Original Article

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DOI: 10.4103/ijh.ijh_5_24

# Detection of active human cytomegalovirus in patients with multiple myeloma

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**Abstract:**

**BACKGROUND:** Human cytomegalovirus (HCMV) infection is ubiquitous and successfully reactivated in patients with immune dysfunction as in patient with multiple myeloma (MM), causing a wide range of life-threatening diseases. Early detection of HCMV and significant advances in MM management has amended patient outcomes and prolonged survival rates.

**OBJECTIVES:** The aim of the study was to estimate the frequency of active HCMV in MM patients.

**MATERIALS AND METHODS:** This is a case–control study involved 50 MM patients attending Hematology Center, Baghdad Teaching Hospital; 25 of them were newly diagnosed and 25 on treatment compared to 50 of apparently healthy control. HCMV-viral load was measured using a real-time polymerase chain reaction (RT-PCR).

**RESULTS:** Active HCMV was detected in 8 patients out of 50 (16%); 6/25 (24%) in newly diagnosed and 2/25 (8%) on treatment and had autologous bone marrow transplant with mean ± standard deviation of $910 \times 10^{10} \pm 210 \times 10^{10}$, and $32{,}000 \times 10^{10} \pm 1500 \times 10^{10}$ IU/mL, respectively. HCMV viremia is equally detected in both remission and relapsed cases.

**CONCLUSION:** RT-PCR detected a significant number of MM patients infected by cytomegalovirus compared to healthy individuals. Further studies are needed to verify if this finding has a relation to etiology or disease progression.

**Keywords:**

Human cytomegalovirus, multiple myeloma, real-time polymerase chain reaction

## Introduction

Human cytomegalovirus (HCMV) is a DNA virus of *Herpesviridae* family. It is one of the most common pathogens in patients with immunocompromised diseases and is attributed to the high rates of morbidity and mortality, especially among hematopoietic diseases and posttransplantation. HCMV coincides throughout the immunosuppressive state of various diseases.[1] It spreads in about 60%–70% in industrial countries, while in developing countries, the rate reaches 100%. Following the acute viral infection, which commonly acquired in early life,[2] HCMV establishes latent phase for life, this occurs due to numerous virus evasion policies that interfere with the host's immune response at various levels.[3]

Multiple myeloma (MM) is a disorder of an immune system characterized by accelerating and irrecoverable hematological neoplasia of B-lymphocyte; it is caused by an unrestricted with a clonal expansion of bone marrow plasma cells. These cells bring about and release monoclonal immunoglobulin (Ig) or its parts; the M-protein.[4] The clinical signs and symptoms of the disease arise from the infiltration of abnormal plasma cells into the bones (that mostly affect pelvis, spine,

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**Submission:** 13-01-2024
**Revised:** 27-01-2024
**Accepted:** 29-01-2024
**Published:** 11-03-2024



**How to cite this article:** Al-Douri AA, Abdullah SF, Al-Mothaffar AM. Detection of active human cytomegalovirus in patients with multiple myeloma. Iraqi J Hematol 2024;13:101-5.

and ribs).[5] In addition, due to excessive production of nonfunctional of incomplete Ig (light chain) that can be excreted in excess by kidneys to urine during which nephropathy may occur. The disease is also manifested due to an immune suppression of normal humoral part of the immunity.[6-8]

Infections are a substantial source of morbidity and the leading cause of death in MM cases, as MM is associated with B-cell dysfunction, as well as abnormalities in T-lymphocytes, dendritic cells, and natural killer (NK) cells. Previous reports demonstrated that anti-MM therapies have an impact on the patient's immunity, leading to increased susceptibility to infections and expanding pathogens.[7,9-11] In addition, other studies have reported that the use of immune-modulators and protease inhibitors are associated with high risk for the appearance of symptoms of reactivated HCMV in MM cases.[12] Of note, a recent study displayed that cytomegalovirus (CMV) reactivation can occur in patients who have been intensively pretreated with daratumumab therapy. The clinical decision and management of adverse effects in medical settings are a concern, suggesting the importance of patient monitoring for the possibility of CMV reactivation.[7]

## Materials and Methods

A case–control study was conducted at the Department of Microbiology, College of Medicine, University of Baghdad. The study extended from September 2022 to December 2023. This study included 50 patients with MM who were currently attending Hematology Center in Baghdad Medical Hospital, between September 2022 and April 2023. The patients were classified into two equal subgroups. Twenty-five patients of newly diagnosed MM before receiving any treatment, and twenty-five patients who were on treatment with Bortezomib, Lenalidomide, and Dexamethasone or Bortezomib, Cyclophosphamide and Dexamethasone plus Acyclovir. All patients gave a history of recurrent blood transfusions; some patients from the second group underwent autologous stem cell transplantation (ASCT). All cases were diagnosed by proper investigation under the care of consultant hematologists. The diagnosis is made by measuring the amounts of different types of antibodies in blood and urine and confirmed by a bone marrow biopsy.

Fifty apparently healthy collected randomly from friends and patient's families. Their age and sex were matched for each of them. From each case involved in this study, 2 mL of blood was drawn aseptically by venipuncture using disposable syringes. The blood was placed in ethylenediaminetetraacetic acid tubes and kept at –20°C till used. The samples were collected after the agreement of each patient to be part of this study. All patients were subjected to a data form. It includes sociodemographic data such as age and sex. Complete medical history was taken from each patient.

Real-time polymerase chain reaction (RT-PCR) depends on the fluorescence detection generated by the reporter's particle, which upsurges as the reaction progresses. The reporter particle is a double-labeled DNA probe that is fixed exactly to the target area of viral DNA. The signal of fluorescence is increased as the dissolution of fluorescence dye and quencher as a result of exonuclease activity of the Taq DNA polymerase throughout DNA amplification.

DNA was extracted from each patients' plasma and the concentration values were measured using Quantus Fluorometer. DNA concentration was ranged from (1 to 78) ng/μL. The viral quantification method was performed according to the manufacturer's protocols using (RealBest DNA CMV, Russia, Cat.No: C-8881).

### Ethical consideration

Ethical approval for this research was obtained from the Ethical Committee of College of Medicine/University of Baghdad. All patients participating in this study were received a written information sheet and the aims of this study were explained for each of them.

### Statistical analysis

Data analysis was made using the SPSS software (Statistical Package for the Social Sciences, version 20, IBM Corp., Armonk, NY, USA). Results of two groups were related by means of Pearson's Chi-squared and *t*-tests. $P \leq 0.05$ indicates that the difference is statistically significant.

## Results

In this study, a total of fifty patients with MM were divided into two subgroups; newly diagnosed and patients on treatment. Their clinical data and histopathology reports were reviewed. Males represented 28 (56%) of patients and (22) 44% of patients were females. The male-to-female ratio was 1.3:1 with male preponderance. The age range was 30 to 78 years with a mean ± standard deviation (SD) of 61.6 ± 10.709 years. Age- and sex-matched with the control group. Data representing age and sex distribution of study groups are shown in Table 1.

Table 2 shows that 8 (16%) patients were CMV viral load positive with mean viral load ± SD $8.9 \times 10^{13} \pm 2.3 \times 10^{13}$ compared to negative HCMV detection in the control group.

Table 3 demonstrates that the viral load of HCMV in patients who were newly diagnosed was 6 of 25 (24%) with mean ± SD of $910 \times 10^{10} \pm 210 \times 10^{10}$ and in patients

**Table 1: Age and sex distribution of the study groups**

| Study groups (*n*) | Age/year, mean±SD | Age range/year | Sex | | Male: female ratio |
|---|---|---|---|---|---|
| | | | Male, *n* (%) | Female, *n* (%) | |
| Patients (50) | 61.6±10.709 | 30–78 | 28 (56) | 22 (44) | 1.3:1 |
| Controls (50) | 55.24±11.74 | 35–72 | 26 (52) | 24 (48) | 1.1:1 |

SD=Standard deviation

**Table 2: Human cytomegalovirus detection by the real-time polymerase chain reaction among the study groups**

| Study group | HCMV - viral load | | *P* |
|---|---|---|---|
| | Positive, *n* (%) | Negative, *n* (%) | |
| Patient (50) | 8 (16) | 42 (84) | 0.0058 |
| Control (50) | 0 | 50 (100) | |

HCMV=Human cytomegalovirus

**Table 3: Human cytomegalovirus - virus detection by the real-time polymerase chain reaction among patients**

| MM patients (*n*) | HCMV - viral load | | *P* |
|---|---|---|---|
| | Detectable, *n* (%) | Nondetectable, *n* (%) | |
| Newly diagnosed (25) | 6 (24) | 19 (76) | 0.14 |
| On treatment (25) | 2 (8) | 23 (92) | |

HCMV=Human cytomegalovirus, MM=Multiple myeloma

who were receiving treatment was 2 of 25 (8%) with mean ± SD of 32,000 × $10^{10}$ ± 1500 × $10^{10}$. *P* value was more than 0.05 and the differences were not statistically significant.

In RT-PCR only 2 of 7 (4%) bone marrow transplant (BMT) patients had a detectable HCMV viral load compared to 5 (10%) patients who did not undergo BMT, no statistical significance difference was observed with a *P* = 0.07 [Table 4].

HCMV viral load was detected equally in one patient out of 14 (4%) with a remission phase and one out of 11 (4%) patients in a relapsing phase. *P* value was more than 0.05, so the association between two groups was not significant [Table 5].

## Discussion

The threat of infection with HCMV infection is noticeably augmented in patients with hematological disorders, especially proceeds the transplantation of allogeneic or autologous stem cells with CD34þ-certain cells, as well as next to high dose of corticosteroid treatments, also as a result of lower levels and dysfunction of NK cells and CD8þ/CD4þ subset T-cells.[13,14] The susceptibility of MM cases to HCMV infection has been increased with a reduced production of polyclonal Igs,[15] failure of renal function[16] as well as growing immunocompromising effects of anti-MM treatment schedules.[9]

Previous studies have reported that the use of immune-modulatory agents and protease inhibitors in MM patients are associated with the symptoms of viral reactivation.[17] One of the leading causes of death has been found to be HCMV infection.[18,19] Augustson *et al*.[20] observed that nearly half of early deaths of 3000 newly diagnosed MM patients occurs in <6 months and has been shown to be related to infections. Beyond the innate immune disorder, some studies elucidated a fluctuating set of infections in MM, possibly related to the different phases of treatment[21,22] and the more innovative treatment approaches of recent years, such as proteasome inhibitors, immunomodulatory drugs, and ASCT.[23]

HCMV transmission may continue by organ transplants or blood transfusion and continues to pose a challenge in the treatment of immunodeficient patient with CMV-sero negativity, for example, after transplantation of stem cell or following blood transfusion to correct patients' anemia. Measures to diminish the hazard of HCMV were assessed in clinical settings, with leukocyte depletion from whole cellular blood products and/or selecting donors with negative HCMV-IgG because blood donors have been identified to have the highest rate of seroconversions of CMV annually, indicating the most consistent origins of CMV infection.[24] Studies in large groups of blood donor have indicated that donations from new donors with detected CMV-IgG carry the highest risk of transmitting CMV infections because they had highest copies of CMV-DNA viral load, and early antibodies cannot clear the infection as they are poorly neutralizing antibodies.[25] In accordance with this information, coherent approaches can be designed to lessen the outstanding threat of transfusion transmitted CMV using leuko-depleted blood products. Although there is no indication that HCMV is still transmitted by transfusing of leuko-depleted blood units,[26] early diagnosis of HCMV infection can improve the preemptive treatment and optimize outcome. The methodology for quantifying HCMV by RT-PCR is highly valuable in directly observing and distinguishing active viral replication. Although there are many proposed RT-PCR threshold standards, it is widely agreed that these values must be determined within centers where patients are monitored.[27]

In this study, the quantitative measurement of HCMV-DNA in patient's blood samples was recorded in 8 of 50 (16%) cases with high viral load; moreover,

**Table 4: Human cytomegalovirus viremia in association with bone marrow transplant patients**

| MM patients | Viral load | | *P* |
|---|---|---|---|
| | Detectable, *n* (%) | Nondetectable, *n* (%) | |
| Autologous BMT | | | |
| Yes | 2 (4) | 5 (10) | 0.0700 |
| No | 0 | 18 (36) | |

BMT=Bone marrow transplant, MM=Multiple myeloma

**Table 5: Human cytomegalovirus viremia in association with disease progression in myeloma patients**

| Phase of disease progression | PCR results | | *P* |
|---|---|---|---|
| | Detectable, *n* (%) | Nondetectable, *n* (%) | |
| Remission (14) | 1 (4) | 13 (52) | 1.00 |
| Relapse (11) | 1 (4) | 10 (40) | |
| Total (25) | 2 | 23 | |

PCR=Polymerase chain reaction

the viral load of HCMV in patients who were newly diagnosed more than those patients who were receiving treatment, indicating that the role administrating acyclovir with the usual treatment regimen have a role in controlling CMV disease. It was recently reported that preemptive administered to asymptomatic patients with CMV positive by screening tests. Some researchers have found that a prophylaxis with higher dose of acyclovir or valacyclovir significantly diminishes the rate of HCMV infection in allo-hematopoietic stem cell transplantation recipients.[28] Previous study conducted in Rome revealed a roughly same percentage of CMV reactivation of 12 of 78 patients (15%).[29] Furthermore, in China, a study reported that 20 out of 131 (33.5%) MM patients had a reactivated CMV infection.[30] In Canada, a total of 27/189 (14.3%) of patients had active CMV–DNA anemia.[31]

A recent NY study determined that 414 MM patients had been sent once for CMV-PCR, and 46 cases of CMV infection were identified in viremic state with viral load of more than 500 IU/mL, 46% of infected patients had symptoms; pneumonitis in one case, 15% of MM patients had CMV related end-organ disease, 43% of cases had progressive disease status, 73% of those received autologous stem cell transplant and 85% were scheduled with a corticosteroids in 30 days of CMV infection, 63% of patients had simultaneous infections within 30 days of viral infection, including infection of bloodstream, bacterial and viral infection of respiratory tracts, colitis, and mycosis, concluding that infection with HCMV in MM patients is associated with high rates of morbidity and mortality. Further studies are mandatory to assess the clinical relevance of CMV reactivation in these cases.[32] CMV was detected in 39% of patients treated with bortezomib in comparison to none of the CMV sero-negative patients. Indicating that the detectable CMV viremia can be developed during the first two cycles of treatment in 83% of MM patients.[33] In support of this study, it was found that RT-PCR test that carried out on whole blood samples are more sensitive than those on plasma or leukocyte.[34] Measurement of HCMV-DNA on whole blood was found to appear ahead and can be sensitive to detect a huge copy number of cytomegalo-viral loads.[35] On the other hand, the presence of HCMV-DNA in blood cells, especially in monocytes may reflect low-level or inactive viral replication but may also merely imitate that the CMV infection in the latent phase and certainly not an active CMV infection.[36]

## Conclusion

In conclusion, RT-PCR detected a significant number of MM patients actively infected by HCMV possibly from recurrent blood transfusion compared to healthy individuals. Further studies are needed to verify if this finding has a relation to etiology or disease progression.

### Acknowledgment

The authors are grateful to all MM patients who participated in this study.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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