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Detection of Cytomegalovirus in Post Hematopoietic Stem Cells Transplant Recipients by Real-Time PCR

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ABSTRACT

Objective: To evaluate the frequency of Cytomegalovirus in Post Hematopoietic Stem Cell Transplant Recipients by molecular method.

Study Design: Cross-sectional study.

Place and Duration of Study: Virology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan Jan to Dec 2021.

Methodology: One hundred and twenty-six hematopoietic stem cell transplant (HSCT) recipients were included in this study and were separated into various age groups. All included patients were CMV IgG positive. Real-time polymerase chain reaction (PCR) was performed on post-HSCT recipients to detect CMV.

Results: Out of 126 post-HSCT recipients, CMV was detected in 80(63%) patients and was not found in 46(36%) patients. Among positive patients, 60(75%) were males, and 20(15%) were female. Maximum cases of CMV detection in post-HSCT were found in 23 patients aged 1-10 years.

Conclusion: A high frequency of CMV in post-HSCT recipients was observed in this study. CMV cause major complications in immunocompromised patients, such as in transplant recipients. Prevention of CMV infection is a main part of post-transplant monitoring and management.

Keywords: Cytomegalovirus, Hematopoietic stem cell transplant, Real-time polymerase chain reaction.

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INTRODUCTION

In adults, CMV infects up to 60-100% of individuals and is a foremost agent causing complications after transplantation, either due to primary infection or latent virus reactivation.[1] The majority of CMV reactivation occurs after a few days of hematopoietic stem cell transplant (HSCT) and may cause bone marrow graft failure.[2]

In HSCT, CMV reactivation or reinfection usually occurs within the first 100 days post-transplantation but may be reactivated throughout the entire immunosuppressive treatment.[3]

Risk factors for CMV infection include chronic neutropenia and transplant of a CMV-positive graft.[4] Primary CMV manifest as CMV mononucleosis-like syndrome that includes malaise, fever and mild liver function abnormalities. Generally, CMV infection is asymptomatic or can present only with non-specific symptoms.[5]

CMV causes latent infection, similar to other members of the Herpes virus family. The exact site of latency of CMV has yet to be discovered.6 Various studies show that granulocyte monocyte lineage is the site of latency and persistence. In immune-competent individuals, disease manifestation is rarely encountered, but CMV can exert its pathogenic potential in an immunocompromised state.[7]

According to a study, subclinical CMV viral reactivation is restricted with active immune surveillance and increased HCMV T-cells in healthy seropositive individuals.[8] On the contrary, reactivation from latency in immunocompromised and immunosuppressed individuals causes significant morbidity and mortality in HSCT recipients, AIDS patients and developing fetuses.[9]

CMV reactivations in HSCT recipients may lead to complications like pneumonia, gastrointestinal infections or central nervous system infections; these are major health problems and impact morbidity and mortality in these patients.[10] Early monitoring and treatment of CMV reactivation in HSCT can significantly improve overall outcomes.

Nucleic acid testing (NAT) by polymerase chain reaction is an extremely specific and sensitive modality for detecting CMV infection in HSCT recipients. Various other assays are also used to detect CMV-

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specific IgM antibodies, indicating recent infection with CMV, and CMV IgG shows past exposure with CMV.

This study is carried out to determine the frequency of CMV infection in Post-HSCT recipients in our community. It will help adopt better management plans and preventive measures necessary to minimize the risk of disease and its associated complications.

METHODOLOGY

The cross-sectional study was conducted at the Virology Department, Armed Forces Institute of Pathology, Rawalpindi Pakistan, from January to December 2021. Ethical approval was taken from the Institutional Review Board (BS AHS/VIR-4/READ-IRB/21/923). The WHO sample size calculator was used to determine a sample size with anticipated population proportion CMV infection of 40 %.[11]

Inclusion Criteria: Patients of either gender, aged 1-40 years with were hematopoietic stem cell transplant recipients were included in this study.

Exclusion Criteria: Patients with debilitating illness or pregnant ladies were excluded from the study

Blood samples of transplant recipients reported in AFIP were collected and transported to the Virology Department for CMV detection. CMV was detected by Real-Time PCR using a hybridization fluorescent detection technique.

Informed consent was taken from all patients, and demographic and clinical data was collected by consecutive non-probability technique on predesigned patient proforma. Sample extraction was carried out using TANBEAD nanotechnology along with internal control, and amplification was done using the Sacace Biotechnologies (CMV Real TM QNT) kit. As per kit literature, quantitative PCR levels or viral load are reported in copies per ml of plasma.

Data were analyzed using Statistical Package of Social Sciences (IBM-SPSS version 26). Qualitative variables were expressed as frequency and percentages.

RESULTS

Out of 126 patients, CMV was detected in 80(63%) patients and not in 46(36.5%), as shown in Table. CMV was considered positive in those patients, with PCR results greater than 200 copies/ml. Among 90 male patients, CMV was detected in 80 patients. Out of 36 females, CMV was found in 26 females. All patients were CMV IgG positive before HSCT. Most of the patients for HSCT were diagnosed with leukaemia in 64 patients, 41 had anaemia, and 21 had thalassemia. The detection day of CMV after Hematopoietic Stem Cell Transplant Recipient is shown in Figure-1

Table: Frequency of Cytomegalovirus detection in Post Hematopoietic Stem Cell Transplant (n=126)

Parameters	Frequency (%)
CMV Positive	80(63.5%)
CMV Negative	46(36.5%)

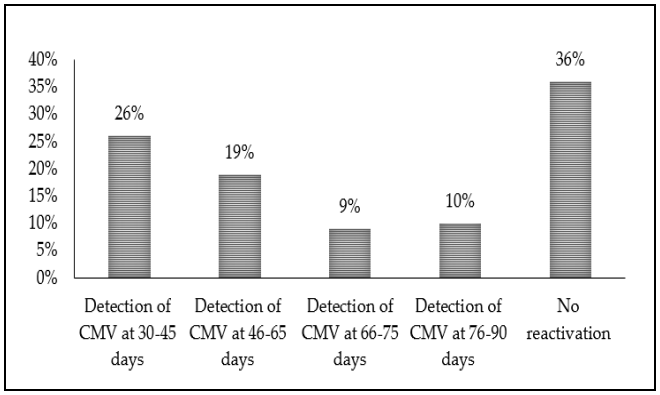

Figure-I: Detection day of CMV after Hematopoietic Stem Cell Transplant Recipient (n=126)

CMV was found more in younger ages, 1-10 years. Mainly, cytomegalovirus detection was found in HSCT recipients 45 during the initial days after HSCT, and the median duration was between 30-45 days post-HSCT. Patients were followed up for 100 days, and CMV PCR was carried out at 30-90 days of the post-transplant period (Figure-2)

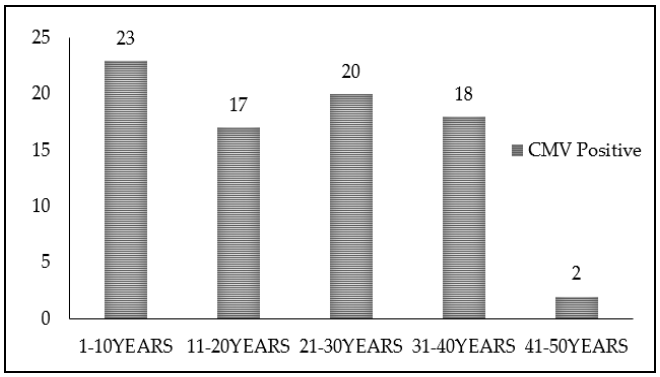

Figure-II: Age distribution of patients in which Cytomegalovirus was detected (n=126)

DISCUSSION

This study determines the frequency of infection caused by CMV in post-HSCT recipients. CMV infection is the main cause of mortality and morbidity in Post HSCT recipients. Patients are at high risk of having CMV infection during their early days of the post-transplant period.[11,12] Immediately after

engraftment, viral infections that modulate the immune system, such as Cytomegalovirus, cause disruption of the mucosal barrier and cellular immune impairment. The practice of Allogeneic HSCT has increased as graft-versus-host disease prophylaxis has been implemented to prevent the disease.[13]

In our study, 126 patients were included. All patients were CMV IgG positive before HSCT. Most of the patients for HSCT were diagnosed with leukaemia in 64 patients, 41 had anaemia, and 21 had thalassemia. Patients of all ages were included in the study. Real-time PCR detected CMV. CMV was considered positive in those patients, with PCR results greater than 200 copies/ml. Viral load of CMV >200 copies/ml was seen in 80 (63%) patients. Results show that most CMV was found positive at an average of 30-45 days.

Our findings are consistent with a study conducted at the Armed Forces Bone Marrow Transplant Centre in Rawalpindi Pakistan, from Jan 2017- Mar 2020. CMV IgG positive were assessed in the study from the first day of HSCT to day 100 of post-HSCT. Patients were observed twice weekly for CMV reactivation using PCR CMV DNA quantitative. Reactivation of CMV was found among 152(66 %) out of a total of 230 patients.[14] A study was conducted by Devasia *et al*. to observe the possibility of reactivation of CMV Post HSCT and its morbidity. CMV reactivation was investigated in 136 hematopoietic stem cell transplant individuals. The CMV was detected by PCR. CMV reactivation was found in 13(9.5%) out of 136 recipients. The median onset of CMV reactivation was 52.5 days post-transplantation.[15]

Our study results correlate with a study carried out by B. George *et al*. in Australia; CMV was found at 50 days in Post-HSCT among 123 patients (39.1%). CMV reactivation was observed 100 days in 11 patients (3.4%) post HSCT. Post HSCT CMV reactivation was detected at 48.9% in CMV IgG-positive patients, and the incidence of reactivation was 56.5% among CMV seronegative patients.[16]

Like our study, one study detected CMV reactivation by RT PCR in BMT recipients. Detection of CMV was carried out among BMT recipients; 415 blood samples were collected weekly from 43 patients up to day 100 post-transplantation. Viral reactivation was detected in 51% of recipients.[17] A comparative study was carried out for detecting CMV by real-time PCR in HSCT patients and its detection significance in immunocompromised patients**.** A total of 197 samples were collected from 60 BMT recipients and haematological malignancies. CMV PCR was performed for the detection of CMV reactivation. CMV was found in 56 samples by PCR studies.[18] The results of this study were different from our study.

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CONCLUSION

Detection of CMV in allogeneic bone marrow transplant recipients showed a higher frequency of 63%. Prevention and early diagnosis of CMV is an important component of post-transplant monitoring and management as CMV increases mortality and morbidity in HSCT patients.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SKN: & NG: Conception, study design, drafting the manuscript, approval of the final version to be published.

EG: & FA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MAR & NS: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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