

Prognosis of Human Cytomegalovirus in Cancer Patients Undergoing Chemotherapeutic Treatment in Egypt and an Emergent Prevalence of Glycoprotein B-5

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Abstract

The human cytomegalovirus (HCMV) is a global opportunistic β -herpes virus causing severe diseases in immune-compromised patients, such as malignant tumor patients, especially those undergoing chemotherapeutic treatment. This study aimed to determine the prevalence of HCMV-DNA in chemotherapeutic treatment naive cancer patients, and after chemotherapy, to compare between conventional nested PCR and ELISA techniques for the detection of HCMV, and to detect glycoprotein B genotypes. Plasma and serum samples before and after three chemotherapy cycles were collected from 49 chemotherapy-naive cancer patients. DNA was extracted from plasma samples using QIAamp[®] DNA Mini kit. HCMV-DNA was detected using a nested PCR technique. Multiplex nested PCR was used for HCMV-glycoprotein B (gB) genotyping. HCMV-IgG and -IgM were detected using ELISA technique. Thirty one (63.3 %) of the 49 plasma samples of the chemotherapy-naïve cancer patients were positive for HCMV-DNA; 21 of which remained positive after chemotherapy. However, 18 samples were negative of which 16 became positive after chemotherapy. gB-5 was the most common glycoprotein genotype detected (80.6 %), followed by gB-1, gB-3, gB-4, and gB-2. HCMV IgG was detected in the 49 serum samples of chemotherapy-naïve patients, and after exposure to chemotherapy. HCMV-DNA is commonly identified in cancer patients. Its detection after chemotherapy exposure may suggest HCMV reactivation. The most common genotype detected in cancer patients in Egypt is gB-5 in contrast to earlier research. IgG was detected in all patients. This indicates that HCMV is endemic in Egypt, necessitating the development of public awareness campaigns about HCMV infection and preventive strategies.

Keywords: Human Cytomegalovirus, Genotyping, Cancer, ELISA, Nested PCR

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INTRODUCTION

Human cytomegalovirus (HCMV) is a global β -herpes virus that is highly pervasive. It is also known as human herpes virus 5 and belongs to the *Herpesviridae* family. The genus cytomegalovirus consists of a genome of approximately 240 kb encoding 165 genes, which is thought to be the biggest herpes virus infecting humans.¹ HCMV is a well-known opportunistic pathogen that can cause severe diseases in immune-compromised patients. HCMV prevalence varies geographically and socioeconomically. Individuals from lower socioeconomic levels have a higher HCMV prevalence rate.² Most primary infections caused by HCMV are either asymptomatic or subclinical, which mainly occur in childhood. Cytomegalovirus then enters into a latent state in both monocytes and macrophages.^{3,4} HCMV abandons latency and reactivates when the immune system of patients is compromised. HCMV reactivation prevalence in severely ill patients is very high, maybe up to 71%.^{4,5} Immune-compromised individuals, such as AIDS patients, recipients of organ transplantation or blood transfusion, newborns, and neonates often contract severe diseases caused by HCMV.^{6,7} Individuals experiencing HCMV reactivation may experience generalized symptoms, such as malaise, fever, leukopenia, as well as HCMV-associated syndromes, such as hepatitis, meningitis, pneumonitis, encephalitis, enterocolitis, gastroenteritis, nephritis, or retinitis.⁸⁻¹⁰ It may also cause severe fatal systemic infections in immune-compromised patients. Malignant tumor patients, especially those undergoing chemotherapeutic treatment, are an important sector in immune-compromised individuals who are vulnerable to HCMV infection and reactivation.¹¹⁻¹⁴ It was demonstrated that HCMV has oncogenic transforming potential *in vitro*.^{15,16} HCMV diseases incidence and prognosis are frequently related to the immunosuppression level, host susceptibility factors, and the virulence of different HCMV strains.¹⁷ Genetic diversity is found among genes involved in tissue tropism and cell penetration or replication affecting HCMV strains virulence.¹⁸ HCMV pathogenicity varies according to the viral genetic variation within viral genes.¹⁹ Gene coding for the viral envelope glycoproteins express great genetic diversity

among HCMV genes, e.g., UL55 which encodes glycoprotein B (gB).²⁰ gB genotyping is composed of five genotypes, numbered 1–5.²¹ The main component of viral envelopes are glycoproteins. They are important for viral penetration into the cell and viral transmission from cell to another.²² A person can be infected by several HCMV strains during their lifetime.²³

People of all ages can acquire HCMV infection according to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO). It was reported in the United States that more than half the adults infected with HCMV aged 40 years, and nearly 33% of children infected with HCMV aged five years. The most common infectious cause of birth defects in the United States is HCMV. The CDC made June as “National CMV Awareness Month” to increase awareness of congenital cytomegalovirus. In Africa, HCMV is neglected due to a widespread belief that HCMV is endemic from childhood, there is less possibility of maternal reactivation or reinfection during pregnancy, and as a consequence, there is a lower incidence for severe congenital infection to occur compared to primary infection.²⁴⁻²⁶ Maternal reactivation or reinfection causes majority of congenital HCMV infections even in high-income populations.²⁷ In addition, African patients might not receive immunosuppressive therapy that may cause HCMV diseases, which is becoming increasingly obsolete as several advanced therapies for non-transmissible diseases become more widely available.^{28,29} Finally, diagnosis and treatment of HCMV-related diseases are largely ignored.³⁰ In literature, much of the recent research in Egypt addresses infections in those undergoing transplantation, AIDS/HIV, and pregnancy. However, there is limited data on HCMV DNAemia in cancer patients undergoing chemotherapeutic treatment.

Aim of the Study

The current study aimed to determine the prevalence of HCMV DNAemia in chemotherapeutic treatment naïve cancer patients. Moreover, it aimed to investigate the effect of chemotherapeutic treatment on HCMV DNAemia and to compare between conventional nested PCR and ELISA techniques

Table 1. Primer's sequences used in HCMV glycoprotein b genotyping by multiplex nested PCR

	Gene	primer sequence	product size (bp)
PCR for HCMV detection	UL55	UL55-F: 5'-TTTGGAGAAAACGCCGAC- 3'	751
		UL55-R: 5'- CGCGCGCAATCGGTTTGTGTA- 3'	
PCR for Glycoprotein B genotyping	gB1	gB1-F: 5'-ATGACCGCCACTTTCTTATC- 3'	420
		gB1-R: 5'- GTTGATCCACRCACCAGGC- 3'	
	gB2	gB2-F: 5'- TTCCGACTTTGGAAGACCCAACG- 3'	613
		gB2-R: 5'- GTTGATCCACRCACCAGGC- 3'	
	gB3	gB3-F: 5'- TAGCTCCGGTGTGAACTCC- 3'	190
		gB3-R: 5'- GTTGATCCACRCACCAGGC- 3'	
	gB4	gB4-F: 5'- ACCATTCTGTTCCGAAGCCGAGGAGTCA -3'	465
		gB4-R: 5'- GTTGATCCACRCACCAGGC- 3'	
	gB5	gB5-F: 5'- TACCTATCGCTGGAGAAC-3'	139
		gB5-R: 5'- GTTGATCCACRCACCAGGC- 3'	

for HCMV reactivation detection. In addition, it aimed to detect glycoprotein B genotypes and the prevalence of HCMV-IgG antibodies in these patients.

MATERIALS AND METHODS

Patients and Sample Collection

Plasma and serum samples were collected from 49 chemotherapy-naïve cancer patients before and after three chemotherapy cycles (each cycle = 21 days) from December 2018 to August 2019 at the Damanhur Oncology Center. Plasma and serum samples were kept in a -80°C freezer until the DNA was extracted for PCR processing and ELISA, respectively.

DNA Extraction and Nested PCR for HCMV Detection

The QIAamp® DNA Mini kit (QIAGEN, Hilden, Germany) was used according to the manufacturer's instructions to extract DNA from plasma samples and then were kept at -80°C until PCR processing. HCMV-DNA was identified using a nested PCR technique in both chemotherapy-naïve and post-three-cycle-chemotherapy samples.³¹ Two sets of primers were designed to target the fourth exon of the HCMV Immediate Early gene.^{31,32} The primer sequences were as follows: the external primers: 1a: 5'-GGTCACTAGTGACGCTTGATGATGA-3' and 1b: 5'-GATAGTTCGCGGGTACA GGGGACTCT-3'; the internal primers: 2a: 5'-AAGTGAGTT CTGTCCGGGTGCT-3' and 2b: 5'-GTGACACCAGAGAATCAGAGGA-3'. A thermal

cycler (BOECO, Hamburg, Germany) was used for PCR amplification. Briefly, the first step of PCR was performed in a 25 µL total volume comprising 1 µL of forward and reverse primers (10 pmol/µL), 3 µL of plasma DNA extract, 7.5 µL of free RNase water, and 12.5 mL of MyTaq™ HS Red Mix. The thermal cycling conditions were an initial denaturation at 95°C for 1 min, followed by 35 cycles [denaturation: 95°C for 15 s, annealing: 55°C for 15 s, and extension: 72°C for 10 s], and followed by final extension at 72°C for 10 min. The second step of PCR was similar to the first, but with the following differences: an initial denaturation step of 95°C for 3 min, 3 µL of the first step amplicon as a template, an annealing temperature of 53°C for 15 s 10 µL of PCR products were visualized via ultraviolet illumination (Analytik Jena, Germany) of 2% agarose gel (GeneDireX, USA) stained with ethidium bromide (Sigma-Aldrich, US).

Glycoprotein B Genotyping

HCMV DNAemia positive samples of chemotherapy-naïve cancer patients were subjected to the multiplex nested PCR (M-nPCR) assay for gB genotyping.³³ The M-nPCR for gB genotype used primers illustrated in (Table 1). The PCR technique was carried out as reported by Pignatelli et al. and Tarrago et al.^{34,35} A PCR master mix [One PCR, Master mix (GENEDIREX)] was used for the amplification. Concisely, the first PCR step was performed in a 25 µL total volume containing 1 µL of each forward and reverse primers (10 pmol/µL), 3 µL of the DNA extract, 7.5 µL of free RNase water and 12.5 µL of master mix. The thermal

cycling condition for the first PCR step was as follows: denaturation: 94°C for 4 min, followed by 35 cycles of [denaturation: 94°C for 30 s, annealing: 60°C for 1 min, extension: 72°C for 1 min], and final extension at 72°C for 10 min. The second PCR step was performed in 20 µL containing 10 µL of master mix, 5 µL of the first step amplicon as a template, 0.5 µL of the five forward primers, and 2.5 µL reverse primer (10 pmol/µL). The second step was performed at the same thermal cycling conditions. However, the annealing temperature was at 58°C. Ultraviolet illumination (Analytik Jena, Germany) of 2.5% agarose gel (GeneDireX, USA) stained with ethidium bromide (Sigma-Aldrich, US) were used to visualize PCR products.

Serology

Serum samples were collected from each chemotherapy-naïve patient and after three cycles of chemotherapy for serological analysis. Samples were subsequently kept at -80°C until ELISA processing. Prechek® (HCMV IgG EIA test kit, Prechek Inc, USA) and a Microtitre plate ELISA reader (BioTek Instruments, Inc, USA) were used to identify IgG antibodies against HCMV. Absorbance was measured at 450 nm. IgM antibodies to HCMV were identified in serum samples after chemotherapy treatment using a commercially

available HCMV-IgM ELISA kit (EIA KIT, Prechek, Inc, USA) according to the manufacturer's instructions, and Microtitre plate ELISA reader (BioTek Instruments, Inc, USA). Absorbance was measured at 450 nm.

Statistical Analysis

The IBM SPSS software program version 20.0 was used for all statistical analyses (IBM Corporation, Armonk, New York). A p-value below 0.05 was considered statistically significant.

RESULTS

Patients and Sample Collection

Plasma and serum samples were collected from 49 chemotherapy-naïve cancer patients aged 25–65 years old. A total of 44 patients were females and 5 were males having different types of cancers (Table 2).

Detection of HCMV DNAemia using Nested PCR Before and After Chemotherapy

A total of 31 (63.3 %) of the 49 plasma samples taken from the chemotherapy-naïve cancer patients involved in this research were positive for HCMV DNAemia by conventional nested PCR, and 18 (36.7%) were negative. The bands were detected at 293 bp, as anticipated (Figure 1). A total of 17 (55%) out of the 31 HCMV-DNA-positive patients, had breast cancer, 10 (32%) had NHL, 2 (6.5%) had colorectal cancer, and 2 (6.5%) had ovarian cancer.

Following three cycles of chemotherapy, samples were obtained from the same patients

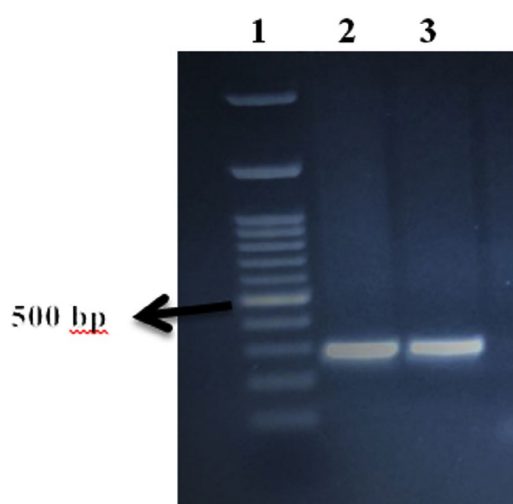


Figure 1. Detection of HCMV DNAemia using nested PCR. PCR products were visualized in 2% agarose gel with ethidium bromide staining. Lane 1: a 100 bp DNA ladder, Lanes 2 and 3: positive samples detected at 293 bp

Table 2. Characteristics of patients enrolled in this study

Demographic Data		No. (%)
Gender	Male	5(10.2%)
	Female	44(89.8)
Age (yrs)	25 – <45	16 (32.7%)
	45 – <65	29(59.2%)
	≥65	4(8.2%)
Cancer types	Breast	27(55.1%)
	NHL	13(26.5%)
	Ovary	4(8.2%)
	Colorectal	4(8.2%)
	Uterus	1(2.0%)

for HCMV DNAemia detection. A total of 10 samples out of the 31 positive HCMV DNAemia were negative (became latent), 21 samples remained positive after chemotherapy exposure. On the other hand, out of the 18 negative HCMV DNAemia chemotherapy-naïve, 16 samples were positive (reactivation or reinfection), and two remained negative after chemotherapy exposure. Therefore, 16/49 (32.7%) represented reactivation or reinfection, and 10/49 (20.4%) entered into latency stage (Figure 2).

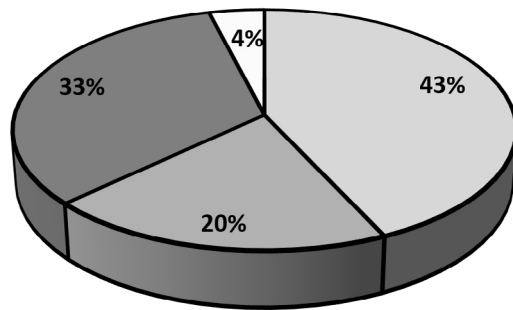
Glycoprotein B (gB) Genotyping

Multiplex nested PCR was used to detect gB genotypes in the 31 HCMV positive

samples collected from the chemotherapy-naïve cancer patients. Various HCMV genotypes were detected as follows: gB-5 was the most common glycoprotein genotype accounting for 25/31 (80.6%), followed by gB-1: 7/31 (22.6%), gB-3 6/31 (19.4%), gB-4 2/31 (6.5%), and gB-2 1/31 (3.2%) (Figure 3). Out of the 31 samples, seven (22.5%) samples harbored a mixture of two or three genotypes. All specimens of mixed genotypes surprisingly belonged to breast cancer patients (Table 3).

ELISA

Remarkably, all the 49 (100%) chemotherapy-naïve patients' serum samples



- positive before and after chemotherapy
- positive before chemotherapy and negative after it
- negative before chemotherapy and positive after it
- negative before and after chemotherapy

Figure 2. Detection of HCMV using nested PCR before and after chemotherapy

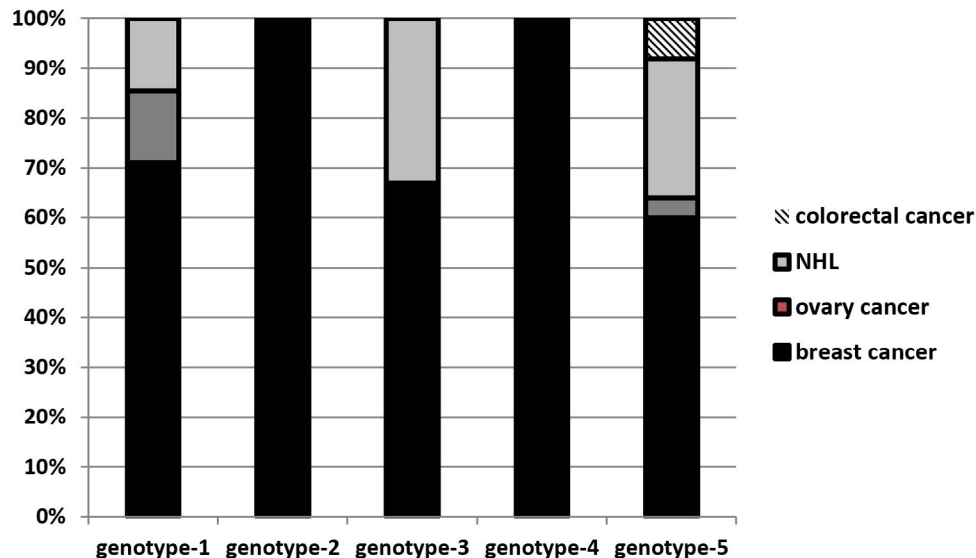


Figure 3. Distribution of HCMV glycoprotein B genotypes among the 31 PCR positive chemotherapy naïve patients with different cancer types

were positive for HCMV-IgG antibodies using the ELISA technique. All samples results also came out positive for HCMV-IgG antibodies after exposure to chemotherapy. HCMV-IgM was also detected in three (6.1%) of the 49 samples after exposure to chemotherapy. Unfortunately, there was no significant relationship ($X^2 = 0.135$, $p = 1.000$) between the results of IgM detection by ELISA and HCMV detection by PCR (Table 4).

DISCUSSION

HCMV is a prominent human infection that affects the vast majority of the world's population. Examples of instances where HCMV reactivation is intentionally sought include pregnancy, hematopoietic stem cell and solid organ transplantation, HIV infection, and recipients on immunosuppressive medications.^{35,36} Immunosuppression-induced HCMV reactivation results in a severe clinical presentation. HCMV has been shown to have oncogenic transforming potential *in vitro*.^{16,37} In addition, HCMV has been studied as an oncomodulatory virus and is an important factor in prognosis and survival in immune compromised patients. Preventive/prophylactic methods have resulted in a considerable decrease in HCMV-related mortality and morbidity. It was reported that chemotherapy causes immunosuppression in cancer patients.³⁸ Unfortunately, very few studies regarding HCMV infections and related morbidities due to reactivation were reported in the literature. There is no documented report regarding HCMV reactivation among cancer patients undergoing chemotherapeutic treatment in Egypt. This study aimed to determine the incidence HCMV

reactivation after chemotherapy, glycoprotein (gB) genotypes among cancer patients, and serological prevalence of HCMV antibodies.

In this study, we collected 49 samples from chemotherapy-naïve cancer patients and after chemotherapy exposure in Egypt. Our results revealed that HCMV DNAemia was detected in 31/49 (63.3%) chemotherapy-naïve patients using conventional nested PCR. On the other hand, El Shazly et al. reported that 20% of their chemoradiotherapy naïve breast cancer patients had HCMV-DNA in their blood samples.³⁶ In 2020, Lv et al. reported that 35.66% of the gastrointestinal cancer patients and 9.74% of the control individuals were HCMV-DNA positive. Therefore, they supposed that HCMV infection is related with an increased risk of gastrointestinal risk.³⁷ In addition, Handous et al. reported that 19.35% of patients with lymphoma revealed presence of HCMV-DNA with a significant higher frequency than the control group.³⁸ Torres et al. reported that HCMV-DNA was significantly associated with non-Hodgkin's lymphoma especially at active and late-stage lymphoma in another study on lymphoma patients.³⁹ Several studies had detected HCMV in malignant glioma, glioblastoma, colon cancer, salivary gland cancer, and prostate carcinoma.⁴⁰⁻⁴⁴ In contrast, El-Shinawi et al. were unable to detect HCMV-DNA in the peripheral blood of their 77 breast cancer patients. However, they detected it in the breast cancer tissue of 62.3% tested females with a higher percent in inflammatory breast cancer revealing HCMV contribution in cancer pathogenesis and prognosis.²⁹ A possible explanation for the oncogenic potential of HCMV is that the virus has the power to manage apoptosis and evade immune surveillance, providing an advantage of

Table 3. Distribution of mixed glycoprotein B genotypes among 7 breast cancer chemotherapy naïve patients

Isolate No.	Glycoprotein B genotypes
1	gB-2, gB-4, gB-5
10	gB-1, gB-5
14	gB-4, gB-5
15	gB-1, gB-5
19	gB-1, gB-3, gB-5
31	gB-1, gB-3
40	gB-1, gB-3, gB-5

Table 4. Comparison between IgM detection by ELISA and HCMV detection by PCR after chemotherapy exposure

		ELISA		Total
		ELISA negative	ELISA positive	
PCR	PCR negative	11	1	12
	PCR positive	35	2	37
Total		46	3	49

survival to the infected cells. Viral proteins were identified in several cells, such as smooth muscle cells, inflammatory cells, tumor cells, epithelial cells, and blood vessel walls.⁴⁵ HCMV can infect different cell types and enter the latency phase in myeloid progenitor cells, specifically CD34+ cells.⁴⁶⁻⁴⁷

HCMV DNAemia and HCMV-IgM seroprevalence among cancer patients after exposure to chemotherapy in Egypt in this study were detected using both PCR and ELISA, respectively. Conventional nested PCR analysis revealed that 37/49 (75.5%) cancer patients were HCMV DNAemia positive after chemotherapy exposure. In terms of prognosis, 16/49 (32.7%) of cancer patients turned from negative HCMV DNAemia before chemotherapy exposure to positive after exposure which may be due to either reactivation or reinfection. On the other hand, 10/49 (20.4%) cancer patients turned from HCMV DNAemia positive before chemotherapy exposure to negative after exposure which may be due to the viral latency. In addition, 21/49 (42.9%) of cancer patients were positive for HCMV DNAemia before and after chemotherapy exposure, and 2/49 (4.1%) cancer patients were negative for HCMV DNAemia before and after chemotherapy exposure. HCMV-DNA presence in plasma indicates human cell disturbance due to viral replication. Besides, it is thought that the presence of DNA in serum is closely linked to symptomatic infection.^{48,49} Similar to our results, HCMV reactivation upon chemotherapy exposure was reported in a previous pilot study on 15 cancer patients.⁵⁰ HCMV reactivation was observed in all their patients except for one throughout the chemotherapy treatment course with viral load peaking during the third treatment session. In addition, Schlick et al. reported that HCMV reactivation was observed in 12% of participants in another retrospective analysis of hematological and oncological patients.⁵¹ Besides, 41% of adult patients with solid tumors showed positive HCMV-DNA after chemotherapy exposure.⁵² It was reported that HCMV reactivation was not only present in terminally advanced cancer patients, but with immune-competent ones as well making it necessary to suspect HCMV with pyrexia of unknown origin.^{39,53-56} HCMV reactivation could not be detected in any of the 93 cancer patients with

mild to moderate level of immunosuppression after chemotherapy exposure, which is contrary to our results.⁵⁷ Schlick et al. reported the case study of a patient with fever, pancreatic adenocarcinoma, and metastasis to the liver, lungs, and peritoneal cavity. He succumbed with sepsis after chemotherapy, and HCMV was the only pathogen detected in post-mortem blood cultures.⁵¹ Previous findings showed that cancer patients are at an increased HCMV reactivation risk and its associated mortality.^{14,58}

In this study, HCMV-specific IgM was detected in 3/49 (6.1%) of cancer patients after chemotherapy exposure. Notably, all these positive HCMV-IgM patients were NHL patients. Similarly, Salman et al. reported that 8.45% of the breast cancer patients under their study were HCMV-IgM positive.⁵⁹ In addition, El Shazly reported that none of their breast cancer patients were HCMV-IgM positive.³⁶ Moreover, IgM was not detected in any of the patients in a study of 130 patients with benign and malignant breast tumors.⁶⁰ In addition, Rådestad et al. reported that 12% of ovarian cancer patients were HCMV-IgM positive in comparison to 3% of benign tumor patients.⁶¹ About 76.6% of the HCMV-IgM positive patients had breast cancer with invasive ductal carcinoma, which is in contrast to our findings.⁶²

In our study, one of the three positive HCMV-IgM samples (33.3%) was considered false positive as they were HCMV-IgM positive and HCMV-DNA negative. The difficulty in detecting HCMV-DNA in IgM positive patients could be related to the persistence of IgM antibodies for a long time after primary infection.^{63,64} HCMV-specific IgM can be detected for an average of 6 to 9 months after the initial infection has resolved.⁶⁵ The presence of HCMV-IgM in serum samples may be due to reinfection or reactivation, and not restricted to primary infection. Therefore, a follow-up test as molecular tests should be performed to ascertain the time of HCMV infection when IgM is detected.⁶⁶ In contrast, conventional nested PCR revealed positive HCMV DNAemia in 35/46 of the negative HCMV-IgM patients, indicating that IgM tests may be negative or under-detectable in immune-compromised patients who are actively infected⁶⁷ or re-infected and peaks within few weeks after infection.⁶⁸ Therefore, serological techniques alone are not reliable in detecting

active cytomegalovirus infection. Molecular approaches should be performed for accurate diagnosis.⁶⁹

Previous research in literature revealed that HCMV strains differ genetically, which greatly affects disease pathogenesis and progression. Furthermore, the HCMV potential to infect numerous organs and cells have been linked to diversity in gene sequence between strains.^{23,70,71} Certain areas exhibit higher rates of mutation, although the genomes of different HCMV strains are 95% identical.^{72,73} One of the most well-studied polymorphic gene is UL55, encoding the viral gB which is required for viral entry, cell fusion, and the key motive for neutralization.^{73,74} Our results showed that the genotype gb-5 is the most predominant in contrast to previous literature; 80.6% of HCMV-DNA positive patients in this study. In contrast to our findings, Mohamed et al. reported the prevalent distribution of gB-1 in their breast cancer patients.⁷³ In addition, Nogueira et al. also reported the prevalence of gB-1 in kidney transplant recipients, and solid organ transplant recipients with accelerated development of invasive diseases in Brazil.⁷⁵ On the other hand, the prevalence of gB2 among AIDS patients was reported by other studies in Canada, Brazil, United States, and Austria.^{21,76-79} Moreover, Dieamant et al. reported that hematopoietic stem cell transplantation patients with gB-3 genotype had higher morbidity and mortality than patients with the gB-1, gB-2, and gB-4 genotypes.⁸⁰ The existence of HCMV-gB-3 aggressive genotype in IBC patients may alter the disease's poor prognosis and morbidity.⁷³ The aggressive activity of HCMV-gB-3 could be attributed to its unique biological pathways involved in host-virus interactions.⁸¹

Mixed HCMV infection with more than one genotype of HCMV-gB was detected in seven samples of HCMV-DNA positive chemotherapy-naïve cancer patients in this present study. Remarkably, all detected mixed genotypes were breast cancer patients. In fact, it was reported that mixed infections of different HCMV genotypes were detected in both healthy and immune-compromised individuals, including pulmonary transplanted patients,⁸² AIDS patients,^{73,74} and inflammatory breast cancer patients.⁷³ The mixture of strains was related with higher morbidity and mortality in solid organ transplant patients,^{83,84}

higher disease progression in inflammatory breast cancer.⁷³, fetal death during pregnancy,⁸⁵ higher viral load, postponed viral eradication, and a higher rate of viral recurrence following treatment in both transplant recipients and AIDS patients.⁸⁶ The increased pathogenesis of HCMV caused by mixed infection of different strains may cause the secretion of chemokines, cytokines, and growth factors as a result of distinct viral strain replication.⁸⁷ In this study, out of 31 samples, 7 (22.5%) had mixed genotypes with the following distribution: 2 samples had (gB-1+gB-5), 2 had (gB-1+gB-3+gB-5), 1 had (gB-1+gB-3), 1 had (gB-4+gB-5), and 1 had (gB-2+gB-4+gB-5). Notably, six of these seven samples with mixed genotypes contained the gB-5. Wu et al. reported that a mixture of genotypes gB-1 and gB-3 was more commonly detected in hematopoietic stem cell transplant patients, and gB-3 was linked to the incidence of pneumonia.⁸⁸

Surprisingly, serological detection of IgG antibodies for HCMV in chemotherapy-naïve cancer patients' samples in this study revealed 100% HCMV-IgG prevalence. All patients except one were HCMV-IgG positive after exposure to chemotherapy. Similarly, both Mohammed et al. and el Shazly et al. reported 100% HCMV-IgG prevalence among invasive ductal carcinoma patients.^{36,62} There were significant changes when they compared ELISA readings of IgG optical densities between breast cancer patients and healthy women ($p = 0.05$). Similarly,³² Fagundes et al. reported that the HCMV antibody titer was considered to be an important objective indicator of fatigue in their study.⁸⁹ HCMV-IgG antibodies were detected in 49 (70%) of their 70 serology samples in another study reported by Richardson et al.; however, HCMV-DNA was not detected in any of the tumor samples by QPCR.⁹⁰ Furthermore, IgG levels were raised to 81.69% in breast cancer patients, compared to only 40% of the control group in another study reported by Salman et al.⁵⁹ Radestad et al. reported that a higher HCMV-IgG level was linked to a higher disease stage as HCMV-IgG level was greater in ovarian cancer patients ($p = 0.002$) or benign cystadenoma patients than control groups ($P.0001$).⁶¹

In contrast, Pandey et al. demonstrated that HCMV-IgG level was much greater in cancer-free people than in breast cancer patients,

supporting the idea that HCMV host immunity may play a role in keeping people cancer-free.⁹¹ Moreover, 96.6 % of the subjects tested positive for HCMV-IgG in a study of voluntary blood donors from the Alexandria Regional Blood Transfusion Centre.⁹² In addition, in a study of 130 patients that had breast swelling and either malignant or benign surgical excision, Surendran & Chisthi reported that HCMV-IgG antibodies were detected in all investigated patients. Therefore, they inferred that there was no correlation between HCMV-IgG seropositivity and breast cancer.⁶⁰ Moreover, HCMV-IgG antibodies were detected in 67/71 (94.36 %) Iraqi breast cancer patients, and in 19/20 (95%) of the control group in the immunological phase of a study assessing HCMV seroprevalence. On the other hand, in a study of 37 patients with colon adenocarcinoma, Avni et al. reported that an increased HCMV-IgG antibody titer only in patients treated with chemotherapy. This was often due to secondary infections due to the chemotherapy immunosuppression and is not associated with the colorectal cancer. Numerous detection methods, such as immunohistochemistry and DNA hybridization revealed that HCMV was not discovered at a significantly greater incidence in cancer tissue than normal tissue in subsequent studies.⁹³

CONCLUSION

HCMV-DNA is commonly identified in cancer patients. Its detection after chemotherapy exposure may suggest HCMV reactivation or reinfection. The most common genotype detected in cancer patients in Egypt is gB-5 in contrast to earlier research, indicating that the prevalence of this genotype requires additional investigations. IgG was detected in all patients indicating that HCMV is endemic in Egypt, necessitating the development of public awareness campaigns about HCMV infection and preventive strategies. HCMV-specific IgM was detected in 6.1% of patients after chemotherapy. Therefore, we recommend HCMV screening for cancer patients suffering from a fever of unknown etiology. Larger investigations are needed to determine the risk factors for HCMV infection. The rising number of older people getting chemotherapy, along with the fact that HCMV prevalence rises with age, predicts

that HCMV response and disease will become more common in the future.

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AUTHORS' CONTRIBUTION

ISS, RA, KB, AAS and SMA contributed to the study concept and design. ISS and KB prepared material, collected data and performed analysis. ISS drafted the manuscript. All authors read and approved the final manuscript for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Research Ethics Committee of the Faculty of Pharmacy, Damanhur University, Egypt with approval reference number 109PM12.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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