Original Article

Polymorphism Detection of TNF-α-857C/T and HHV-8 in Patients with Acute Myeloid Leukemia

Luma Amer Yasir[1], Zahraa Hamza Merza[2], Ruqaya Munther J. Ewadh[3], Shakir H. M. Al-Alwany[4], Zahraa Ali Abdullah[4]

[1]Department of Medical Microbiology, College of Medicine, Mustansiriyah University, Baghdad, Iraq, [2]Department of Radiology Techniques, College of Health and Medical Techniques, Al-Mustaqbal University, Hillah, Babylon, Iraq, [3]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Hillah, Babylon, Iraq, [4]Department of Biology, College of Science, University of Babylon, Hillah, Babylon, Iraq

Abstract

Background: Human herpesvirus 8 (HHV-8) has been linked to the pathogenicity of Kaposi's sarcoma (KS) and a number of other hematologic malignancies. However, it is still unknown what part HHV-8 plays in acute leukemia patients. **Objectives:** The objective of this study is to examine the occurrence of HHV-8 and the variation in tumor necrosis factor (TNF)-α-857C/T in patients with acute myeloid leukemia (AML) from a specific subset of the Iraqi population. **Materials and Methods:** Seventy-five whole blood samples were obtained from patients with AML enrolled in this study. Polymerase chain reaction (PCR) was used to identify HHV-8, whereas the sequencing method was applied to analyze the TNF-α-857C/T gene polymorphism. Viral and total DNA genomic extraction procedures were conducted as part of these analyses. **Results:** The positive rate of viral genome extraction was found 41.3% (31 out of 75 specimens with viral genome), while 59.7% (44/75) specimens did not contain viral genome. The PCR results showed that in the AML patient group, the rate of human herpesvirus-8 infection was 35.4% (11 out of 31 cases). The results showed that DNA polymorphism distribution was according to CT, CC, and TT genotypes of TNF-α-857C/T polymorphism: 50%, 33.3%, and 16.7% in the AML patient group, respectively, and 20%, 65%, and 15% in the control group, respectively. **Conclusion:** Considering the limited sample size of our investigation, the current findings suggest that HHV-8 and TNF-α-857C/T may have a function in the tumor biology of the specific subset of AML that was studied, perhaps contributing to their development.

Keywords: Acute myeloid leukemia, HHV-8, PCR, sequencing, TNF-α-857C/T

Introduction

Acute myeloid leukemia (AML) is distinguished by an impaired ability to produce normal hematopoiesis and an abnormal development of clonal neoplastic myeloid hematopoietic progenitor cells. According to reports, AML patients have immune system impairment, and the most crucial component of the immune system, T cells, is shown to be numerically and functionally deficient. The impact of these abnormalities on the regulatory T cells (Tregs), which are responsible for inhibiting the proliferation and activity of T helper (Th) cells, has been documented.[1] Patients with AML demonstrate significantly elevated levels of Tregs in both their peripheral blood and bone marrow in comparison with healthy donors.[2] Kaposi's sarcoma-associated herpesvirus (KSHV), often referred to as HHV-8, is recognized as the causative agent responsible for the development of KS.[3] HHV-8 has been implicated in several malignant conditions, such as multicentric Castleman's disease, pleural effusion lymphoma, and lymphoproliferative diseases.[4] In contrast to other human herpesviruses, HHV-8 exhibits a limited distribution within the human population; the seroprevalence of HHV-8 exhibits geographical and sub-population variations.[5]

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_994_24

Address for correspondence: Dr. Shakir H. M. Al-Alwany, Department of Biology, College of Science, University of Babylon, Hillah, Babylon 51002, Iraq.
E-mail: sci.shakir.hammad@uobabylon.edu.iq

Submission: 24-Oct-2024 **Accepted:** 09-Jun-2025 **Published:** 23-Jul-2025

How to cite this article: Yasir LA, Merza ZH, Ewadh RMJ, Al-Alwany SHM, Abdullah ZA. Polymorphism detection of TNF-α-857C/T and HHV-8 in patients with acute myeloid leukemia. Med J Babylon 2025;22:S102-8.

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 through 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 due to 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. Additionally, there is a paucity of information about HHV-8 infection in patients receiving treatment for acute leukemia (AL) who are not transplanted.[7-9] Tumor necrosis factor (TNF)-α is a multifunctional cytokine that has a significant impact on the growth and advancement of cancerous diseases. It actively contributes to multiple stages of leukemogenesis, such as cell growth, transformation, blood vessel formation, inflammation, and infiltration outside the bone marrow. TNF-α is essential for the formation of a tumor microenvironment and aids in the ability of malignant cells to evade the immune system, survive, and resist therapy.[10,11] A diverse range of cell types expresses TNF-R-1, whereas TNF-R-2 is exclusively expressed by immunological and endothelial cells.[12,13] TNF-α is released by activated macrophages and immune cells, such as T lymphocytes, natural killer cells, and neutrophils. It has been observed that immune cells and malignant/leukemic cells produce TNF-α inside the tumor microenvironment, which fosters the growth of tumors and plays a major role in the onset and progression of malignant disease.[9] TNF-α plays a significant part in the pathophysiology of leukemia, including many processes such as cellular transformation, angiogenesis, proliferation, and extra-medullary infiltration. This phenomenon greatly influences the tumor microenvironment, facilitating immune evasion, survival, and resistance to chemotherapy in malignant cells.[14] The association between TNF-α polymorphisms and the severity of various B-cell lymphoproliferative disorders has been established. An unfavorable prognosis in non-Hodgkin lymphoma, chronic lymphocytic leukemia, and AML has been linked to an imbalanced production of interleukin-10 (IL-10) and TNF-α.[15-17]

Materials and Methods

This study is designed as case–control study. The studied blood of AML obtained from patients was related to those aged 2 years to 78 years, while the collected blood from the fresh blood of apparently healthy persons as a control group was aged 12 years to 70 years.

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 PCR analysis for HHV-8

PCR was performed using 500 ng of DNA extracted from fresh frozen tumors to amplify the DNA sequence encoding the KI region. Rigorous precautions were made to prevent any form of contamination during the PCR procedures for HHV-8. PCR reactions included negative controls. Primer sets were used in this study to detect the HHV-8 (IF) CAGTCTGGCGGTTTGCTTTC; (IR) GTAGGTGCGGTTGCAAATGT.

Total DNA extraction

Genomic DNA was isolated from the blood samples of both patients and control groups using a DNA extraction kit (G-SPIN-INTRON/vKOREA). After that, the detection of the TNF-α-857C/T SNP was performed by sequencing.

Sequencing of PCR products

The concept of DNA sequencing pertains to techniques utilized for ascertaining the precise arrangement of nucleotide bases, namely adenine, guanine, cytosine, and thymine, within a DNA molecule. Academic researchers accomplished the initial acquisition of DNA sequences in the early 1970s, employing laboratory techniques grounded in two-dimensional chromatography. The advent of dye-based sequencing methodology coupled with automated analysis has facilitated the process of DNA sequencing, rendering it more efficient and expeditious. Understanding DNA sequences of genes and other components of an organism's genome has become essential in both fundamental scientific investigations of biological processes and practical domains such as diagnostic and forensic research.

TNF-α rs361525: TNF-F: CTTTCTGAAGCCCCTCCCAG and TNF-R: CTGGTCCTCTGCTGTCCTTG.

Table 1: Amplification conditions of HHV-8 and (TNF-α rs361525) gene

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
HHV-8	95C°/5 min	95C°/1 min	53C°/45 s	72C°/1 min	72C°/5 min	40
TNF-α rs361525	95C°/5 min	95C°/1 min	52C°/45 s	72C°/1 min	72C°/5 min	40

Table 2: Distribution of patients with AML and their control according to age

No.	Studied group	No.	Mean of age (years)	S.D	S.E	Range		*P* value
						Minimum	Maximum	
75	Patients with AML	75	48.5	10.23	2.032	2	78	$P = 0.67$
75	AHP	75	46.26	11.21	2.798	12	70	No sign
150		150						$P > 0.05$

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 primers were used to amplify the target regions of HHV-8 and TNF-α rs361525 polymorphism, following the conditions specified in Table 1.

Statistical analysis

The significance of the variables analyzed in this study was evaluated using the chi-square test. The SPSS software, version 23 (Armonk, NY, United States), was used for all statistical analyses. A statistically significant value is defined as $P < 0.05$.

Ethical approval

The ethical guidelines derived from the Declaration of Helsinki were followed when conducting the study. Before the sample was taken, the patient's verbal and analytical consent was obtained. On February 15, 2024, a local ethics committee evaluated and approved the study protocol, subject information, and permission form in accordance with document number M420100.

Results

The distribution of patients with AML according to age

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 (46.26 ± 11.21 years). There was a non-significant statistical difference ($P = 0.67$) between patients with AML and the apparently healthy control groups (AHC).

Distribution of pediatric patients with AML according to their sex

The male sex in this study constituted 56% (42/75) of the patients with AML and AHC, while 44% (33/75) were female patients with AML.

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

Viral genome		AML group	AHC group+	Chi-square
		N = 75	*N* = 75	*P* value
Positive	*N*	31	3	$P = 0.02$
	%	41.30%	4%	$P > 0.05$
Negative	*N*	44	72	
	%	58.70%	96%	
Total	*N*	75	75	
	%	100%	100%	

+AHC means apparently healthy control

Detection rates of HHV-8 by using PCR technique

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

Detection of HHV-8 in samples from patients with AML

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, while 64.6% (20 of 31 cases) had negative results, as shown in Table 4 and Figure 1.

Distribution of patients with AML according to their age

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

PCR results for HHV-8-DNA among patients with AML according to their sex

Table 6 illustrates the results of HHV-8-DNA detection in patients with AML according to their sex. Among

Table 4: The positive results of PCR for HHV-8- infection in patients with AML

Total viral genome	No.	%	*P* value
Positive	11	35.4%	$P = 0.03$ Sign $P > 0.05$
Negative	20	64.6%	
Total	31	100%	

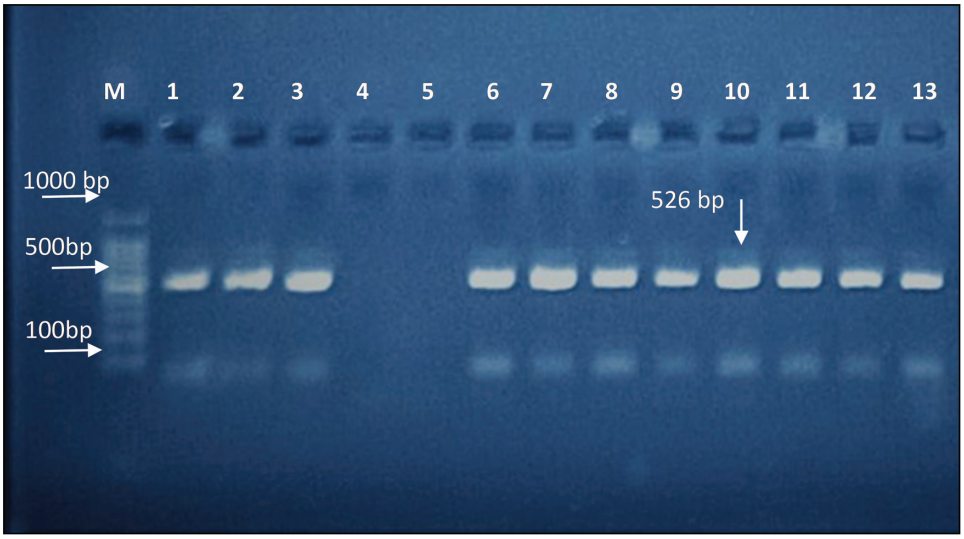

Figure 1: Detection of HHV-8 gene using PCR showed band (526 bp) molecular size in patients with AML. M: DNA ladder 100–1100 bp. The PCR-amplified products migrated into 2% agarose, 75 V, 20 mA for 120 min; 15 μL in each well; stained with ethidium bromide

Table 5: HHV-8-DNA PCR results of pediatric patients with AML according to their age

Age stratum	Years	HHV-8-DNA PCR results			*P* value
		No.	Positive	Negative	
	2–20	16	1	15	Anova test $P = 0.03$ Sign $P < 0.05$
		21.3%	6.2%	93.8%	
	21–40	18	3	15	
		24%	16.7%	83.3%	
	41–60	24	5	19	
		32%	15.6%	79.2%	
	73–120	17	2	15	
		22.7%	11.8%	88.2%	
Total		75	11	64	
		100%	14.7%	85.3%	

Table 6: Percentage of HHV-8 infection in patients with AML according to their sex

Patients with AML	No.	%	HHV-8 infection			
			+	%	–	%
Male	42	56%	7	16.70%	35	83.3
Female	33	44%	4	12. 2%	29	87.88
Statistical analysis			($P > 0.05$) $P = 0.04$			

the patients with AML, 16.7% (seven out of 42 patients) were males and 12.2% (four out of 33 patients) were females. Statistically significant differences were revealed among the sex of patients with AML-positive regarding positive PCR results of HHV-8-DNA detection ($P = 0.04$).

Table 7: Genotyping of TNF-α gene (rs361525) in patients with AML and AHC groups

Zygosity status	AML No. (%)	Control No. (%)	Position in PCR fragment	OR (95%)	SNP type	Sig.
C/T	14 (46.7%)	3 (15%)	349	1.38 (0.55–2.88)	Missense variant	0.02
C/C	11 (36.7%)	13 (65%)	349	1.47 (0.42–3.34)		0.02
T/T	5 (16.6%)	4 (20%)				0.56
Totals	30	20				
Allele						
T	56	30		1.78 (0.75–2. 651)		0.032
C	44	70				

Genotyping of TNF-α (TNF-α rs361525) Polymorphism

The results showed that DNA polymorphism distribution was as follows: C/T, C/C, and T/T were 46.7%, 36.7%, and 16.6%, respectively, in patients with AML, and 15%, 65%, and 20%, respectively, in AHC group. The difference in frequency of genotype distribution of the polymorphism between the patients and control groups was statistically significant [Table 7].

In this study, a new TNF-α (rs361525) has been recorded in GENE BANK and NCBI. LC775561; LC775562; LC775563.

Discussion

The global epidemiological data about HHV-8 in patients with AL is currently poor. So far, there is a lack of reported studies on the prevalence of HHV-8 in patients.[18]

The rate of seroprevalence of HHV-8 infection among healthy blood donors in Tunisia was found to be comparatively lower (7.1%) in comparison with 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.[19] 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, while 64.6% (20 of 31 cases) had negative results.

Soma *et al*.[20] reported that individuals with the TNF-α-857 C/T nonC/C genotype, which is associated with higher production of TNF-α, have an increased risk of AML compared to the control (AML vs. control = 39.6% vs. 28.2%, OR = 1.67, 95% CI = 1.01–2.75, $P = 0.045$). Furthermore, it was observed that the prevalence of the TNF-α-857 C/T T allele, which is associated with higher production of TNF-α, was significantly greater in patients diagnosed with AML when compared to the control group (AML vs. controls = 24.8% vs. 16.8%, OR = 1.625, 95% CI = 1.078–2.451, $P = 0.02$).

Countries with a moderate to high HHV-8 seroprevalence may want to consider screening blood units, especially when destined for immunocompromised patients.[7,8] Previous investigations have revealed that immunosuppression plays a significant role in the pathogenesis of HHV-8 infection.[21,22]

Handous *et al*.[17] conducted a study that found that patients with AL had a higher occurrence of HHV-8 in their blood compared to healthy blood donors (21.4% vs. 7.1%, $P = 0.02$). This suggests that individuals with AL are more vulnerable to HHV-8 infection compared to those with a normal immune system. The seropositivity of HHV-8 among African cancer patients did not differ significantly from the overall seropositivity reported among blood donors, as found in another study.[23] This variation may result from a number of 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 hematologic malignancies (HM). The presence of HHV-8 DNA in the blood was detected in four out of 62 patients with HM, accounting for a prevalence rate of 6.5%. Among these cases, one patient (3.7%) had AML, while three patients (13.6%) had chronic myeloid leukemia. No instances of HHV-8-DNAemia were identified in patients with acute lymphoblastic leukemia (ALL) or lymphoma. On the contrary, Hen *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.[24] While there is evidence associating HHV-8 with several lymphoproliferative illnesses,[4,22] 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 observed in the patient, which has not been reported previously.[25]

Numerous clinical observations have indicated a favorable correlation between TNF-α expression levels and unfavorable clinical characteristics in leukemia. Leukocytosis and TNF-α are significantly correlated in ALL and AML.[26,27] Patients with AML who had higher TNF-α levels experienced more fatigue and a lower quality of life.[28]

The results showed that DNA polymorphism distribution according to C/T, C/C, and T/T were 46.7%, 36.7%, and 16.6%, respectively, in patients with AML and 15%, 65%, and 20%, respectively, in the AHC group.

A previous study[16] found that elevated levels of TNF-α expression exhibited a positive correlation with a greater proportion of CD34þ cells in AML and extra-medullary infiltration. Additionally, this association was found to be indicative of a worse prognosis in the context of AL.[16] Patients who have reduced TNF-α expression often experience greater rates of complete remission and event-free survival (EFS). However, it is important to mention that when analyzed together with other factors in a multivariate analysis, the level of TNF-α expression did not independently predict clinical outcomes.[29] Furthermore, cells that do not express TNF-α have a greater susceptibility to the cytotoxic effects of daunorubicin, doxorubicin, cytarabine, or exogenous TNF-α, as indicated by previous studies.[26,28] Hence, an elevated level of TNF-α expression is commonly linked to adverse clinical features and treatment resistance in cases of leukemia.

A pivotal function of TNF-α is to facilitate the advancement and emergence of cancerous illnesses. TNF antagonists that specifically target TNF-α have shown an objective response to certain solid tumors in phase I and II clinical trials. Prior research has demonstrated that TNF-α plays a part in both the pathophysiology of AML and leukemogenesis, which includes angiogenesis, cellular change, and proliferation. Prior studies have demonstrated that serum TNF-α levels are significantly raised in patients with AML. Moreover, it has been noted that a poor prognostic factor for survival and EFS is linked to a high serum TNF-α level.[30] While a worse prognosis in AML has been linked to elevated TNF-α, the study of intracellular TNF-α in AML is not yet known. It has long been believed that mononuclear cells are the main source of TNF-α.[31-33]

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Ustun C, Miller JS, Munn DH, Weisdorf DJ, Blazar BR. Regulatory T cells in acute myelogenous leukemia: Is it time for immunomodulation? Blood 2011;118:5084-95.
2. Szczepanski MJ, Szajnik M, Czystowska M, Mandapathil M, Strauss L, Welsh A, *et al*. Increased frequency and suppression by regulatory T cells in patients with acute myelogenous leukemia. Clin Cancer Res 2009;15:3325-32.
3. Agut H, Bonnafous P, Gautheret-Dejean A. Human herpesviruses 6A, 6B, and 7B. Microbiol Spectrum 2016;4:DMIH2-0007-2015.
4. Hasan AS, Abdulwahab SA, Lames K. Prevalence of anti-human herpes virus type 7 IgG positivity rate among children with fever and skin rash in diyala province, Iraq. Arch Razi Inst 2023;78:79-86.
5. Abbas Al-Jawdhari AJ, Mohammed Al-Alwany, SH. Association between interleukin-1 receptor polymorphism and human herpesvirus 8 among lymphoma patients. Med J Babylon 2023;20:875-81.
6. Sehrawat S, Kumar D, Rouse BT. Herpesviruses: Harmonious pathogens but relevant cofactors in other diseases? Front Cell Infect Microbiol 2018;8:177.
7. Dixon SB, Lane A, O'Brien MM, Burns KC, Mangino JL, Breese EH, *et al*. Viral surveillance using PCR during treatment of AML and ALL. Pediatr Blood Cancer 2018;65:e26752.
8. Wade JC. Viral infections in patients with hematological malignancies. Hematology 2006;2006:368-74.
9. Zhou X, Li Z, Zhou J. Tumor necrosis factor α in the onset and progression of leukemia. Exp Hematol 2017;45:17-26.
10. Sethi G, Sung B, Aggarwal BB. TNF: A master switch for inflammation to cancer. Front Biosci 2008;13:5094-107.
11. Carpentier I, Coornaert B, Beyaert R. Function and regulation of tumor necrosis factor receptor type 2. Curr Med Chem 2004;11:2205-12.
12. Aggarwal BB. Signalling pathways of the TNF superfamily: A double-edged sword. Nat Rev Immunol 2003;3:745-56.
13. Waters JP, Pober JS, Bradley JR. Tumour necrosis factor and cancer. J Pathol 2013;230:241-8.
14. Lech-Maranda E, Mlynarski W, Grzybowska-Izydorczyk O, Borowiec M, Pastorczak A, Cebula-Obrzut B, *et al*. Polymorphisms of TNF and IL-10 genes and clinical outcome of patients with chronic lymphocytic leukemia. Genes Chromosomes Cancer 2013;52:287-96.
15. Jrad BBH, Chatti A, Laatiri A, Ahmed SB, Romdhane A, Ajimi S, *et al*. Tumor necrosis factor promoter gene polymorphism associated with increased susceptibility to non-Hodgkin's lymphomas. Eur J Haematol 2007;78:117-22.
16. Cunningham LM, Chapman C, Dunstan R, Bell MC, Joske DJ. Polymorphisms in the interleukin 10 gene promoter are associated with susceptibility to aggressive non-Hodgkin's lymphoma. Leuk Lymphoma 2003;44:251-5.
17. Handous I, Hannachi N, Achour B, Marzouk M, Hazgui O, Yacoub S, *et al*. Human herpesvirus-8 infection in Tunisian adult acute leukemia patients. Afr Health Sci 2023;23:504-10.
18. Hannachi N, El Kissi Y, Samoud S, Nakhli J, Letaief L, Gaabout S, *et al*. High prevalence of human herpesvirus 8 in schizophrenic patients. Psychiatry Res 2014;216:192-7.
19. Persson L, Dahl H, Linde A, Engervall P, Vikerfors T, Tidefelt U, *et al*. Human cytomegalovirus, human herpesvirus-6 and human herpesvirus-7 in neutropenic patients with fever of unknown origin. Clin Microbiol Infect 2003;9:640-4.
20. Soma K, Gotoh N, Tetsuhiro K, Yuki M, Rei I, Maaya A, *et al*. Th1 Cytokine polymorphism Gene: TNF-Alpha -857C/T affects the pathogenesis and progression of acute myeloid leukemia. Blood 2019;134:1431.
21. Tsai WH, Lee YM, Kuo BI, Ho CK, Liao PT, Liu MD, *et al*. Increased seroprevalence of human herpesvirus 8 in patients with hematological disorders. Acta Haematol 2005;114:95-8.
22. Cattani P, Capuano M, Graffeo R, Ricci R, Cerimele F, Cerimele D, *et al*. Kaposi's sarcoma associated with previous human herpesvirus 8 infection in kidney transplant recipients. J Clin Microbiol 2001;39:506-8.
23. Sitas F, Carrara H, Beral V, Newton R, Reeves G, Bull D, *et al*. Antibodies against human herpesvirus 8 in black South African patients with cancer. N Engl J Med 1999;340:1863-71.
24. Chen CH, Chang CP, Wu FY, Liu CL, Peng CT, Lin CW, *et al*. Prevalence of human herpesvirus 8 DNA in peripheral blood

mononuclear cells of acute and chronic leukemia patients in Taiwan. FEMS Immunol Med Microbiol 2011;61:356-8.
25. Zhou X, Zhou S, Li B, Li Q, Gao L, Li D, *et al*. Transmembrane TNF-alpha preferentially expressed by leukemia stem cells and blasts is a potent target for antibody therapy. Blood 2015;126:1433-42.
26. Kupsa T, Vasatova M, Karesova I, Zak P, Horacek JM. Baseline serum levels of multiple cytokines and adhesion molecules in patients with acute myeloid leukemia: Results of a pivotal trial. Exp Oncol 2014;36:252-7.
27. Fung FY, Li M, Breunis H, Timilshina N, Minden MD, Alibhai SM, *et al*. Correlation between cytokine levels and changes in fatigue and quality of life in patients with acute myeloid leukemia. Leuk Res 2013;37:274-9.
28. Tsimberidou AM, Estey E, Wen S, Pierce S, Kantarjian H, Albitar M, *et al*. The prognostic significance of cytokine levels in newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndromes. Cancer 2008;113:1605-13.
29. Kobayashi D, Watanabe N, Yamauchi N, Tsuji N, Sato T, Niitsu Y, *et al*. Endogenous tumor necrosis factor as a predictor of doxorubicin sensitivity in leukemic patients. Blood 1997;89:2472-9.
30. Dar LS. Measurement of apoptosis, proliferation and three cytokines in 46 patients with myelodysplastic syndromes. Leuk Res 1996;20:891-900.
31. Watanabe N. Endogenous tumor necrosis factor and doxorubicin sensitivity in leukemic patients. Leuk Lymphoma 1998;30:477-82.
32. Hussein, AHA, Mohammad, NBA, Farhood, RG. Serum erythropoietin level in anemic and non-anemic patients with chronic leukemia. Med J Babylon 2023;20:739-44.
33. Gatea, AK, Al-Jabory, MA, Baiee, NH. Evaluation of imatinib adherence in chronic myeloid leukemia patients in Babylon province, Iraq. Med J Babylon 2023;20:388-92.