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Original Article

Cytomegalovirus Reactivation in Allogenic Haematopoietic Stem Cell Transplant Patients with Haploidentical Versus Fully Human Leukocyte Antigen Matched Donor

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ABSTRACT

Objective: *To determine cytomegalovirus reactivation in hematopoietic stem cell transplant patients with haploidentical versus fully HLA matched donors.*

Methods: *We enrolled 60 patients in this retrospective observational study at Armed Forces Bone marrow transplant centre (AFBMTC) between March 2019 and October 2022. CMV DNA PCR was done weekly from day +14 to day +100 to monitor CMV reactivation during the 100-day post-HSCT period. An Applied Biosystems 7500 real-time PCR machine was used to conduct the PCR analysis.*

Results: *The median age was 15±10.6 years. 39(65%) patients had CMV reactivation. The median time to CMV DNAemia was 19.25 days. 2 out of 39 patients received ganciclovir, while 26 (43.3%) received valganciclovir. The underlying diagnosis had statistically significant correlation with CMV reactivation (p =0.02). Primary graft failure was the most frequent cause of mortality, resulting in neutropenic sepsis in 6 patients (42.85%), but 3 of these patients also had CMV reactivation. The overall survival (OS) was 76.7% and disease-free survival (DFS) was estimated at 71.7%. Neither OS nor DFS in our analysis showed a statistically significant correlation with CMV infection.*

Conclusion: *This study demonstrated a statistically significant association between haploidentical transplant and CMV reactivation, compared to fully matched recipients. Various factors, including underlying diagnosis, conditioning regimen, and GVHD prophylaxis, were also found to be correlated with CMV reactivation. However, while CMV reactivation was associated with specific factors, it did not impact overall survival or disease-free survival in the study population.*

Keywords: *Allogenic HSCT, CMV Reactivation, Fully HLA Matched Donor, Haploidentical.*

1. INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is now widely used as a therapeutic treatment for a number of benign and malignant blood disorders[1]. When perfectly matched donors are not available, the spectrum of stem cell resources has broadened because to the use of hematopoietic stem cells (HSC) from haploidentical donors (HID)[2]. Human cytomegalovirus (HCMV) reactivation, the most frequent viral complication following transplantation and one that is linked to a higher risk of non-relapse mortality (NRM), is a problem that HSCT recipients frequently deal with[2].

There are regional differences in the rates of opportunistic CMV reactivation, with affluent nations like China having reactivation rates ranging from 23.5% in cases of matched sibling donor-stem cell transplant (MSD-SCT) to 81.0% in patients of HID-SCT[3].

Due to inadequate immunological reconstitution, notably a shortage of CD8+ cytotoxic T cells, the first few weeks following transplantation are the most dangerous for HSCT participants in terms of CMV infection (within the first 100 days)[4]. Asymptomatic viremia and CMV end-organ disorders that impact the gastrointestinal system, retina, oesophagus, liver, lungs, and brain are just a few of the different ways that CMV infection can present itself. CMV reactivation might indirectly affect the function of the transplanted graft in addition to directly harming organs, perhaps resulting in concomitant bacterial and/or fungal infections[5]. To detect CMV DNAemia, which indicates CMV DNA is found in the blood, the most sensitive test is CMV DNA quantitative PCR[6].

This study aims to assess the probability of cytomegalovirus (CMV) reactivation of among individuals who underwent hematopoietic stem cell transplantation (HSCT) and received either fully HLA-matched stem cells or stem cells from haploidentically matched donors. To the best of our current understanding, there has been no similar study conducted within our country.

2. METHODOLOGY

We enrolled a total of 60 consecutive patients who had an allogenic bone marrow transplant (BMT) at Armed Forces Bone Marrow Transplant Centre (AFBMTC) between March 2019 and October 2022. The patients were split into two groups for ease of comparison: Group A was made up of patients who had fully matched HLA donors, whereas Group B was made up of patients who had haploidentical donors. Individuals with a history of CMV infection or CMV illness were excluded from the trial, along with individuals of both sexes and of all age categories. The study protocol was reviewed and approved by the Institute's Ethical Review Board and Research Department, an informed consent was obtained from patients' or their legal guardians. CMV seropositivity was determined using enzyme-linked immunosorbent assay (ELISA)-detected CMV IgG antibodies, which denote past exposure to the virus. For the purposes of the study, a viral load of 1000 copies/mL or above in blood, cerebrospinal fluid, urine, stool, or bronchoalveolar lavage fluid was regarded as indicative of CMV DNAemia or infection. The threshold for commencing CMV treatment was 2000 copies/mL or higher, according to institutional standards.

The existence of end-organ involvement, including hepatitis, pneumonia, enteritis, retinitis, or encephalitis, along with general symptoms like fever, malaise, and declining blood counts, was suggestive of CMV disease and was confirmed by histologic or molecular confirmation of the virus. Overall survival (OS) refers to survival

at the time of the most recent follow-up, whereas disease-free survival (DFS) defined survival without relapse or rejection. The probabilities of OS and DFS were calculated using the Kaplan-Meier technique.

CMV DNA PCR analysis was carried out every week from day +14 to day +100 in order to monitor CMV reactivation during the 100-day post-transplantation period. Two millilitres of blood collected in EDTA tubes were subjected to the procedure using the SaMag viral nucleic acid extractor system (Sacace Biotechnologies, Como, Italy) in order to obtain the DNA. The lower limit of detection for the kit was 5 copies/mL for the serum and blood and 500 copies/mL for additional body fluids. The PCR analysis was performed on an Applied Biosystems 7500 real-time PCR apparatus (Thermo Fisher Scientific, Waltham, MA).

To identify the presence of CMV disease, patients with CMV infection received a thorough physical examination and biochemical evaluation. Those who had a CMV quantitative PCR viral load greater than 2000 copies/mL underwent preemptive treatment with Valganciclovir or ganciclovir. After two consecutively negative PCR results, antiviral therapy was stopped.

The information was entered into SPSS version 25 and analysed. A few categorical factors that were expressed as frequencies and percentages were sex, CMV reactivation, antiviral therapy, acute GVHD, steroid use, conditioning regimen, use of ATG, GVHD prophylaxis, ABO mismatch, and gender mismatch. For the purpose of determining statistical significance, both the Student t-test and the Chi-square test were used. In univariate analysis, which was used to assess the relevance of various variables, a p-value of less than 0.05 was utilised as the cutoff for statistical significance.

3. RESULTS

The age range from 0.6 to 53 years ago was a median of 15±10.6 years. The average time between diagnosis and HSCT was 30.5 months. Aplastic anaemia (35% of cases), beta thalassemia major (27%), and acute leukaemia (17%) were the most common reasons for HSCT in our study. The predominant source of stem cells was bone marrow harvest (BMH), which accounted for 82 percent of the total. Table 1 provides an overview of patient, donor, and transplantation information. The average time frame for neutrophil engraftment was observed to be 14 days, 49 (82%) patients were able to successfully engraft. Six patients (10% of the overall research population) did not successfully engraft neutrophils. Details on the patient and the transplant are provided in table 1.

Table 1: Transplant characteristics

Characteristics	n(%)
Age	
Upto 10 years	28 (47%)
10 – 20 years	14 (23%)
20-30 years	12 (20%)
30-40 years	6 (10%)
Mean ± St.d	15±10.6
Gender	
Male	38 (63.3%)
Female	22 (36.6%)
Donor Relationship	
Brother	26 (43.3%)
Sister	19 (31.6%)
Father	8 (13.3%)
Mother	7 (11.6%
Gender Mismatch	
Yes	34 (56.6%)
No	26 (43.3%)
Type of transplant	
Allo	30 (50%)
Haplo	30 (50%)
ABO Mismatch	
Major	14 (23.3%)
Minor	8 (13.3%)
No mismatch	38 (63.3%)
Type of conditioning	
MAC	21 (35%)
RIC	1 (1.6%)
NMA	7 (11.6%)
RIC with PTCy	19 (31.6%)
MAC with PTCy	12 (20%)
ATG	
Yes	50 (83.3%)
No	10 (16.6%)
GVHD Prophylaxis	

CSA+MMF,+PTCy	28 (46.6%)
CSA+MTX	20 (33.3%)
CSA	9 (15%)
CSA+MMF	1 (1.6%)
CSA+PTCy	2 (3.3%)

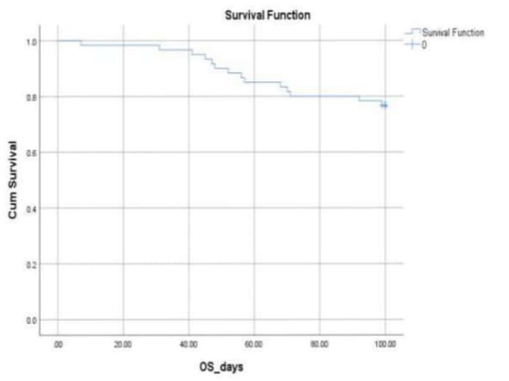
39 patients had CMV reactivation (65%). With a range of 13 to 66 days, the average duration for the occurrence of CMV DNAemia was found to be 19.25 days. The viral load ranged from 1300 to 192476 copies/mL, with 1995 copies being the median value. Only two (3.3%) of the 39 patients received ganciclovir, while 26 (43.3%) received valganciclovir. 11 patients (28.2%) were not given antiviral medication. Patients with aplastic anaemia had the highest rate of CMV infection (41%), followed by those with ALL (20.5%), AML (12.8%), and primary immunodeficiency (10.2%). A statistically significant connection between the underlying diagnosis and CMV infection was seen (p value = 0.020). With statistically significant (p value = 0.0000), CMV infection was higher in haplo identical transplant recipients (66.6%) than in completely matched recipients (33.3%). A statistically significant link exists between the use of steroids during conditioning and CMV reactivation. (P value= 0.019). Table 2 lists the correlations between CMV reactivation and several factors.

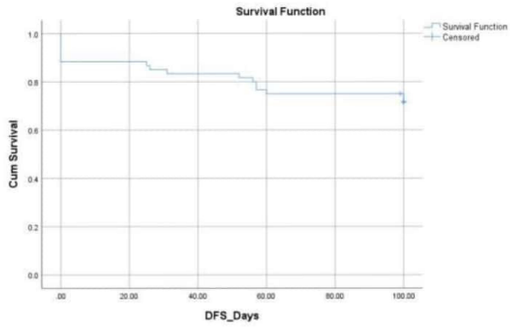
Table 2: CMV correlation with different factors

Characteristic	**CMV Reactiva (N= 39; 64%)**	**No CMV Reactiva (N= 21 ; 35%)**	***P*Valu**
Underlying Diagno			
AA	**16(41.0%)**	**5**(23.8%)	
BTM	**4(10.2%)**	**9(42.8%)**	
B-ALL	**8(20.5%)**	**2(9.5%)**	***0.02***
AML	**5(12.8%)**	**0**	
FA	**0**	**2(4.76%)**	
MDS	**1(2.56%)**	**1(9.5)**	
PID	**4(10.2%)**	**2(9.53)**	
HL	**1(2.56%)**	**0**	
Transplant Type			
Matched related	**13(33.3%)**	**17(81%)**	
Haploidentical	**26(66.6%)**	**4(19%)**	***0.000***
ATG			
Yes	**33(84.60)**	**17(81%)**	***0.717***
No	**6(7.6%)**	**4(19)**	
Conditioning Proto			
MAC	**9(23.0%)**	**8(38.0%)**	***0.000***
RIC	**0**	**5(23.8%)**	
NMA	**3(7.69%)**	**5(23.8%)**	
RIC with PTCy	**15(38.4%)**	**2(9.525)**	
MAC with PTCy	**12(30.7%)**	**1(4.76%)**	
			0.412
GVHD	**12 (30.7%)**	**8 (42.1%)**	
No GVHD	**27 (69.2%)**	**11 (57.8%)**	

Out of 60 patients,14 patients died yielding a mortality rate of 23.3%. Primary graft failure was the most frequent cause of mortality, resulting in neutropenic sepsis in 6 patients (42.85%), but 3 of these patients also had CMV reactivation. Secondary graft failure occurred in 4 patients (28.57%) after this, one of whom had CMV reactivation. Acute respiratory distress syndrome occurred in 2(14.28%) patients, while transplant-associated thrombotic microangiopathy (TA-TMA) struck one patient. One patient (7.14%) demise was secondary to CMV colitis.

The rate of overall survival (OS) was found to be 76.7%. The rate of disease-free survival (DFS) was estimated at 71.7%. Notably, neither OS nor DFS in our analysis showed a statistically significant connection with CMV infection.





4. DISCUSSION

Although there is a significant occurrence of CMV positive serology among both donors and recipients we observed a comparable incidence of CMV reactivation in our study population (65%) compared to the rates reported in other medical centers (ranging from 40% to 70%) by day 100 post-transplantation.[7-9.] The results of our study indicate that the transplantation of hematopoietic stem cells from haplo identical donor (HID) was a statistically significant and distinct factor in predicting the reactivation of cytomegalovirus (CMV) following transplantation(p=0.000). However, we did not find a significant correlation between CMV reactivation and disease relapse in our study. However, statistically significant associations were observed between CMV reactivation and the underlying disease, preparing regimen, and graft-versus-host disease (GVHD) prophylaxis utilizing post-transplant cyclophosphamide (PTCy). Importantly, CMV reactivation did not impact overall survival (OS) or disease-free survival (DFS) in our study group. The study revealed a notable prevalence of CMV reactivation (66.1%) was comparable to that reported in other studies (63.9%) involving HID-SCT.[9] Contrary to our findings, several studies have reported a higher incidence of CMV reactivation in (HID) transplantation compared to fully matched donor transplantation. These studies have documented CMV reactivation rates of 81% in HID transplantation, whereas fully matched donor transplantation had a significantly lower incidence (23%)[10.] In addition to the aforementioned findings, another study specifically focusing on acute leukemia patients individuals who underwent hematopoietic stem cell transplantation (HID) exhibited a greater occurrence of cytomegalovirus (CMV) reactivation. This study demonstrated an incidence rate of 85.7% for CMV reactivation in HID-SCT recipients with acute leukemia. The findings of this study provide additional evidence to substantiate the proposition that hematopoietic stem cell transplantation from a haploidentical donor entails a greater likelihood of cytomegalovirus reactivation in comparison to transplantation from a fully matched donor[11.]

A higher degree of immunosuppression might be the key factor for the increased CMV infection in HID-HSCT which can be seen in our study with statistically significant result(p=0.000) while using PTCy in conditioning regimen for GVHD prophylaxis. Another retrospective study has reported that incidence of CMV reactivation after HID-SCT was higher in patients using a both T cell-replete and T cell-deplete approach with ATG prior to transplantation than those using T cell-replete

approach with high-dose post transplantation cyclophosphamide for GVHD prophylaxis.[12] Another study has shown a dose dependent correlation of CMV reactivation with ATG[13]. The minimum dose of ATG in our study was 5mg/kg and maximum dose of 20mg/kg, however our study did not established a significant correlation of CMV reactivation with higher ATG dose (p=0.222). The functional recovery of CMV specific T cell immunity is a major contributing factor in controlling CMV infection after allo-HSCT[14].Use of ATG delays the immune reconstitution of T cells and thereby increases the risk of CMV reactivation after transplantation.[15] In our study we found significant correlation of CMV reactivation with underlying diagnosis (p=0.02) with highest incidence in aplastic anemia (41%). T cell and natural killer cell malfunction, as well as underlying immunological abnormalities, may be to blame for this [16] and increased alloreactivity as a result of acquired aplastic anemia's higher transfusion burden.

We found that lymphoid malignancies (20.5%) reactivated CMV at a higher rate than myeloid malignancies (12.8%) in patients with cancer. This result deviates from a prior epidemiologic analysis of CMV infection, which found no correlation between the categories of underlying illnesses and the frequency of CMV reactivation[17]. After looking into the relationship between gender and CMV reactivation and analyzing the literature on the subject, we discovered that there is no proven connection between patient gender and CMV reactivation in the context of HID-SCT. However, one retrospective cohort study carried out by Lin et al. revealed an interesting result. Their investigation found that the rate of CMV antigenemia was substantially higher in females undergoing allo-HSCT than in males (HR = 1.2, p = 0.006)[18]. After looking into the relationship between gender and CMV reactivation and reading the literature on the subject, we discovered that no conclusive correlation has been found. It is significant to stress that additional investigation is necessary to confirm this conclusion and examine the underlying mechanisms. Recent research from a review article has suggested that patients with CMV reactivation might have a higher risk of developing acute GVHD[19].

Nevertheless, contrary findings were seen in another trial, where CMV reactivation did not seem to significantly affect the future emergence of acute GVHD[16]. These contradictory results emphasize the need for more study to clarify the connection between CMV reactivation and the development of acute GVHD in transplantation settings. In our investigation, we discovered that there was no discernible difference in the incidence of later development of acute GVHD between individuals with and without CMV DNAemia (p=0.412). However, some research have suggested that CMV reactivation in allo-HSCT may increase the risk of leukemia relapse, while other studies have found no protective effect of CMV reactivation on post-transplantation relapse of hematologic illness[20].

5. CONCLUSION

The findings demonstrated a statistically significant association between haploidentical transplantation and CMV reactivation, with higher rates observed in the haploidentical group compared to fully matched recipients. Various factors, including underlying diagnosis, conditioning regimen, and GVHD prophylaxis, were also found to be correlated with CMV reactivation. However, while CMV reactivation was associated with specific factors, it did not impact overall survival or disease-free survival in the study population.

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