagnosis of the studied groups according to patients pre-transplantation

Patients	CMV Reactivation				Test of sig.	P
	NR (n= 11)		R (n= 14)			
	No.	%	No.	%		
Sex						
Male	7	63.6	8	57.1	χ^2= 0.108	FEp= 1.000
Female	4	36.4	6	42.9		
Diagnosis						
AML	4	36.4	1	7.1	χ^2= 1.576	MCp= 0.159
ALL	1	9.1	1	7.1		
BTM	3	27.3	2	14.3		
CML	0	0.0	2	14.3		
DC	0	0.0	1	7.1		
FA	0	0.0	3	21.4		
MDS	0	0.0	2	14.3		
PRCA	0	0.0	1	7.1		
SAA	3	273	1	7.1		

χ^2: Chi-square test, FE: Fisher Exact, MC: Monte Carlo, p: p-value for comparing between the two groups, NR: Not reactivated, R: reactivated, AML, acute myeloid leukemia, ALL, acute lymphoblastic leukemia, βTM, beta-thalassemia major, CML, chronic myeloid leukemia, MDS, myelodysplastic syndrome, PRCA, Pure red cell aplasia, SAA, severe aplastic anemia, DC, Dyskeratosiscongenita, FA, Fanconi Anemi

Table 2. Demographic data and virology screening of the donors.

Donors' Characteristics	CMV Reactivation				Test of sig.	P. value
	NR (n= 11)		R (n= 14)			
	No.	%	No.	%		
Gender						
Male	6	54.5	8	57.1	χ^2= 0.017	FEp= 1.000
Female	5	45.5	6	42.9		
Virology						
HCV Ab	0	0.0	0	0.0	-	-
HCV PCR	0	0.0	0	0.0	-	-
HBsAg	0	0.0	0	0.0	-	-
HBsAb	4	36.4	3	21.4	χ^2=0.682	FEp=0.656
HBcAb	0	0.0	0	0.0	–	-
HSV	0	0.0	0	0.0	–	-
CMV IgG	9	72.7	14	100	χ^2=2.767	FEp=0.183
CMV IgM	0	0.0	0	0.0	-	-
ToxIgG	2	18.2	2	14.3	0.070	FEp=1.000
ToxIgM	0	0.0	0	0.0	-	-

χ^2: Chi-square test, FE: Fisher Exact, p: p-value for comparing between the two groups, *: Statistically significant at p ≤ 0.05, NR: Not reactivated, R: reactivated, HCV Ab: Hepatitis C antibody, HCV PCR: Hepatitis C polymerase chain reaction quantitative, HBsAg: Hepatitis B Surface antigen, HBsAb: Hepatitis B Surface antibody, HBcAb: Hepatitis B core antibody, HSV: Herpes simplex virus, CMV IgG: Cytomegalovirus Immunoglobulin G antibody, CMV IgM: Cytomegalovirus Immunoglobulin M antibody, ToxIgG: Toxoplasmosis Immunoglobulin G antibody, ToxIgM : Toxoplasmosis Immunoglobulin M antibody

Table 3. Matching in sex and blood group between donors and recipients

	CMV Reactivation				X^2	p
	NR (n= 11)		R (n= 14)			
	No.	%	No.	%		
Sex						
Matching	6	54.5	6	42.9	0.337	0.561
Non-matching	5	45.5	8	57.1		
Female to male	3	60.0	4	50.0	0.124	FEp=1.000
Male to female	2	40.0	4	50.0		
Blood group						
Matching	7	63.6	6	42.9	1.066	0.302
Non-matching	4	36.4	8	57.1		

χ^2: Chi-square test, FE: Fisher Exact, p: p-value for comparing between the two groups, *: Statistically significant at p ≤ 0.05

Table 4. Source of stem cells, dose of stem cells and hospital stay in the studied groups

Variable factors	CMV Reactivation				Test of sig	P
	NR (n= 11)		R (n= 14)			
	No	%	No	%		
Stem cells source					χ^2=0.711	FEp=0.565
P.B	9	81.8	13	92.9		
B.M	2	18.2	1	7.1		
Dose of stem cells (×10^6 / kg)						
Min. – Max.	3.80 – 12.0		2.80 – 24.90		U=67.50	0.813
Mean ± SD.	6.75 ± 2.16		8.94 ± 6.24			
Median	7.0		7.0			
Hospital stay						
Min. – Max.	27.0 – 62.0		22.0 – 55.0		U=72.0	0.783
Mean ± SD.	35.36 ± 9.33		34.14 ± 8.47			
Median	34.0		32.50			

χ^2: Chi-square test, FE: Fisher Exact, U: Mann Whitney test, p: p-value for comparing between the two groups, NR: Not reactivated, R: reactivated, P.B = Peripheral blood, B.M = Bone marrow harvest

Table 5. The degree of kinships in study groups

	CMV Reactivation				χ^2	p
	NR (n= 11)		R (n= 14)			
	No.	%	No.	%		
the degree of kinship						
Sister	6	54.5	4	28.6	4.378	MCp=0.239
Brother	5	45.5	5	35.7		
Father	0	0.0	3	21.4		
Mother	0	0.0	2	14.3		

χ^2: Chi-square test MC: Monte Carlo, p: p-value for comparing between the two groups

Table 6. Condition regimens, their related toxicity and infections in the two studied groups

Conditioning regimen & related toxicity	CMV Reactivation				χ^2	MCp
	NR (n= 11)		R (n= 14)			
	No.	%	No.	%		
BU/CY	8	72.7	4	28.6	5.263	0.142
FLU/ALK	0	00	2	14.3		
FLU/BU	3	27.3	1	7.1		
FLU/CY	0	0.0	2	14.3		
FLU/CY/ATG	0	00	3	21.4		
BU/CY/ATG	0	00	2	14.3		
Oral Mucositis						
No	3	27.3	1	7.1	2.784	0.512
Mild	6	54.5	7	50.0		
Moderate	1	9.1	4	28.6		
Severe	1	9.1	2	14.3		
Infection						
No	8	72.7	7	50.0	2.531	0.617
Bacterial	3	27.3	4	28.6		
Fungal	0	0.0	1	7.1		
Bacterial/Fungal	0	0.0	2	14.3		

χ^2: Chi-square test, MC: Monte Carlo, p: p-value for comparing between the two groups

The primary study aim was to estimate some factors that affected initial CMV reactivation (RA) in allogeneic hematopoietic cell transplantation patients (alloHCT) and to assess their influence on CMV reactivation.

Ljungman et al.,2002based on the diagnosis of CMV infection and disease on the mentioned criteria. In short, CMV infection is characterized as virus isolation or viral protein or nucleic acid detection in any sample of body fluid or tissue.

The occurrence of clinical signs and/or symptoms of the end-organ disease combined with the identification of CMV infection in a biopsy or bronchoalveolar lavage fluid in the event of pneumonia has been identified as CMV disease. After day 100, CMV disease was described as late CMV disease. When at least one positive stained cell was observed on the slides, the CMV antigenemia test was considered positive. In the first 3 months following allogeneic but not autologous HCT, regular screening for CMV viremia is mandatory (Lin and Liucorresponding, 2013).

In this study, there were no differences in the clinical features between the two groups (reactivation and non-reactivation groups), including gender, diagnosis and matching in sex and blood group between donors and recipients. No differences were found regarding the dose of stem cells infused and their source. Also, no differences were observed in the demographic data of donors between the two groups, including sex and Microbial screening (HBsAb., CMV IgG and toxoplasma IgG). Regarding the degree of kinships and CMV IgG status of donors and their effect on CMV reactivation were no different in the two studied groups. Also, no differences were observed in the two studied groups according to the conditioning regimens, their related toxicity (Oral mucositis and bacterial and fungal infection).

Pinheiro et al. (2013) carried one study on Cytomegalovirus infection after hematopoietic stem cell transplantation and found that CMV infection post SCT usually occurs as a consequence of CMV reactivation in patients previously exposed to CMV as indicated by positive antibody titers (CMV+vepatients). Also, after HSCT Cytomegalovirus (CMV) reactivation used to be a major cause of pneumonia and death in HSCT recipients. The incidence of reactivation range from 40% to 60% in the allogeneic setting and <5% in the autologous setting.

One wide research on factors associated with cytomegalovirus infection in children receiving allogeneic hematopoietic stem cell transplantation was carried by Jaing et al. (2019). They confirmed that while ganciclovir (GCV) preventive therapy is used following allogeneic hematopoietic stem cell transplantation (HSCT) for cytomegalovirus (CMV) infection, risk factors for CMV reactivation are poorly understood in children undergoing HSCT. In contrast to our result, no significant differences were observed in the two studied groups according to the conditioning regimens, this may be due to the small number of patients studied.

Yanada and his colleagues (2003) agreed with the current findings. To determine the relationship between cytomegalovirus antigenemia, ganciclovir (GCV) treatment and outcome, 241 consecutive cytomegalovirus-risk patients who underwent allogeneic hematopoietic stem cell transplantation were retrospectively evaluated. They found that there were no significant differences between the two patients categories (with cytomegalovirus reactivation and without cytomegalovirus reactivation) as regard to the incidence of graft versus host disease, the results in this point were Compatible with our results.The risk of CMV is highly dependent on the serostatus of the donor (D) and recipient (R) (D/R+ > D+/R+ >D+/R > D/R). Not all seropositive individuals with these risk factors produce CMV, which strongly indicates that host factors play a major role in CMV predisposition in HCT recipients, such as those controlling CMV specific T cell responses(Camargo and Komanduri,2017; Paourietet al., 2018).

Angela et al. (2013) studied the effect of patient age on selection for transplantation and their data analysis has shown that patients under 40 years of age gain greatly from transplantation relative to patients over 40 years of age, this may be due to increased transplant-related mortality in patients more than 40 years. On the contrary, in our study, we found that the age of the patients did not affect CMV reactivation.

Kim and his colleagues (2016) studied the effect of donor and recipient sex in allogeneic stem cell transplantation. They find the opposite of our results, that when a female sibling donates to a male transplant recipient, sibling gender affects survival. The risk of relapse is decreased in these female-to-male transplants, but the risk of mortality from transplant-related

complications such as graft versus host disease is increased, thus decreasing overall survival. In female recipients, the gender of the sibling donor has no major effect. But Compatible with our study, they did not mention any effect of donor and recipient sex in CMV reactivation.

Magdalena et al. (2017) study the risk factors for cytomegalovirus infection after allogeneic hematopoietic cell transplantation in malignancies. They concluded to CMV reactivation should be regarded as a continuous function of the recipient and donor CMV-seropositivity and recipient immune suppression, caused by conditioning, immunosuppressive therapy and HLA disparity between donor and recipient.D−/R+ CMV serostatus, acute or chronic GVHD, and unrelated or mismatched stem cell donor are the major risk factors for CMV reactivation and disease after allo-HSCT, contrary to our results.

Leonardo and his colleagues (2020) studies the impact of anti-CMV IgG titers and CD34 count before hematopoietic stem cell transplantation from alternative donors on CMV reactivation. In this study, they explored the role of anti-CMV antibody titers in HSCT from alternative donors and compare the risk of CMV reactivation between post-transplant cyclophosphamide-based haploidentical HSCT and antithymocyte globulin-based unrelated donor (URD) HSCT. they included 98 CMV-positive patients, 30 undergoing haploidentical HSCT and 68 undergoing URD HSCT. The majority of patients had a malignant disease (84%), received a myeloablative conditioning regimen (78%), and received a bone marrow graft (90%). The median pre-transplantation anti-CMV IgG level was 109 U/mL. With a median follow-up of 2.2 years, a total of 72 CMV reactivations occurred in 50 patients. There was no difference in CMV reactivation pattern between haploidentical HSCT recipients and URD HSCT recipients. They concluded to pre-transplantation cytomegalovirus (CMV) IgG titers predict the risk of CMV reactivation in hematopoietic stem cell transplantation recipients. An infused CD34 cell dose >1.6 × 10^6 cells/kg reduces the risk of CMV reactivation. Also, they found CMV reactivation risk is not different between recipients of unrelated donor transplants and recipients of haploidentical transplants. They find that the use of reduced-intensity conditioning regimens is associated with an increased risk of CMV. In the contrast, our study explained that either the conditioning regimen or CD34 dose did not affect CMV reactivation.

Masoud et al. (2020) retrospectively analyzed the data of reactivation 145 CMV-seropositive cases out of a total of 201 allo-HSCT patients, including age, gender, underlying disease, conditioning regimen, prophylaxis regimen and occurrence of acute graft-versus-host disease (aGVHD) to evaluate their roles in CMV reactivation. their result showed that a conditioning regimen containing Busulfan and Fludarabine or Cyclophosphamide significantly decrease the early CMV reactivation. Patients who developed aGVHD and those who received anti-thymocyte globulin (ATG) as a prophylaxis regimen, had 1.84 and 2.63 times higher risks of CMV reactivation, respectively. Also, Masoud and his colleagues' results differ from ours in this regard, as we did not find any effect of the conditioning regimen on CMV reactivation.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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