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Importance of Cytomegalovirus Mir-UL-148D Expression Pattern in Sexually Transmitted Diseases

Buthana Thijeel Shibeeb
Department of Biology, College of Science, Kirkuk University, Kirkuk, Iraq, scbd24018@uokirkuk.edu.iq

Asma Sumiea Karomi
Department of Biology, College of Science, Kirkuk University, Kirkuk, Iraq, dr.asmakaromi@uokirkuk.edu.iq

Yasser Hamad Humada
Department of Biology, College of Science, Kirkuk University, Kirkuk, Iraq, yasirhamad1974@uokirkuk.edu.iq

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Abstract: Introduction & aim: Small, non-coding RNA molecules known as microRNAs (miRNAs) control post-transcriptional gene expression in almost all eukaryotic organisms as well as certain viruses, especially herpesviruses. Since the primary cause of morbidity in immunocompromised people is the human cytomegalovirus (HCMV), HCMV miRNAs could be useful targets for novel, much needed antiviral medications. Therefore, the current study aimed to evaluate the importance of Cytomegalovirus miR-UL-148D Expression Pattern in sexually transmitted diseases.

Materials & methods: From January to May 2025, A total of 400 blood samples were collected from100 blood donors at the main blood bank in Kirkuk, 100 samples from people preparing to get married at the public health laboratory in Kirkuk city and 200 samples were collected from external dermatology and venereology clinics. 5 ml

of blood was withdrawn and placed in a 2 ml TRIzol collection tube for molecular diagnosis and microRNA isolation. The remaining 3 ml was collected in a gel tube for serological examinations.

Results: Among 400 samples tested using several methods for CMV detection, Table 1 shows that the CMV infection rate was 42.8% using the Rapid Strip, while the infection rate using ELISA was 20.3% for IgG and 2.5% for IgM. On the other hand, the rate using PCR was 3.3%, while the rate was 4.3% using RT-PCR. For gene expression, folding changes ranged from (0.768 to 1.164) in the control group, with an average of 1.009, while in patients, they ranged from (1.765 to 11.471) with an average of 3.808. The results showed a significant (P=0.001) increase in gene expression in patients compared to the control group.

Conclusions: The significance of this viral miRNA as a biological biomarker of HCMV latent infection is demonstrated by the validation of the elevated miR-UL-148D expression level in the patients when compared to the control.

Keywords:STIs, HCMV, MicroRNAs, UL-148D, gene expression.

Introduction

Sexually transmitted illnesses are caused by many bacteria, viruses, and parasites and are primarily transferred by oral, anal, or vaginal sexual contact. Sexually transmitted infections (STIs) can occur concurrently or spread concurrently, and the presence of one STI raises the risk of contracting another. The majority of STDs merely exhibit oligo-symptoms or no symptoms at all (1). People of all ages can contract the herpes virus known as cytomegalovirus (CMV). Sexual contact is one of the key ways that CMV is spread, and it can spread through blood, tissue, and bodily fluids including urine and saliva (2,3,4). It has been shown that this happens between males and women. Although sexual contact is a danger for this transmission, kissing and other salivary

exchanges can also promote cross-infection. Sexual transmission may result in reinfection by another strain or strains of CMV (5). The prevalence of CMV infection is significantly higher in South America, Asia, and Africa (>90%) than it is in the Americas and Western Europe (6). 82% to 89% of blood donors, 23% to 37% of pregnant women, and 95% of healthy people tested positive for CMV IgG in different regions of Iran. In contrast, positive CMV IgG findings were seen in Saudi Arabia (90.8), France, Turkey (90.6%–99%), India (97%), Brazil (96%–98%), and Jordan (77%). As with other herpes viruses, the mature envelope is acquired in the cytoplasm, while virus DNA replication occurs in the nucleus. Following HCMV entry, the viral genomes are deposited at PML nuclear domains in the host cell, where they undergo circularization (8,9). Cell death and infectious virions are the results of CMV's 96-hour lytic replication cycle in fibroblasts (10). Six essential replication proteins—pUL54 (DNA polymerase), pUL44 (DNA polymerase processivity factor), pUL57 (single-stranded DNA binding protein), pUL70 (primase), pUL102 (primase-associated factor), and pUL105 (helicase)—are needed for viral genome replication to begin at the origin of lytic replication (11). In a flow cascade, the human cytomegalovirus expresses genes for virion assembly and release that are immediate-early (IE), early, and late. Amplification of the DNA comes after early gene expression. Within 24 to 72 hours of infection, in vitro DNA replication starts (12). Therefore, the current study aimed to evaluate the importance of Cytomegalovirus miR-UL-148D Expression Pattern in sexually transmitted diseases.

Materials & Methods

Sample collection

From January to May 2025, A total of 400 blood samples were collected from100 blood donors at the main blood bank in Kirkuk, 100 samples from people preparing to get married at the public health laboratory in Kirkuk city and 200 samples were collected from external dermatology and venereology clinics. 5 ml of blood was withdrawn and placed in a 2 ml TRIzol collection tube for molecular diagnosis and microRNA isolation. The remaining 3 ml was collected in a gel tube for serological examinations.

Human Cytomegalovirus pp65 (CMV pp65) ELISA Kit

SUNLONG Biotech's Human Cytomegalovirus pp65 (Cat.No. SL2117Hu) ELISA kit is used to qualitatively determine the amount of CMV pp65 in human serum or plasma. By comparing with the CUTOFF value, the CMV pp65 is qualitatively determined.

Real-Time PCR Amplification

RNA Extraction According to the manufacturer's recommendations, RNA was extracted utilizing the TransZol Up Plus RNA Kit.

Primers

PCR was performed by using UL-148D, F (5’-TCG TCC TCC CCT TCT TCA CCG-3’), and R (5’- CAG AGA GTA GAT GAG CGT GCA GT-3’), and U6 was used as housekeeping gene F (5’- GAC GGT GAG ATC CAG GCT TGA GAG C-3’) and R (5’- CAG AGA GTA GAT GAG CGT GCA GT -3’) (13).

RT-PCR Amplification

The EasyScript® First-Strand cDNA Synthesis SuperMix is used to convert RNA to cDNA. Initial denaturation took place at 95°C for 10 minutes, followed by 40 denaturation cycles at 95°C for 20 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 20 seconds. The formula for the 2-ΔΔCt expression was used to determine the values for the relative quantification.

Statistical analysis

Statistical analysis SPSS was used to analyze the results of UL-148D gene expression (14).

Results & Discussion

Among 400 samples tested using several methods for CMV detection, Table 1 shows that the CMV infection rate was 42.8% using the rapid Strip, while the infection rate using ELISA was 20.3% for IgG and 2.5% for IgM. On the other hand, the rate using PCR was 3.3%, while the rate was 4.3% using RT-PCR.

Table (1): the number and percentage of CMV infections in the studied group

Results		No.	%
Rapid strip		171	42.8%
ELISA	IgG	81	20.3%
	IgM	10	2.5%
PCR		13	3.3%
RT-PCR		17	4.3%

The current study's methods demonstrate that the infection rate varied according to the method employed. Rapid stripping had the highest rate, whereas PCR had the lowest rate. A more accurate detection technique, like an ELISA test, is necessary for a proper diagnosis because of the extensive dispersion of CMV, especially in immunocompromised individuals. Because ELISA tests employ an enzyme substrate reaction and washing steps to eliminate any nonspecific antibodies that might be present in patient samples, they are highly sensitive and specific. These tests require validation using more sophisticated techniques such ELISA techniques that provide wide-ranging detection, in contrast to the Rapid test, which is a simple screening tool used in the early diagnosis of a range of disorders. Because it yields highly quantitative and consistent results, ELISA is a crucial tool for clinical diagnoses and scientific research (15, 16). Using the ELISA approach, we found that 2.5% of the 128 patients had positive IgM tests. Our findings are in line with recent research that looked at the prevalence of CMV in pregnant women in Southern Ethiopia and found it to be 8.2% (17). By using the ELISA test, Sharghi et al. discovered that the IgM positive rate for deceased women was 0.06% (18). IgM Abs differences between our data and other studies may be caused by differences in sample sizes, biased patient selection, or the fact that CMV tends to induce dormant status and that reactivation of the virus is more likely to occur in pregnant or other immunocompromised individuals when the immune status is weakened, allowing the virus to proliferate and flourish (16). According to Parmigiani et al. (19), the polymerase chain reaction was more accurate than serological tests in diagnosing CMV infection, which is consistent with our findings. Shams et al. (20) came to the conclusion that PCR was a more sensitive, dependable, and accurate approach for detecting CMV infection in pregnant women, which is also consistent with our data. Numerous studies that calculated the incidence of CMV in women found that the disease was highly prevalent in both industrialized and developing nations, including the United States, Europe, Pakistan, India, Saudi Arabia, and Africa, with rates ranging from 30 to 90 percent (21). Numerous investigations have been conducted in Iraq to determine the prevalence of CMV in women; the results range from 77.3% in Babylon to 95.7% in Kirkuk, whereas the prevalence was 100% in Erbil (22, 23). The greater prevalence rate in this study is in line with Iraq's high prevalence rate of over 95%. While screening for CMV antibodies is still controversial, distinct prevalence rates in different regions, even within the same country, may indicate the need for diverse approaches for the control and management of CMV infection (7). This could draw attention to the unnecessary routine CMV screening for patients, with the exception of immunocompromised women and those who have had contact with someone who has presented with suspected CMV symptoms or who has a confirmed acute infection (24).

Table (2) shows that gene expression in positive samples, based on ΔCT, ranged from (6.5 to 7.3), while if ΔΔCT was used, its values ranged from (-0.22 to 0.38). Meanwhile, in patients, ΔCT ranged from (3.4 to 6.2), and ΔΔCT ranged from (-3.52 to -0.82). Folding changes ranged from (0.768 to 1.164) in the control group, with an average of 1.009, while in patients, they ranged from

(1.765 to 11.471) with an average of 3.808. The results showed a significant (P=0.001) increase in gene expression in patients compared to the control group. In Figure (1) Curves of amplification were the X-axes represent the number of cycles while Y-axes represent in testing Florence. Positive samples showed curve shape of amplification at different cycle number on roy channel, while negative specimens display know curve amplification at any point of the cycle number and will remain flit blow the threshold level of amplification.

Table (2): The expression level of the UL-148D gene in patients and control.

Groups	miR148D	GAPDH	ΔCT	ΔΔCT	2-ΔΔCt	Folding	Mean	P value
Control	32.2	25.3	6.9	-0.02	1.01395948	1.01395948	1.009565	0.001
	31.9	25.1	6.8	-0.12	1.086734863	1.086734863		
	31.7	25	6.7	-0.22	1.164733586	1.164733586		
	32.5	25.2	7.3	0.38	0.768437591	0.768437591		
	32	25.1	6.9	-0.02	1.01395948	1.01395948		
Patients	31.1	24.6	6.5	-0.42	1.337927555	1.337927555	3.808801	
	31.2	25.2	6	-0.92	1.892115293	1.892115293		
	31	24.8	6.2	-0.72	1.647182035	1.647182035		
	30.2	25.9	4.3	-2.62	6.147500725	6.147500725		
	31.2	25.9	5.3	-1.62	3.073750363	3.073750363		
	30	25.8	4.2	-2.72	6.588728138	6.588728138		
	31.1	25.8	5.3	-1.62	3.073750363	3.073750363		
	31.2	25.5	5.7	-1.22	2.329467173	2.329467173		
	31.1	25	6.1	-0.82	1.765405993	1.765405993		
	30.4	25.3	5.1	-1.82	3.530811985	3.530811985		
	31.2	25.7	5.5	-1.42	2.67585511	2.67585511		
	29.2	25.8	3.4	-3.52	11.47164198	11.47164198		
	30.3	25.7	4.6	-2.32	4.993322196	4.993322196		
	29.8	24.7	5.1	-1.82	3.530811985	3.530811985		
	30.1	24.8	5.3	-1.62	3.073750363	3.073750363		

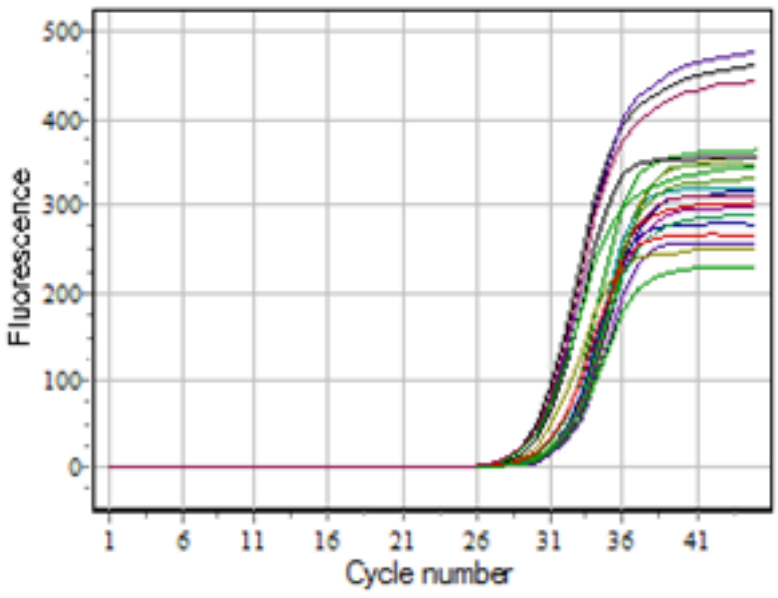

Figure (1): amplification of UL-148D gene on Cy5 channel indicating positive sample with curve shape at different cycle number.

Human cytomegalovirus adopts effective methods by encoding multiple immuno-modulatory genes that can influence host immune response pathways (25). Among these tactics, the miRNA-based mechanism has received experimental validation, which is noteworthy. Numerous vital functions of cells and tissues have been linked to HCMV miRNAs (26). The miRNAs HCMV miRUL-112, -UL-22A, -US-25-1, and -UL-148D are essential for HCMV replication and immune system evasion. For the first time, we examined the expression levels of HCMV miR-UL-148D in healthy individuals and STD patients. Although HCMV miRUL-148D is essential to the pathogenicity of HCMV strains (27), the attenuated AD169 strain's lack of this miRNA suggests that its accumulation occurs during the latent phase of HCMV infection (28). Although the target genes for HCMV miRNAs have not been thoroughly studied, it has been found that miRUL-148D targets the chemokines IEX-1 and RANTES during viral infection (29). Pan et al. (30) demonstrated that HCMV miRUL-148D targets the activin A receptor (ACVR1B) in infected monocytes, resulting in a decrease in IL-6 (30,31). Lau et al. (32) also demonstrated that by targeting CCL5 (32,33), the expression of the aforementioned viral miRNA is elevated during latent infection. An essential HCMV glycoprotein that is more highly expressed during the viral lytic stage and is crucial for virus replication is the UL-148D gene. Previous research indicates that the expression of the UL-148D gene plays a crucial function in the viral lytic stage and prepares the required capsid glycoproteins (34). The recent study indicated that miRUL-148D rises in the latent stage of the viral life cycle. Thus, it can be concluded that this miRNA is one of the molecular biomarker candidates for identifying the viral latent state based on the findings of this study and others. Other HCMV miRNAs, however, require further investigation. Lastly, by altering the host immune evasion mechanisms, viral miRNAs promote viral infection. In addition to helping researchers discover more effective and less harmful therapeutic approaches, they can serve as a targeted plan for future individualized therapy and diagnostic techniques. The UL-148D gene's expression levels were examined between sick and healthy groups in the current investigation. According to the findings, this miRNA was more highly expressed during the latent period than the gene under study. Figure 1 illustrates the substantial association between the expression levels of UL-148D in the control and sick groups. These results validate the significantly altered expression of the UL-148D gene. The results of the current study are in line with previous research that demonstrated that the regulation of miRNA expression differs significantly from that of genes (35). At every stage of the virus's life cycle, the pattern of UL-148D gene expressions varied. The current study's findings demonstrated that the patient group's expression of this miRNA was higher.

Conclusions

The significance of this viral miRNA as a biological biomarker of HCMV latent infection is demonstrated by the confirmation of the elevated miR-UL-148D expression level in the patients when compared to control.

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