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# Pathogenicity role of human herpesvirus-8 in patients with acute myeloid leukemia

**Zahraa Ali Abdullah, Luma Amer Yasir[1], Ruqaya Munther J. Ewadh[2], Shakir H. Mohammed Al. Alwany**

**Abstract:**

**BACKGROUND:** Certain hematologic cancers, including Kaposi's sarcoma (KS), have been associated with the pathogenicity of human herpesvirus-8 (HHV-8). HHV-8's involvement in acute leukemia patients is yet unclear, nevertheless. The diagnosis, categorization, and course of treatment for acute myelogenous leukemia, an aggressive heterogeneous hematologic malignancy, have changed dramatically in recent years.

**OBJECTIVE:** This study aims to investigate the pathogenicity role of HHV-8 in patients with acute myeloid leukemia (AML) of a group of the Iraqi population.

**MATERIALS AND METHODS:** Case–control research has been carried out on 75 fresh blood samples recruited from the Mirjan Teaching Hospital in Al-Hilla City. The studied blood samples were obtained from patients with AML enrolled in this study, whereas control groups in the current study included 75 fresh whole blood. The specimens were collected during the period from June 2023 to February 2024. Conventional polymerase chain reaction (PCR) was used to identify HHV-8.

**RESULTS:** The results show that the mean age of the patients with AML (48.5 ± 10.23 years) was more than that of the apparently healthy control (46.26 ± 11.21 years). There was a nonsignificant difference between patients with AML and the control group. In addition, the male in this study group constituted 56% (42/75), whereas 44% (33/75) were female. Furthermore, the positive of viral genome extraction was found in 41.3% (31 out of 75 of the specimens with viral genome), whereas 59.7% (44/75) specimens did not contain viral genome. The PCR results showed that in the AML patient group, the rate of HHV-8 infection was 35.4% (11 out of 31 cases).

**CONCLUSION:** Considering the relatively small numbers included in our results, the positive results lead to the idea that HHV-8 works as a cofactor in the tumor biology of the AML subset under consideration and may have contributed to its development.

**Keywords:**

Acute myeloid leukemia, human herpesvirus-8, polymerase chain reaction

*Department of Biology, College of Science, University of Babylon, [1]Department of Medical Microbiology, College of Medicine, University of Al-Mustansiriyah, [2]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Iraq*

**Address for correspondence:**
Dr. Shakir H. Mohammed Al. Alwany, College of Science, University of Babylon, Hillah, Iraq.
E-mail: 3taa2.5alil@gmail.com

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## Introduction

Acute myeloid leukemia (AML) is characterized by aberrant growth of clonal neoplastic myeloid hematopoietic progenitor cells and a decreased ability to produce normal hematopoiesis. T-cells, the most important part of the immune system, are reported to be both functionally and quantitatively inadequate in AML patients, and data indicate that these individuals have immune system impairment. It has been reported how these anomalies affect regulatory T-cells (Tregs), which are in charge of preventing T helper (Th) cell growth and activity.[1] Peripheral blood and bone marrow from AML patients show abnormally high numbers of Tregs in comparison to healthy donors.[2] The responsible agent for Kaposi's sarcoma (KS) is identified as KS-associated herpesvirus (KSHV), also known as human



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herpesvirus-8 (HHV-8).[3] Numerous cancerous disorders, including multicentric Castleman's disease, pleural effusion lymphoma, and lymphoproliferative illnesses, have been linked to HHV-8.[4] As contrasted to other HHVs, HHV-8 only resides in a small portion of the human population; its seroprevalence varies by region and subpopulation.[5]

HHV-8 latency is enabled through its complex immune system manipulation. The incidence of HHV-8 infection and subsequent occurrence of KS varies between 0.6% and 5.3%. The clinical manifestations of KS encompass both mucocutaneous and visceral symptoms. The occurrence of disseminated disease accompanied by visceral involvement is considered atypical and linked to an adverse outcome.[6]

HHV-8 mostly spreads by the exchange of saliva between individuals. However, transmission can also occur through blood transfusion, organ transplantation, and sexual contact. In immunocompetent individuals, HHV-8 infections typically manifest as asymptomatic. HHV-8 has the potential to undergo reactivation, leading to the development of symptomatic infections. In particular, individuals with compromised immune systems, such as those with immunodeficiency or immunosuppression, are at a higher risk of experiencing severe and potentially life-threatening complications because of HHV-8 reactivation. Several studies have documented a higher prevalence of HHV-8 infection, particularly among organ transplant recipients. However, HHV-8 infection is infrequently observed in individuals who have undergone allo-hematopoietic stem cell transplantation. Moreover, scant information exists on HHV-8 infection in nontransplanted patients receiving treatment for acute leukemia (AL).[7-9]

## Materials and Methods

### Study design

This study is designed as a case-control study. Included 75 patients with recently diagnosed AML were included in the study.

### Studied blood of acute myeloid leukemia cases and their control

In this case–control study, (75) fresh blood samples were obtained from patients with AML enrolled in this study from the Mirjan Teaching Hospital in Al-Hilla City, whereas control groups in the current study included 75 fresh whole blood, during the period from May 2023 to January 2024. The patient with an AML was confirmed and diagnosed by the physicians. The studied blood samples of AML obtained from patients were related to those aged 2 to 78 years, whereas the collected blood from the fresh blood of apparently healthy persons as a control group aged 12 to 70 years. Patients were classified into three age groups: 2–20, 21–40, 41–60, and 61–78 years. The study included the patients with AML in this study with newly diagnosed and excluded the patient with treatment.

### Viral DNA extraction

Viral genomic DNA was extracted from the blood samples of both patients and control groups using a commercially available kit specifically designed for viral DNA extraction (PathoGene-Intron/Korea). Subsequently, the detection of HHV-8 was accomplished using the polymerase chain reaction (PCR) technique.

### The polymerase chain reaction analysis for human herpesvirus-8

Five hundred nanograms of DNA from fresh frozen tumors were used for PCR of DNA sequence encoding for the *KI* region. Great care was undertaken to avoid contamination during the PCR reactions for HHV-8. Negative controls were also run in all the PCR reactions. Specific HHV-8 primers (IDT/USA) with product size (522 bp) were used in this study to detect the HHV-8 and nucleotide sequence HHV-8 (IF) CAGTCTGGCGGTTTGCTTTC; (IR) GTAGGTGCGGTTGCAAATGT.

### Total DNA extraction

Genomic DNA was extracted using a DNA extraction kit (G-SPIN-INTRON/KOREA) from blood samples belonging to both the patient and control groups, according to the manufacturer's instructions.

### Thermal cycle conditions

The reactions were conducted in a Biometra Germany Thermal Cycler that was preheated to a temperature of 94°C. The thermal cycler was previously programmed with the necessary cycling parameters. The particular HHV-8 primers (IDT/USA) with product size (522 bp) were used to amplify the target regions of HHV-8. s follows, PCR amplification was carried out. Two microliters of template DNA were added to the PCR Master Mix tubes. 1.5 µL of forward and reverse primers were added to each PCR Master Mix tube. To PCR PreMix tubes, distilled water was added to a total volume of 25 µL., following the conditions specified in Table 1.

### Statistical analysis

The Chi-square test was employed for assessing the variables' significance, utilizing Version 23 (IBM, Chicago) of SPSS software for statistical analyses. Results with $P < 0.05$ are deemed statistically significant.

### Ethical approval

The study was conducted according to the ethical principles in the Declaration of Helsinki. It was done with

the patient's verbal and analytical approval before taking the sample. To get this approval, the study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number M221204 on December 6, 2022.

## Results

### The distribution of age in patients with acute myeloid leukemia and their control subjects

Table 2 shows that the mean age of the patients with AML (48.5 ± 10.23 years) was more than that of the apparently healthy control (AHC) (46.26 ± 11.21 years). There was a nonsignificant statistical difference ($P = 0.67$) between patients with AML and AHC groups.

### Distribution of patients with acute myeloid leukemia according to their sex

This study constituted 56% (42/75) of male patients with AML and AHC, whereas 44% (33/75) were female patients with AML [Figure 1].

### Detection rates of human herpesvirus-8 using polymerase chain reaction technique

*Extraction of viral genome*

It was found that 41.3% (31 out of 75 of the AML specimens) were having DNA viral genome, whereas in the control group, 3 out of the 75 (4%) blood specimens were having DNA/RNA viral genome [Table 3]. There is a statistically significant difference seen between the results of the study groups ($P = 0.02$).

### Amplification detection of human herpesvirus-8 by polymerase chain reaction technique in samples from patients with acute myeloid leukemia

The positive result according to amplification detection of HHV-8 by PCR technique in samples from patients with AML showed 35.4% (11 out of 31 cases) positive results, whereas 64.6% (20 of 31 cases) have negative results, as shown in Table 4 and Figure 2.

### The results of human herpesvirus-8 infection in patients with acute myeloid leukemia according to their age strata

In patients with AML group, the most common age group infected with HHV-8 was (41–60 years) (constituted 15.6%; 5 out of 24 patients), whereas the age group 2–20 years, 21–40 years, and 61–78 years were constituted 6.2%; 16.7%, and 11.8%, respectively. Statistically significant differences revealed $P < 0.05$ [Table 5].

### The polymerase chain reaction results for human herpesvirus-8-DNA among patients with acute myeloid leukemia according to their sex

Table 6 illustrates the results of HHV-8-DNA detection in patients with AML according to their sex. Among the patients with AML, 16.7% (7 out of 42 patients) were males and 12.2% (4 out of 33 patients) were females. Statistically significant differences were revealed among the sex of patients with AML positive about positive PCR results of HHV-8-DNA detection ($P = 0.04$).

## Discussion

There is currently a lack of global epidemiological data about HHV-8 in individuals with AL. Studies on the

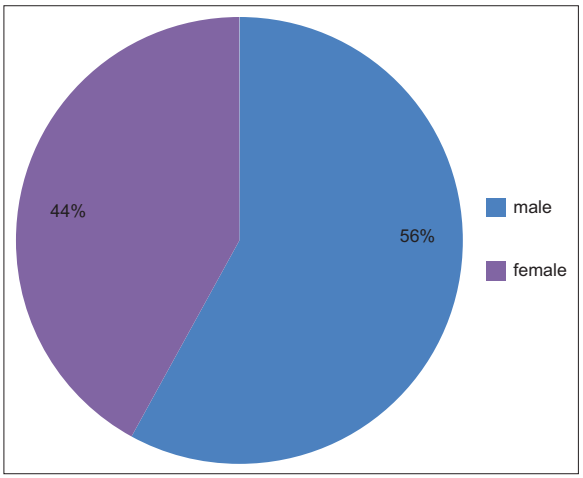


**Figure 1:** The distribution of gender within the study population

**Table 1: Amplification conditions of human herpesvirus-8 gene**

| Gene | Initial denaturation | Denaturation | Annealing | Extension | Final extension | Number of cycles |
|---|---|---|---|---|---|---|
| HHV-8 | 95C°/5 min | 95C°/1 min | 53C°/45 s | 72C°/1 min | 72C°/5 min | 40 |

HHV-8=Human herpesvirus-8

**Table 2: Distribution of patients with acute myeloid leukemia and their apparently healthy control according to the age**

| Studied group | *n* | Mean of age (years) | SD | SE | Range | | *P* |
|---|---|---|---|---|---|---|---|
| | | | | | Minimum | Maximum | |
| Patients with AML | 75 | 48.5 | 10.23 | 2.032 | 2 | 78 | *P*=0.67 |
| AHC | 75 | 46.26 | 11.21 | 2.798 | 12 | 70 | No significant (*P*>0.05) |
| Total | 150 | | | | | | |

SD=Standard deviation, SE=Standard error, AML=Acute myeloid leukemia, AHC=Apparently healthy control

**Table 3: The viral genome in blood specimens within the study groups**

| Viral genome | AML group (*n*=75), *n* (%) | AHC group (*n*=75), *n* (%) | $\chi^2$ (*P*) |
|---|---|---|---|
| Positive | 31 (41.3) | 3 (4) | 0.02 (>0.05) |
| Negative | 44 (58.7) | 72 (96) | |
| Total | 75 (100) | 75 (100) | |

AML=Acute myeloid leukemia, AHC=Apparently healthy control

**Table 4: The positive results of polymerase chain reaction for human herpesvirus-8 infection in patients with acute myeloid leukemia**

| Total viral genome | *n* (%) | *P* |
|---|---|---|
| Positive | 11 (35.4) | *P*=0.03 |
| Negative | 20 (64.6) | Significant >0.05 |
| Total | 31 (100) | |

**Table 5: Human herpesvirus-8 - DNA polymerase chain reaction results of patients with acute myeloid leukemia according to their age**

| Age stratum (years) | HHV-8-DNA PCR results | | | *P* |
|---|---|---|---|---|
| | *n* (%) | Positive, *n* (%) | Negative, *n* (%) | |
| 2–20 | 16 (21.3) | 1 (6.2) | 15 (93.8) | ANOVA test |
| 21–40 | 18 (24) | 3 (16.7) | 15 (83.3) | *P*=0.03 |
| 41–60 | 24 (32) | 5 (15.6) | 19 (79.2) | Significant |
| 61–78 | 17 (22.7) | 2 (11.8) | 15 (88.2) | (*P*<0.05) |
| Total | 75 (100) | 11 (14.7) | 64 (85.3s) | |

HHV-8=Human herpesvirus-8, PCR=Polymerase chain reaction

**Table 6: Percentage of human herpesvirus-8 infection in patients with acute myeloid leukemia according to their sex**

| Patients with AML | *n* (%) | HHV-8 - infection | |
|---|---|---|---|
| | | Positive, *n* (%) | Negative, *n* (%) |
| Male | 42 (56) | 7 (16.7) | 35 (83.3) |
| Female | 33 (44) | 4 (12.2) | 29 (87.88) |
| Statistical analysis | | *P*>0.05=0.04 | |

HHV-8=Human herpesvirus-8, AML=Acute myeloid leukemia

prevalence of HHV-8 in patients have not yet been published.[10]

The rate of seroprevalence of HHV-8 infection among healthy blood donors in Tunisia was found to be comparatively lower (7.1%) in comparison to previous studies. These studies reported a seroprevalence rate of 13%–15% in blood donors,[7-9] 13% in pregnant women,[7] and 12% in children.[7] This indicates that Tunisia is within the category of regions with intermediate prevalence of HHV-8 infection. Nevertheless, it is worth noting that there is considerable geographic variance observed across the Mediterranean regions.[11] The positive result according to amplification detection of HHV-8 by PCR technique in samples from patients with AML showed 35.4% (11 out of 31 cases) positive results, whereas 64.6% (20 of 31 cases) had negative results.

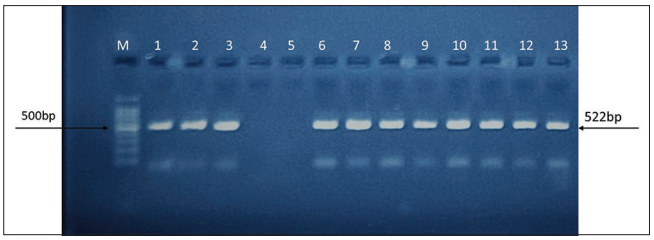


**Figure 2:** Human herpesvirus-8 gene detection using polymerase chain reaction showed band (526 pb) molecular size in patients with acute myeloid leukemia. M: DNA ladder 100–1000 bp. The amplified products migrated into 2% agarose, 20 mA for 120 min; 75V, 15 µL in each well; stained with ethidium bromide. The positive results (1–3, 6–13), whereas the negative results (4 and 5)

In nations with a moderate-to-high HHV-8 seroprevalence, blood units meant for immunocompromised individuals would want to be checked.[7,8] Previous investigations have demonstrated that immunosuppression plays a crucial role in the development of HHV-8 infection.[12,13] According to a study by Handous *et al*.,[10] subjects with AL had a higher seroprevalence of HHV-8 than healthy blood donors (21.4% vs. 7.1%, *P* = 0.02). This finding suggests that patients with AL are more vulnerable to HHV-8 infection than immunocompetent people. The HHV-8 seropositivity among African cancer patients did not significantly differ from the overall seropositivity among blood donors, as compared to another study.[14]

This variation may result from several variables, such as methodological modifications, technique sensitivity, geographic location, and subgroups.

Comparable results were observed in a study conducted on individuals from Iran who had hematological malignancies. The presence of HHV-8 DNA in the blood was detected in 4 out of 62 patients with HM, accounting for a prevalence rate of 6.5%. Among these cases, 1 (3.7%) patient had AML, whereas 3 (13.6%) patients had chronic myeloid leukemia. No instances of HHV-8 DNA were identified in patients with acute lymphoblastic leukemia or lymphoma. On the other hand, Chen *et al*. have documented a comparatively elevated occurrence of HHV-8 DNA, namely, in 10.29% of leukemia patients from Taiwan, as observed in peripheral blood mononuclear cells.[15] While there is evidence associating HHV-8 with several lymphoproliferative illnesses,[4,13] its potential involvement in AL patients has not been definitively established. The detection of HHV-8 DNA could potentially be attributed to the reactivation of latent viruses, particularly in individuals with impaired immune systems.

The incidence of viral infections has emerged as a significant obstacle for individuals undergoing stem cell transplantation, leading to a considerable mortality rate. Sequential infection of HHV-1/VZV (HHV-4)/CMV (HHV-3)/EBV (HHV-8)/BKV (HHV-5)/KSHV

was noted in the patient, which has never been documented previously.[15]

## Conclusion

Our results showed that HHV-8 acts as a cofactor in patients suffering from AML, which can start at any age. Nevertheless, as we get older, our risk of developing most cancers increases. We indicated that Ki genes might be associated with risk in patients with AML and may have contributed to its development.

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### Conflicts of interest

There are no conflicts of interest.

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