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Association between increased incidence of human cytomegalovirus (HCMV) DNAemia and acute myeloid leukemia among adult patients

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Background
Acute myeloid leukemia (AML) is a severe hematological malignancy and constitutes the common type of leukemia in adults, and FMS-like tyrosine kinase 3 (FLT3) mutations represent a prognostic marker in AML. Furthermore, human cytomegalovirus (HCMV) is a globally spread opportunistic pathogen that has been implicated in different malignancies.

Objective
This study aimed to describe the incidence of HCMV DNAemia in AML and to investigate the associations between HCMV DNAemia and AML risk, demographic parameters, and the FLT3 internal tandem duplication (ITD) mutation in adult AML patients.

Materials and methods
This study involved a total of 104 subjects (52 healthy control subjects and 52 adult AML patients). Baseline hematological measurements were recorded for all subjects. All of adult AML patients underwent FLT3-ITD mutation evaluation. By application of real time PCR, HCMV-DNA level was determined in all subjects.

Results and conclusion
The chi-square result revealed a significant increase in the incidence of HCMV DNAemia in adult AML patients 28/52 (53.846%) compared to controls 3/52 (5.769%) (P < 0.001). Moreover, the logistic regression analysis referred to an association between the increased incidence of HCMV-DNAemia and AML risk (Odd's ratio 19.055, 95% confidence intervals: 5.2615 to 69.0129, and P < 0.001). Also, a significantly higher incidence of HCMV DNAemia was observed in adult AML male patients and those ≥ 45 years old compared to females and those < 45 years old (P < 0.001 and 0.006, respectively). Additionally, the chi-square result revealed no significant change in the incidence of HCMV DNAemia among adult AML patient groups based on AML subtypes (P 0.086). Moreover, HCMV DNAemia was not found in adult AML patients with positive FLT3-ITD mutation compared to those with negative FLT3-ITD mutation (P 0.016). Furthermore, the logistic regression results revealed the absence of an association between the incidence of HCMV DNAemia and either AML subtypes or the incidence of FLT3-ITD mutation in adult AML patients. It is concluded that the increased incidence of HCMV DNAemia is well established in adult AML patients association with AML risk. Moreover, the increased incidence of HCMV DNAemia is associated with males and older age, but not with AML different subtypes and FLT3-ITD mutation in the studied cohort of adult AML patients.

Keywords: Acute myeloid leukemia, human cytomegalovirus, DNAemia, FLT3-ITD mutation, prognostic marker

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Introduction

Uncontrolled bone marrow stem cell proliferation is a hallmark of acute myeloid leukemia (AML), a type of bone marrow malignancy [1,2]. AML originates in the bone marrow and is considered a clonal disease that is genetically heterogeneous [3,4]. The disorder is characterized by abnormal proliferation and halted differentiation of primitive cells in the bone marrow, impairing normal hematopoietic function which results in potentially fatal cytopenia and transfusion dependence [4,5]. Although AML can affect individuals of any age, it is most prevalent in adults, with incidence rates

increasing with age [4,6]. Due to the aging tendency, the occurabce of AML is predicted to rise yearly [4,7]. AML is the sixth most common cause of cancer-related mortality in men and makes up 25% of all leukemia types [8,9]. AML has the greatest fatality rate among all types of leukemia. Un 2020, It was anticipated that there would be 19,940 new cases of AML diagnosed in the United States, with 11,180 deaths. [2,10−12]. In Egypt, Leukemia constitutes 10% of all cancers with AML accounting for 16.9% of cases [9,13]. Children have a much better prognosis than adults, and survival rates for AML patients significantly decreases with age at diagnosis [9,14]. AML is associated wiyh a wide range of molecular and cytogenetic abnormalities, such as alterations in chromosomal number and structure, mutations, and the development of fusion genes [4,15−17]. Diagnosing AML requires extensive multilevel testing, including cytogenetic, cytomorphological, and molecular genetic evaluation, to define its genetic heterogeneity for determining the exact therapy [4,18]. AML patients may have mutations in many genes, such as the FMS-like tyrosine kinase 3 (FLT3) mutation, which typically foretell a worse prognosis and necessitate the inclusion of appropriate targeted therapies or a more aggressive treatment regimen during upcoming therapy [4,19−21].

Of newly diagnosed AML cases, between 25 and 30% may have a FLT3 mutation [3,22−24]. In general, the extracellular region of FLT3 is made up of immunoglobulin-like domains, which include the juxtamembrane domain and the tyrosine kinase domain (TKD) separated by the kinase insert domain, whereas the intracellular region contains the C-terminal. Normal hematopoietic progenitor and stem cells can express FLT3, while bone marrow stroma cells express their ligand in soluble or membrane-bound form [25−27]. Activated FLT3 promotes various signaling pathways intracellularly, resulting in hematopoietic cell proliferation, differentiation, and survival [27,28]. In fact, most cells of acute leukemia can express FLT3. Moreover, the stimulation of FLT3 ligands increases proliferation and decreases apoptosis [27,29].

FLT3 gene mutations may appear by internal tandem duplication (ITD) at or near the receptor's juxtamembrane region, or by the point mutation affecting the TKD that results in the substitution of a single amino acid inside the activation loop [19,24,30]. The incidence of FLT3-ITD and FLT3-TKD in AML is roughly 20 and 10%, respectively [27,31,32]. In AML patients, having a FLT3-ITD mutation is associated with a generally poor prognosis [19,24,30]. Indeed, human cytomegalovirus (HCMV) may interfere with the action of tyrosine kinases. HCMV triggers cellular tyrosine kinase signaling and activation, leading to increased glioma cell invasiveness [33]. Likewise, FLT3, a kind of tyrosine kinase, may be supposed to be affected by HCMV infection.

It is known that HCMV infection promotes the growth, development and progression of various malignancies [34]. HCMV belongs to the Herpesviridae family with a double-stranded DNA genome of 236 kbp in size [35,36]. Most organs and tissues of the human body can be infected by HCMV [36]. HCMV is an opportunistic virus that causes significant morbidity and mortality in immunocompromised populations, particularly organ transplants, AIDS, and those with malignant diseases. Infection with HCMV is prevalent worldwide. Approximately, the virus infects 70–90% of the world's population. After primary infection, HCMV can remain latent for the host's lifespan, then reactivate to produce severe illness under certain conditions [34,37,38]. The oncogenic potential of HCMV was demonstrated in 1973 when the virus was observed to transform the fibroblast cells of a hamster embryo. These results indicated that HCMV was included in the category of viruses that may cause malignant transformation [34,39]. Despite the incidence of HCMV DNAemia being identified in different kinds of cancers [40], the reports regarding the incidence of HCMV DNAemia and its association with AML risk in adult patients are limited. The current study was designed to demonstrate the incidence of HCMV DNAemia in AML and to investigate the associations between HCMV DNAemia and AML risk, demographic parameters, and the FLT3-ITD mutation as a prognostic marker in adult AML patients.

Materials and methods

Study population

The study involved a total of 104 Egyptian participants, who were divided into two groups: 52 patients with acute myeloid leukemia (AML) and 52 control subjects.. The diagnosis of AML was performed at the National Cancer Institute. Prior to blood sampling, written informed consent was taken from each subject. All procedures in the study followed the World Medical Association's Declaration of Helsinki guidelines, declared in 1975 and revised in 2008 for studies involving human subjects, as well as the guidelines of the National Cancer Institute ethics committee, approval number CP2302-503-043. According to the French-American-British (FAB) classification, AML patients were classified into subtypes that included M1, M2, M4, and M5. Control subjects and patients were evaluated by routine clinical hematological parameters, including total leucocyte count (TLC), hemoglobin (HB), hematocrit [packed cell volume

(PCV)], red blood cell (RBC) count and RBC indices [mean-corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)], and finally platelet count (PLC). Adult AML patients were evaluated by FLT3-ITD mutation. All subjects were investigated for the presence of HCMV DNAemia.

Inclusion criteria

All subjects in the study cohort were seropositive for HCMV IgG antibodies. We selected positive cases of HCMV DNAemia to represent HCMV reactivation after the diagnosis of (AML) in adult patients of both sexes. The group of patients included those who were AML-naïve, while the control group consisted of healthy adults of both sexes with no history of malignant disease.

Exclusion criteria

Immunocompromised subjects and AML patients under treatment were excluded. Furthermore, subjects with serological confirmation of viral diseases, including hepatitis B, hepatitis C, and others with human immunodeficiency virus infection, were also not included. Finally, children were excluded from the sample recruitment.

Deoxyribonucleic acid (DNA**) extraction from whole blood**

From each subject, 10 ml of whole blood was collected in EDTA vacutainer tubes, and the plasma was separated. Using a genomic DNA extraction kit (Qiagen, Milan, Italy), the genomic DNA was extracted following the manufacturer's instructions. A Thermo Scientific NanoDrop™ Spectrophotometer was used to measure the purity of genomic DNA samples by ultraviolet absorbance at 260 nm. At -20 °C, the DNA was kept.

Determination of FMS-like tyrosine kinase 3 - internal tandem duplication (FLT3-ITD) mutation

FLT3-ITD mutation was detected by nested polymerase chain reaction (PCR) amplification procedure using the Techne Thermal Cycler (Cole-Parmer, Germany). Briefly, the master mix was prepared by the addition of 200 mM dNTPs (Qiagen, Santa Clarita, CA) to 0.5 μM of each of the forward and reverse primers for FLT3-ITD mutation (Thermo Fisher Scientific, USA), in addition to 10 mM Tris-HCl, 50 mM KCl, and 1.5 mM $MgCl_2$. 1U of Taq DNA polymerase enzyme (Qiagen, Santa Clarita, CA) was added, followed by 1μl of DNA sample, and completed with distilled water to a final volume of 25μl. The amplification thermal profile started by denaturation at 94°C for 150 sec, then 35 cycles at 94°C for 30 sec, and 57°C for 60 sec, followed by 72°C for 120 sec, then 72°C for 10 min as a final elongation. The gel electrophoresis was performed to analyze the product of the PCR reaction on 3% agarose gel. A wilde type allele was observed at a fragment of 328 base pair. A sample of additional elevated molecular weight was considered positive for the FLT3-ITD mutation. The two primers used for the PCR reaction were forward: 5' −GCAATTTAGG TATGAAAGCCAGC−3' and reverse: 5' −CTTTCAG CATTTTGACG GCAACC−3' [41].

Determination of human cytomegalovirus deoxyribonucleic acid (HCMV-DNA)

HCMV-DNA was detected by real-time PCR using the Artus HCMV PCR assay, as instructed by the manufacturer (Qiagen, Santa Clarita, CA). The amplification reactions were conducted under the following conditions: Initial incubation 10 min at 95°C, then 45 cycles at 95°C for 15 sec and 55°C for 1 min. The real-time Rotor Gene PCR was used for amplification procedures (Qiagen, Santa Clarita, CA). Using amplification standards, the positive results of HCMV-DNA were considered at >150 copies/mL [42].

Statistical analysis

The data was collected, organized, transferred to a Microsoft Excel sheet, and statistically analyzed using statistical computer package (SPSS) software version 25 (IBM SPSS Statistics, version 25; IBM Corp., Armonk, NY, USA). The mean and standard deviation (SD) of the quantitative values were reported, and then an independent *t* test was used for data analysis. The qualitative data was expressed as numbers and percentages, and then a chi-square test was used for data analysis. A logistic regression test was used to analyze the data, determine the association of one dichotomous dependent variable with independent variables, and determine an outcome. A significant threshold of $P \leq 0.05$ was considered.

Results and discussions

AML is a widespread hematological malignancy marked by rapid disease progression [4,43,44]. It poses challenges for public health worldwide, causing continuous debates over its global impact [4,45−47]. The exact mechanisms underlying AML development are not fully understood [1,2]. On the other hand, in vitro studies have demonstrated that HCMV has oncogenic transforming potential [35,40,48], and it has been studied as an oncomodulatory virus that constitutes an important factor in disease prognosis. Unfortunately, there are limited recorded reports on the occurrence of HCMV DNAemia and viral reactivation in cancer patients in general [40] and leukemia patients in particular. The data regarding the incidence of HCMV

DNAemia in AML and its relationship with the disease risk is rare. Therefore, the aim of the current study is to describe the incidence of HCMV DNAemia in AML and to investigate the associations of HCMV DNAemia with AML risk and FLT3-ITD mutation among adult AML patients who were naïve treatment.

Demographic data and hematological clinical features in the studied cohort

The demographic and baseline hematological clinical features of the study cohort, including group 1 (healthy controls, n = 52) and group 2 (adult AML patients, n = 52), were listed in Table 1. A significant increase in age (P < 0.001), HCMV-DNA titre (P 0.007), TLC (P < 0.001), MCV (P 0.001), and MCH (P 0.009) was observed in adult AML patients compared with controls. However, a significant decrease in HB, PCV, RBCs, and PLC was noted among adult AML patients compared with controls (P < 0.001).

Incidence of human cytomegalovirus (HCMV) DNAemia in healthy controls and adult acute myeloid leukemia (AML) patients

The incidence data of HCMV DNAemia (HCMV-DNA) in the entire study cohort (adult AML patients [n = 52] and controls [n = 52]) were listed and compared in Table 2. HCMV-DNA was detected in 28/52 (53.846%) of adult AML patients compared with 3/52(5.769%) of controls. The chi-square result revealed a significant increase in the positivity of HCMV-DNA in adult AML patients compared with controls (p < 0.001). Furthermore, the logistic regression result referred to an association between the increased incidence of HCMV DNAemia and AML risk among adult patients (Odd's ratio 19.055, 95% C.I. 5.2615 to 69.0129, and P < 0.001).

In positive cases of HCMV DNAemia, the quantitative values of HCMV-DNA in mean and standard deviation were recorded as 183,333.33 ± 28,867.513 copy/ml among 3/52 (5.769%) of controls and 814,285.714 ± 30,6671.842 copy/ml among 28/52 (53.846%) of adult AML patients, a significant increase in HCMV-DNA titre was noted among adult AML patients compared with controls (P 0.007). (Table 1).

Table 1 Demographic information and hematological clinical features of the study population

Parameter	Study population						*P*-value
	Group 1 (Healthy controls, n = 52)			Group 1 (Adult AML patients, n = 52)			
Sex (M/F)	36(69.23%)/16(30.77%)			32(64.54%)/20(38.46%)			0.410
Age (years)	30.385	±	7.867	44.077	±	13.671	< 0.001*
HCMV-DNA (copy/ml)	183,333.33	±	28,867.513	814,285.714	±	30,6671.842	0.007*
TLC (10^3/cmm)	7.776	±	1.954	112.614	±	95.990	< 0.001*
HB (g/dL)	14.308	±	1.290	7.650	±	1.598	< 0.001*
PCV (%)	45.561	±	2.921	23.050	±	4.461	< 0.001*
RBCs (10^6/Cmm)	5.296	±	0.718	2.695	±	0.737	< 0.001*
MCV (fl)	81.246	±	8.081	87.950	±	11.157	0.001*
MCH (pg)	27.030	±	2.814	29.037	±	3.803	0.009*
MCHC (g/dL)	33.600	±	0..963	33.087	±	2.690	0.104
PLC (10^3/cmm)	259.846	±	31.565	91.787	±	73.528	< 0.001*

Where: M: male; F: female; TLC: total leucocyte count; HB: hemoglobin; PCV: packed cell volume (hematocrit); RBCs: red blood cells; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLC: platelets count; *: significant value. Normal ranges were as follows: HCMV-DNA detection limit >150 copy/ml (the data represents HCMV-DNA positivity in only 3/52 in group 1 of healthy controls and 28/52 in group 2 of adult AML patients); TLC: from 4 to 11, HB: from 14.00 to 17.5 for males and from 12.5 to 14 for females, PCV: from 41 to 52; RBCs: from 4.5 to 6; MCV: 80 to 100; MCH: 27 to 33; MCHC: 31 to 37; PLC: from 150,000 to 450,000; age ranged from 20 to 41 years in healthy controls and from 29 to 58 years in adult AML patients. The chi-square test analyzed sex data. Except sex, t-test was used to analyze all data, which were expressed as means and standard deviation values (M ± SD).

Table 2 Chi-square and logistic regression analyses of the incidence of human cytomegalovirus (HCMV) DNAemia in healthy subjects and adult acute myeloid leukemia (AML) patients

HCMV DNA	Study population				Chi-square		Logistic regression		
	Group 1 (Healthy controls, n = 52)		Group 2 (Adult AML patients, n = 52)						
	n	*%*	*n*	*%*	X^2	*P*-value	Odd's ratio	95% C.I.	*P*-value
Positive	3	5.769	28	53.846	28.722	< 0.001*	19.055	5.2615 to 69.0129	< 0.001*
Negative	49	94.231	24	46.154					
Total	52	100	52	100					

Where: C.I.: confidence intervals; *: significant value.

Regarding the incidence of HCMV DNAemia among adult AML patients, our findings were in agreement with those of Chanswangphuwana *et al*., who reported the incidence of HCMV as a reactivated form among 54.1% of the cohort of 85 AML patients from Thailand [49]. However, HCMV reactivation among AML patients in the previously mentioned study was detected after HLA-matched myeloablative allogeneic stem cell transplantation, which is different from our study. On the other hand, our findings were differed from those of Yuasa *et al.*, who reported the incidence of HCMV as a reactivated form of infection among 10.7% (13/121) of AML patients. Their study was conducted on a cohort of 195 patients with acute leukemia; from them, 121 patients were diagnosed with AML. HCMV was detected among 10% of the entire cohort of patients. However, HCMV reactivation was detected among AML patients who received induction chemotherapy [50]. HCMV reactivation in the previously mentioned study may be attributed to receiving chemotherapy, while our studied AML patients were naïve treatment. Furthermore, our findings regarding the incidence of HCMV DNAemia among controls were consistent with Lv *et al.*, who reported HCMV DNA in 9.74% of the control individuals during their study of the incidence of HCMV DNA among patients with gastrointestinal cancer [51]. Furthermore, the incidence of HCMV was studied in types of leukemia other than AML. Abbas *et al*. reported HCMV reactivation among three cases out of 72 of a cohort of acute lymphoblastic leukemia (ALL) patients. Although HCMV reactivation was reported in a limited number of patients, their study found that all of the three patients with reactivated HCMV had an aggressive clinical course, with two having poor tolerance to chemotherapy [52]. Another study found that individuals with chronic lymphocytic leukemia (CLL) accumulate more HCMV-specific T lymphocytes than age-matched controls in response to persistent HCMV infection [53].

Indeed, the relationship between HCMV and cancer represents definitely a contentious issue [15]. The incidence of HCMV DNAemia was observed in different types of cancers among naïve treatment cancer patients. Shamsia *et al*. reported HCMV DNAemia in 63.3% (31/49) of chemotherapy-naïve cancer patients [40], which is similar to the finding in current study regarding the high incidence of HCMV DNAemia in AML patients. El Shazly *et al*. revealed that 20% of blood samples of the cohort of chemoradiotherapy-naïve treatment patients with breast cancer were positive for having HCMV-DNA [54], which is dissimilar to the finding in our study regarding the higher incidence of HCMV DNAemia in AML patients. Furthermore, El-Shinawi *et al.* did not detect HCMV-DNA from the peripheral blood of a cohort consisting of 77 patients suffering from breast cancer. However, 62.3% of examined females were found to have HCMV-DNA in their breast cancer tissue, with a larger percentage in inflammatory breast cancer, indicating the role of HCMV in cancer etiology and illness prognosis [55].

The results of the current study demonstrated the association between the incidence of HCMV DNAemia and AML risk. Our findings were consistent with those of other studies that revealed an association between the incidence of HCMV DNAemia and other types of cancer. Lv *et al.* reported the incidence of HCMV-DNA in 35.66% of patients with gastrointestinal cancer compared to 9.74% in controls. As a result, they suggested that HCMV infection could increase the risk of gastrointestinal cancer [51]. Likewise, Handous *et al*. revealed that HCMV-DNA was detected in 19.35% of lymphoma patients, with a significantly higher incidence than that of controls [56]. Additionally, Torres *et al*. found an association between HCMV-DNA and non-Hodgkin's lymphoma, particularly in active and late-stage lymphoma [57]. Also, numerous studies have detected HCMV in cancers of the salivary gland, colon, colorectal, and prostate, in addition to malignant glioma and glioblastoma [40,58−61].

A reasonable explanation for HCMV-related oncogenic potential is that the virus can control apoptosis and possesses the property of evading immune surveillance, giving infected cells an edge in survival. Viral proteins could be observed in numerous cells, including inflammatory cells, and tumor cells [40,62]. The direct participation of HCMV in cell transformation and the identification of viral genes that facilitate this transformation could characterize HCMV as an oncovirus [36]. The fact that HCMV can infect several types of cells and enter a latency phase in cells of myeloid progenitor, particularly CD34+ cells [40,63,64], and then reactivate later may represent an increased risk for HCMV oncogenic potential regarding patients with leukemia. The oncogenic potential of HCMV is associated with the presence of a number of oncogenes. Previous studies revealed that HCMV genome could encode several oncogenes, including US28, IE1, IE2, and UL76 [15,58,65], suggesting that HCMV may play an essential

role in cancer development and progression [15]. In tumor cells, it is thought that there are numerous crucial other genes that enhance HCMV major pathways associated with malignancy [15,66]. Therefore, numerous cellular functions that are believed to be involved in tumor development could be targeted by HCMV gene products. Those functions included dysregulation of the cell cycle and immortalization of the cells. Additionally, the mutation of the viral genome could increase immune evasion and cell survival with tumor expansion [36,67−69]. Upon HCMV infection of monocytes, increased production of proinflammatory cytokines could promote the development of cancer [36,70]. Moreover, HCMV could change the surrounding environment of tumor cells, thereby enhancing tumor formation and progression [36].

Incidence of human cytomegalovirus (HCMV) DNAemia in relation to sex and age among adult acute myeloid leukemia (AML) patients

The data on the incidence of HCMV DNAemia in adult AML patients regarding sex was tabulated in Table 3. The chi-square result referred to a significant increase in the incidence of positive HCMV DNAemia among the group of adult AML male patients, 24/32 (75%), compared to the other group of female patients, 4/20 (20%) (P < 0.001). Additionally, the data on the incidence of HCMV DNAemia in adult AML patients regarding age are listed in Table 4. The chi-square result referred to a significant increase in the incidence of positive HCMV DNAemia among the group of adult AML patients with increased age ≥ 45 years old, 20/28 (71.43%), compared to the other group of adult AML patients with decreased age < 45 years old, 8/24 (33.33%) (P 0.006).

Table 3 Chi-square analysis of the incidence of human cytomegalovirus (HCMV) DNAemia in relation to sex in adult acute myeloid leukemia (AML) patients

HCMV DNA	Adult AML patients, n = 52				Chi-square	
	Group 1 (Male patients, n = 32)		Group 2 (Female patients, n = 20)			
	n	%	n	%	X^2	P-value
Positive	24	75	4	20	14.981	< 0.001*
Negative	8	25	16	80		
Total	32	100	20	100		

Where: *: significant value.

Table 4 Chi-square analysis of the incidence of human cytomegalovirus (HCMV) DNAemia in relation to age in adult acute myeloid leukemia (AML) patients

HCMV DNA	Adult AML patients, n = 52				Chi-square	
	Group 1 (≥ 45 years, n = 28)		Group 2 (< 45 years, n = 24)			
	n	%	n	%	X^2	P-value
Positive	20	71.43	8	33.33	7.546	0.006*
Negative	8	28.57	16	66.67		
Total	28	100	24	100		

Where: *: significant value.

Regarding the incidence of HCMV DNAemia in relation to sex among adult AML patients, our finding that demonstrated the increased incidence of HCMV DNAemia in male patients compared with females may be explained to some extent by the effect of sex hormones. Indeed, estrogen as a female sex hormone was found to have a general suppressive effect on HCMV replication [71], whereas testosterone as a male sex hormone was found to have a little effect on HCMV replication *in vitro* [72]. Furthermore, regarding the incidence of HCMV DNAemia in relation to age among adult AML patients, our finding that demonstrated the increased incidence of HCMV DNAemia in adult AML patients with older age (≥ 45 years) may be explained by increasing age, as immune function declines and becomes less effective, which is a phenomenon known as immunosenescence. Large-scale changes in both the innate and adaptive immune system enhance susceptibility to infections, leading to the increased morbidity and mortality [73−75]. Many of these changes are exacerbated by HCMV [73,76,77]. Chronic HCMV infection further impairs immune function and is associated with increased mortality in the elderly [73]. Additionally, AML can lead to the excessive production of white blood cells, resulting in a bone marrow accumulation of young white blood cells,

which in turn causes a decrease in healthy blood cells and inhibits their function [78]. Consequently, a significant drop in immune system functions may be exerted, leading to an increase in the occurrence of HCMV DNAemia over time among adult AML patients.

Incidence of human cytomegalovirus (HCMV) DNAemia in different subtypes of acute myeloid leukemia (AML) among adult patients

The data on the incidence of HCMV DNAemia among adult AML patients regarding different AML subtypes are reported in Table 5. According to AML subtypes, adult AML patients were divided into two groups: group 1 referred to adult AML patients of non-M4 and M5 subtypes, including M1 and M2 (n = 28), and group 2 represented AML patients with M4 and M5 subtypes (n = 24). The chi-square analysis revealed a non-significant change in the incidence of positive HCMV DNAemia between the group of adult AML patients with M1 and M2 subtypes12/28 (42.86%), compared with the group of those with M4 and M5 subtypes 16/24 (66.66%) (P 0.086). However, a tendency toward the increased incidence of HCMV DNAemia was observed among AML patients with M4 and M5 subtypes (group 2). Moreover, the logistic regression analysis revealed no association between the incidence of HCMV DNAemia and the group of patients that included M4 and M5 subtypes of AML.

Table 5 Chi-square and logistic regression analyses of the incidence of human cytomegalovirus (HCMV) DNAemia in groups of acute myeloid leukemia (AML) subtypes among adult patients

HCMV DNA	Adult AML patients, n = 52				Chi-square		Logistic regression		
	Group 1 (M1 and M2, n = 28)		Group 2 (M4 and M5, n = 24)						
	n	%	n	%	X^2	P-value	Odd's ratio	95% C.I.	P-value
Positive	12	42.86	16	66.66	2.948	0.086	2.667	0.8600 to 8.2684	0.089
Negative	16	57.14	8	33.34					
Total	28	100	24	100					

Where: C.I.: confidence intervals.

Generally, the role of HCMV infection in the leukemic cell phenotype is unknown. However, HCMV normally interacts with the host immune system, allowing the virus to remain in a latent condition [79]. Indeed, AML-M4 subtype represents acute myelomonocytic leukemia, and AML-M5 represents acute monocytic leukemia, whereas AML-M1 refers to acute myeloblastic leukemia with minimal maturation, and M2 refers to acute myeloblastic leukemia with maturation [80,81]. Considerably, our findings revealed no association between the incidence of HCMV DNAemia and AML different subtypes.

Relationship between incidence of human cytomegalovirus (HCMV) DNAemia and FMS-like tyrosine kinase 3 -internal tandem duplication (FLT3-ITD) mutation in adult acute myeloid leukemia (AML) patients

The data on the incidence of HCMV DNAemia in adult AML patients regarding the occurrence of FLT3-ITD mutation are listed in Table 6. FLT3-ITD mutation was detected in 6/52 (11.538%) of adult AML patients. HCMV-DNAemia was not found in the group of adult AML patients with positive FLT3-ITD mutation 0/6 (0%), compared to the other group of those with negative FLT3-ITD mutation 24/46 (51.174%), with a chi-square significant result (P 0.016). Furthermore, the logistic regression analysis demonstrated no association between the incidence of HCMV DNAemia and positive FLT3-ITD mutation in adult AML patients.

Table 6 Chi-square and logistic regression analyses of the incidence of human cytomegalovirus (HCMV) DNAemia among adult acute myeloid leukemia (AML) patients in relation to the occurrence of FMS-like tyrosine kinase 3 - internal tandem duplication (FLT3-ITD) mutation

HCMV DNA	Adult AML patients, n = 52				Chi-square		Logistic regression		
	Group 1 (Negative FLT3-ITD mutation, n = 46)		Group 2 (Positive FLT3-ITD mutation, n = 6)						
	n	%	n	%	X^2	P-value	Odd's ratio	95% C.I.	P-value
Positive	24	51.174	0	0	5.814	0.016*	0.071	0.0038 to 1.3268	0.076
Negative	22	47.826	6	100					

Total	46	100	6	100					

Where: C.I.: confidence intervals; *: significant value.

Indeed, FLT3 is a type III receptor tyrosine kinase that plays a crucial role in hematopoietic cell survival, cell proliferation, and cell differentiation. The most clinically crucial finding is that the FLT3 gene mutation is a common genetic change and is considered a prognostic marker that is associated with poor prognosis in AML patients [27] and serves as a promising molecular target for the treatment of AML [27,82,83]. Our finding referred to the occurrence of the positive FLT3-ITD mutation in 11.538% of the studied cohort of adult AML patients. However, the previous reports revealed that the incidence of FLT3-ITD mutation among AML patients was supposed to be about 20% [27,31,32]. This difference may be attributed to the differences in the cohort characteristics of the studied adult AML subjects.

The limitations of our study included the unavailability of measuring HCMV serologically for the incidence of the corresponding immunoglobulin IgM antibodies side by side with HCMV DNAemia, the relatively small sample size that hindered us from balancing the groups of AML subtypes, and the unavailability for evaluation of other prognostic markers regarding adult AML patients.

Conclusion

The increased incidence of HCMV DNAemia is well established in adult AML patients associated with AML risk. Moreover, the increased incidence of HCMV DNAemia is associated with males and older age, but not with AML different subtypes and FLT3-ITD mutation in the studied cohort of adult AML patients. Future studies are recommended to reveal the associations of HCMV DNAemia with several prognostic markers of AML patients, including FLT3 mutation, with a larger cohort of adult AML patients and in relation to different AML subtypes.

Abbreviations

AML: Acute Myeloid Leukemia
HCMV: Human Cytomegalovirus
FLT3: FMS-like Tyrosine kinase 3
ITD: Internal Tandem Duplication

Author contributions

A.K.: Conceptualization, methodology, data collection and analysis, data interpretations, writing the manuscript, and revision of important intellectual content. R.S.A. and E.M. R.: Patient recruitment, and participation in methodology, data collection and analysis. R.D.: Conceptualization, data interpretations, and revision of important intellectual content. All authors approved the manuscript.

Conflicts of interest

The authors declare there are no conflicts of interest.

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