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Impact of a polymorphism on transcriptional activation in the human tumor necrosis factor α promoter

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Abstract

Human cytomegalovirus (HCMV), a common herpesvirus, usually causes a quiet infection but can cause serious problems in immunocompromised individuals. The immune response, especially inflammatory cytokines, plays a major role in controlling the virus. TNF-α gene polymorphisms, such as G>A changes in the promoter region, can change TNF-α production and impact susceptibility to HCMV infection or reactivation. These polymorphisms can be used to explain individual differences in the immunological response to the virus. The purpose of this study was to investigate the effects of cmv infection on TNF gene expression in thalassimia patients. between 50 healthy blood donor controls and 50 thalassemia patients (31 men and 19 women) in Al-Muthanna Governorate, Iraq, between November 2024 and April 2025.Five individuals carried the TNF α –308 G>A (rs1800629) mutation, according to the examination of TNF-α polymorphisms. Two of these had high TNF-α levels and tested positive for HCMV, while three had low TNF-α levels and tested negative for HCMV. Based on the results, it can be concluded that there is a relationship between the elevation of TNF – α level in patients in compare with control ,in addition to the mutant (A) allele, found more frequent in cmv infection of patients with thalassemia.

Keywords: TNFα, genes expression, ELIZA ,thalassimia ,cmv

تأثير تعدد الأشكال الجينية على تنشيط النسخ في مُحفِّز عامل نخر الورم ألفا البشري

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الملخص

يُعدّ الفيروس المضخم للخلايا البشري(HCMV) ، وهو فيروس هربس شائع، مُسبِّباً عادةً لعدوى كامنة، ولكنه قد يُسبِّب مشاكل خطيرة لدى الأفراد الذين يعانون من نقص المناعة. وتلعب الاستجابة المناعية، وخاصة السيتوكينات الالتهابية، دوراً رئيسياً في السيطرة على الفيروس. ويمكن أن تُغيِّر تعددات الأشكال الجينية في جينTNF-α ، مثل تغيير G>A في منطقة المُحفِّز، إنتاج TNF-α وتؤثر على قابلية الإصابة بعدوى HCMV أو إعادة تنشيطه. ويمكن استخدام هذه التعددات لتفسير الاختلافات الفردية في الاستجابة

المناعية للفيروس. هدفت هذه الدراسة إلى بحث تأثير عدوى الفيروس المضخم للخلايا (CMV) على التعبير الجيني لعامل نخر الورم (TNF) لدى مرضى الثلاسيميا. شملت الدراسة 50 متبرعًا سليمًا بالدم كمجموعة ضابطة، و50 مريضًا بالثلاسيميا (31 رجلًا و19 امرأة) في محافظة المثنى، العراق، خلال الفترة من نوفمبر 2024 إلى أبريل 2025. أظهر فحص تعدد أشكال جين TNF-α أن خمسة أفراد يحملون طفرة TNF-α –308 G>A (rs1800629). اثنان منهم لديهما مستويات عالية من TNF-α وكانت نتيجة فحصهم إيجابية لفيروسHCMV ، بينما كان لدى الثلاثة الآخرين مستويات منخفضة من TNF-α وكانت نتيجة فحصهم سلبية لفيروس HCMV. بناءً على النتائج، يمكن استنتاج وجود علاقة بين ارتفاع مستوى TNF-αلدى المرضى مقارنةً بالمجموعة الضابطة، بالإضافة إلى وجود الأليل الطافر(A) ، الذي وُجد أنه أكثر شيوعًا في عدوى الفيروس المضخم للخلايا لدى مرضى الثلاسيميا.

الكلمات المفتاحية: عامل نخر الورم ألفا، التعبير الجيني، إليزا، الثلاسيميا، الفيروس المضخم للخلايا

Introduction :

The Herpesvirales order, Herpesviridae family, and Betaherpesvirinae subfamily include the human cytomegalovirus (HCMV). Humans and other primates are natural hosts. Among the 11 species in this genus, human betaherpesvirus 5 (also known as human cytomegalovirus, or HHV-5) is the one that infects humans (Anshuet al., 2017).[1].Globally, between 40 and 100 percent of persons have HCMV. The majority of the infected population is still present due to the effective immune response, which has an impact on symptoms. Like other herpesviruses, HCMV establishes a lifelong latency following the resolution of the initial infection. However, a primary infection or viral reactivation can cause severe multiorgan illness in immunocompromised individuals. Many risk categories, such as fetuses and neonates, individuals with acquired immunodeficiency syndrome (AIDS), transplant recipients, Globally, between 40 and 100 percent of persons have HCMV. The majority of the infected population is still present due to the effective immune response, which has an impact on symptoms. Like other herpesviruses, HCMV establishes a lifelong latency following the resolution of the initial infection. However, a primary infection or viral reactivation can cause severe multiorgan illness in immunocompromised individuals. Due to the compromised immune response, a number of risk groups, including fetuses and newborns, patients with acquired immunodeficiency syndrome (AIDS), transplant recipients, and intensive care unit patients, are vulnerable to developing HCMV-mediated disease. In 2021, Krstanovicet al.[2 During infection, the human cytomegalovirus (HCMV) modifies most of the host cell's environment, including the proteome, secretome, and interactome. Interactions between the virus and the

host are required for virion replication and release (Vensko, 2024).[3] During pregnancy or the postpartum period, HCMV infections can be transmitted through contaminated maternal breast milk. Functional immune suppression results from the changed cytokine profile of pregnancy, which makes it easier for viruses to spread from mother to fetus. Through the placenta, the virus can infect the developing fetus during primary maternal infection, reactivated infection, or reinfection. The next signs of a localized immune response include the production of cytokines, fetal and transplacental IgM and IgG, and cytotoxic natural killer (NK) cell responses. On the other hand, once the virus penetrates the fetal compartment, underdeveloped foetal CD4+ T-cells are unable to respond to the invasion by proliferating correctly, compromising the foetal immune response. Abuhakim (2019)[4] The main risk factors for HCMV transmission include bodily fluid transfers, such as saliva, urine, and placental cell transfer, as well as behaviors that are thought to spread fluids and infected cells, such as breastfeeding, blood transfusions, transplants, and sexual activity (Prendergast et al., 2019).(5) Thus, it appears that the variation in the TNF-α-like receptor that CMV encodes is associated with congenital CMV illness. Further research is necessary to determine the potential impact of additional CMV polymorphisms for neonatal infection, transplantation, and CMV illness associated with acquired immunodeficiency syndrome (Arav-Bogeret al., 2010).[6]

Materials and Methods

Study Population and Sample Collecting

Between November 2024 and April 2025, 100 blood samples (4 mL each) were taken in EDTA tubes from the Women and Children Hospital in Al-Samawah, Al-Muthanna Governorate. Fifty of the samples came from thalassemia patients, and the other fifty came from healthy blood donor controls. Every sample was split into two sections: Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) was used to genotype the TNFb-α polymorphism. Two milliliters of blood were centrifuged to obtain serum, which was then stored at -20°C for the detection of Human TNF-a concentrations in serum by ELISA and two milliliters of blood for the extracted DNA.

Human nucleic acid extraction from the research population

The Wizard® Genomic DNA Purification Kit (Promega, USA) was used to extract genomic DNA from whole blood samples in accordance with the manufacturer's

instructions. To put it briefly, once the red blood cells were lysed and removed, the white blood cell pellet was treated with Nuclei Lysis Solution. DNA was rehydrated in DNA Rehydration Solution after being precipitated with isopropanol and cleaned with 70% ethanol. Proteins precipitated and were eliminated. The pure DNA was stored between 2 and 8 °C until it was needed again.

Primers

The sequences of primers used in this study showed in table (1) and (2)

Table (1) Primers used for amplification of TNF-α gene (rs1800629) -308

Primer designation	Sequence (5′-3′)	Size(bp)	Company
Primer F1	ATAGGTTTTGAGGGGCATGG	184	Bioneer/ KOREA.
Primer F2	AATAGGTTTTGAGGGGCATGA	184	Bioneer/ KOREA.
Primer R	TCTCGGTTTCTTCTCCATCG	184	Bioneer/ KOREA.

Table (2) Primers used for amplification of TNF-α gene (rs361525) -238

Primer designation	Sequence (5′-3′)	Size(bp)	Company
Primer F1	TCACACTCCCCATCCTCCCTGCTCC	349	Bioneer/ KOREA.
Primer F2	TCACACTCCCCATCCTCCCTGCTCT	349	Bioneer/ KOREA.
Primer R	AGCCTTTCCTGAGGCCTCAAGCC	349	Bioneer/ KOREA.

Polymerase Chain Reaction (PCR) and ARMS-PCR

PCR amplification was carried out in a 20 µl reaction mixture including 10 µl of 2× PCR Master Mix, 1 µl of each primer, 2 µl of template DNA, and nuclease-free water using a conventional heat cycler (Biometra, Germany). TNF-α polymorphisms (–308 G>A, rs1800629 and –238 G>A, rs361525) were amplified

using the following cycling conditions: initial denaturation at 95 °C for 5 minutes; 35 cycles of denaturation at 95 °C for 30 seconds; annealing at 57 °C (for –308 G>A) and 61 °C (for –238 G>A) for 30 seconds; extension at 72 °C for 30 seconds; and a final extension at 72 °C for 5 minutes.

ELISA measurement of serum levels of human TNF-a

Certain wells of a commercial CMV ELISA kit (Abia, Germany) were filled with 100 µL of diluted sera and control samples. The plates were cleaned five times following an hour of incubation at 37 °C. A 100 µL aliquot of HRP-conjugate was added to each well, and the mixture was then incubated at 37 °C for 30 minutes. After washing, 100 µL of substrate solution was added, and after 15 minutes of dark incubation at 37 °C, the reaction was stopped with 100 µL of stop solution. Absorbance was measured at 450 nm.

Statistical analysis

To determine the significance of the findings, a number of statistical tests, such as the t-test and chi-square, were used to the examined data. The significance level for the tests was set at 0.05, and the data was analyzed using SPSS statistical software, version 20.

Results

Extraction of DNA ,Using 1% agarose gel electrophoresis stained with ethidium bromide and visible under UV transillumination, the integrity and quality of isolated genomic DNA from human blood samples were evaluated.

Distribution of TNF-α (rs361525) polymorphism according to demographic and clinical factors

Using the ELISA method 50% of patients had HCMV infections found in compared to 14% in the control group.as shown in table .1.This study is the first to show a connection between the CMV infection and higher titeration of TNF-**α**. The TNF-α −238G/A (rs361525) variation showed significant associations with demographic traits. Since all positive cases were discovered in male patients and none in controls, there might be a sex-related effect. Furthermore, the favorable

genotype was only seen in younger patients (1–20 years old). Additionally, a noteworthy association with dwelling was found.

Table (1): Distribution of TNF-α (rs361525) polymorphism according to demographic and clinical factors

Characteristics		Patient				Control			
		Positive	%	Negative	%	Positive	%	Negative	%
Sex	Male	3	6	28	56	0	0	49	98
	Female	0	0	19	38	0	0	1	2
Tableted Chi-square = 6.63, df = 1, Calculated Chi-square = 11.44, P≤0.001									
Age	1-10	1	2	13	26	0	0	0	0
	11-20	2	4	25	50	0	0	11	22
	21-30	0	0	8	16	0	0	15	30
	31-40	0	0	1	2	0	0	17	34
	41-45	0	0	0	0	0	0	7	14
Tableted Chi-square = 15.09, df = 4, Calculated Chi-square = 26.87, P≤0.001									
Site	Rural	3	6	26	52	0	0	21	42
	Urban	0	0	21	42	0	0	15	30
Tableted Chi-square = 6.63, df = 1, Calculated Chi-square = 12.99, P≤0.001									

Correlation of TNF-alpha Titer with rs1800629 SNP and Viral Positivity

TNF-α titers (290-505 pg/mL) were markedly elevated in males aged 11-20 who had both virus positivity and the positive rs1800629 SNP. The extremely low

levels (5–18 pg/mL) seen in SNP-negative and/or virus-negative individuals stand in stark contrast to this. As shown table .2

Table (2) Correlation of TNF-alpha Titer with rs1800629 SNP and Viral Positivity

Positive snp(rs1800629)	Positive virus	Tnf-a titer	sex	site	Age group
1	Negative	5	Male	Rural	1-10
2	Positive	290	Male	Rural	11-20
3	Positive	505	Male	Rural	11-20
4	Negative	8	Male	Rural	11-20
5	Negative	18	Female	Urban	1-10

Correlation of TNF-alpha Titer with rs361525 SNP and Viral Positivity

Table (3) demonstrates that individuals with the positive rs361525 SNP who were also virus-positive had elevated TNF-α titers, reaching up to 530 pg/mL, particularly in younger participants (1–10 years). Participants who were SNP-negative and virus-negative showed lower TNF-α levels, around 47 pg/Ml

Table (3) Correlation of TNF-alpha Titer with rs361525 SNP and Viral Positivity

Positive snp (rs361525)	Positive virus	Tnf-a titer	Sex	site	Age
1	Negative	47	Male	Rural	11-20
2	Positive	290	Male	Rural	11-20
3	Positive	530	Male	Rural	1-10

Discussion

The TNF-α polymorphisms and the susceptibility of thalassemia patients to infection were found to be strongly correlated.Environmental and population factors appear to further influence this genetic effect. TNF-α expression and immune response may be influenced by age, sex, and rural versus urban residency disparities (Duanet al., 2022) [7], indicating that environmental and genetic factors impact disease vulnerability .The −308 G>A polymorphism appears to play a

crucial role in modulating TNF-α activity, promoting inflammatory responses, and predisposing thalassemia patients to chronic viral infections, including HCMV, highlighting the clinical significance of this genetic marker for infection risk assessment (Ali et al., 2022; Kasztelewiczet al., 2017).[8][11].

The TNF-α polymorphisms and the susceptibility of thalassemia patients to infection were found to be strongly correlated. Environmental and population factors appear to further influence this genetic effect. TNF-α expression and immune response may be influenced by age, sex, and rural versus urban residency disparities (Duanet al., 2022) [7], indicating that environmental and genetic factors impact disease vulnerability. The −308 G>A polymorphism appears to play a crucial role in modulating TNF-α activity, promoting inflammatory responses, and predisposing thalassemia patients to chronic viral infections, including HCMV, highlighting the clinical significance of this genetic marker for infection risk assessment (Ali et al., 2022; Kasztelewiczet al., 2017).[8][11]. TNF-α polymorphisms may affect immune responses and interact with environmental exposures such as rural life and viral burden. All things considered, the A-allele-related variations appear to impact TNF-α transcription and may contribute to disease susceptibility or protection based on immunological environment and genetic background.

TNF-alpha Titer Correlation with Viral Positivity and rs1800629 SNP The results were in line with Luaibi & Mohammed (2024) [12]. who found that β-thalassemia patients had considerably higher TNF-α serum levels (137.894 ± 4.216 pg/ml), whereas the healthy group and people over the age of 18 had the lowest values (89.870 ± 3.644 pg/ml). In terms of the impact of TNF-α-308 genotypes on serum TNF-α levels in patients with β-thalassemia major, the GA genotype had the greatest level (136.560 ± 2.694 pg/ml) in comparison to the GG genotype (106.746 ± 2.235 pg/ml).These results are consistent with those of Malallah et al. (2023) [9], who discovered that thalassemic patients had considerably greater TNF-α levels than controls. Chronic pancreatitis and liver cirrhosis have also been linked to elevated TNF-α levels (Szuster-Ciesielska et al., 2000). [19] Hepatocyte apoptosis and LPS-mediated hepatotoxicity are both significantly influenced by TNF-α (Wroblewskiet al., 2016).[20] Shimizu et al. (2005) [21] state that increased TNF-α levels in thalassemia patients are indicative of persistent inflammatory reactions, which are probably caused by chronic inflammatory processes.The TNF-α

(rs1800629) polymorphism, which raises TNF-α gene expression and hence increases protein production, can account for the association between TNF-α-308 genotypes and TNF-α blood levels. Increased TNF-α mRNA synthesis may result from the –308 polymorphism, which is located inside the promoter region and may change transcription-factor binding, including the restrictive transcription factor AP-2 (Wilson et al., 1997).[22].Furthermore, although neither factor alone demonstrated a significant effect, Vacheret al. (2025) [23] indicated that the TNF-308*2 genotype and CMV antibody levels may work in concert to protect cognitive function in younger people, especially those without the APOE ε4 allele. Although elevated CMV antibody levels are frequently thought to be a sign of viral burden, there is evidence to show that they may actually be a sign of successful viral replication control in older persons. Age-related maintenance of CMV-specific memory B-cell responses often supports ongoing antibody production (Frasca, 2018).[25] and CMV usually remains dormant (Poole & Sinclair, 2015).[26] TNF-alpha Titer Correlation with Viral Positivity and rs361525 SNP The results were consistent with (Luaibi & Mohammed, 2024) [12], which demonstrated that the control group had the lowest gene expression and thalassemia patients with liver disorders had higher folding than other groups based on TNF α gene expression assessment. TNF-α plays a variety of complex biological roles, including both preventing sickness and causing pathological issues. Genetic differences in the genes governing TNF-α production and impact may play an opposing function (Elahiet al., 2009) [16].

Reactive Oxygen Species (ROS) are free radicals that might cause further liver damage and genomic instability when TNF-α is overproduced (Wang et al., 2016) [22]. Additionally, a number of authors have suggested that the activation of macrophages brought on by an excess of iron and the antigenic stimulation from long-term transfusion treatment is the main source of the rise in TNF-α (Lombardi et al., 1994) [23]. Crespo et al. (2001) [29] .discovered that patients with nonalcoholic steatohepatitis had significantly higher TNF-α mRNA expression. Furthermore, individuals with cardiac disease had higher levels of TNFα, according to research by Eskandari et al. (2018) [25]. The proinflammatory cytokine TNFα production is a major predictor of the severity and prognosis of individuals with chronic heart disease, according to the study's findings. TNFα is engaged in defensive reactions because of its dual characteristics as an anti-inflammatory and pro-inflammatory cytokine. Organ harm, however, may result from an imbalance. TNFα overexpression is thought to play a role in a number of

illnesses. Iron overload and antigen stimulation from ongoing transfusion therapy may be the cause of elevated TNFα levels in thalassemia patients (Hassoon, 2025) [26]. Excessive iron buildup and macrophage activation from long-term transfusion therapy-induced antigen stimulation could be the cause of the rise in TNFα. Ineffective erythropoiesis resulted from activated macrophages consuming apoptotic erythroid precursors (Angelucci et al., 2002). [27] The results of the current study showed a significant upregulation of TNF α gene expression in all patient groups, which closely matched the findings of a study by Nasser et al. (2023) [28] that found a significant and statistically significant increase in TNF-α levels in patients with beta-thalassemia major (β-TM) compared to healthy individuals. Increased vulnerability to some viral infections and symptoms, like symptomatic dengue fever and severe influenza, is connected to the s361525 SNP in the TNF gene, which is linked to higher TNF-α production (and consequently higher inflammation). Although its precise function varies depending on the virus and population under study, research indicates that the A allele of this SNP is more prevalent in people with viral infections and may be a risk factor for more severe consequences (Villanueva-Aguilar et al., 2023). [14]

Conclusion

Patients with promoter polymorphisms (−308 G>A and −238 G>A) exhibited significantly higher serum TNF-α levels, according to molecular genomic analysis of the TNF-α gene. Overall, the findings demonstrate how important molecular screening is. Genetic profiling for TNF-α polymorphisms and early viral infection identification may improve patient outcomes and reduce chronic inflammation.

Ethical Approval

This study was conducted after obtaining official approvals from the College of Science, Al-Muthanna University, and the Center for Training and Human Development, Al-Muthanna Health Directorate, (Approval No. 102 in 20.02.2025.)

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