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MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF CYTOMEGALOVIRUS IN DIWANIYAH, IRAQ

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ABSTRACT

Background and Objectives: Human cytomegalovirus (HCMV) infection is the most common cause of congenital malformations worldwide. There is no vaccine or specific drug therapy to prevent congenital HCMV infection. Congenital transmission of HCMV during pregnancy is a major concern, as it causes a wide range of clinical outcomes in fetuses and infants, ranging from asymptomatic infection to severe malformations. Vertical transmission of HCMV, which results in congenital HCMV infection (cHCMV), can have disabling and potentially fatal effects on the fetus or newborn. The increased risk of vertical transmission is thought to be due to reactivation or reinfection of HCMV during pregnancy. In this study, we focused on the genomic sequencing and viral dendritic branching of HCMV-infected pregnant women in Diwaniyah City. **Material and Methods**: 600 blood samples were collected from pregnant women in different hospitals and private clinics in Diwaniyah Governorate during the period from December 2021 to June 2022, and information about the pregnant women's ages (28-45) and their place of residence (urban or rural) was collected. All samples were tested for the presence of both IgG and IgM antibodies against HCMV using rapid test and enzyme-linked immunosorbent assay (ELISA). Materials and Methods: Six hundred blood samples were collected from pregnant women from various hospitals and private clinics in Diwaniyah Governorate during the period from December 2021 to June 2022. Information was collected on the pregnant women's ages (18–45 years) and place of residence (urban or rural). All samples were tested for the presence of IgG and IgM antibodies against human cytomegalovirus (HCMV) using rapid tests and enzyme-linked immunosorbent assay (ELISA). Results showed a high positive rate for IgG (96.2%) and IgM (1.8%) antibodies using ELISA. 11 Samples positive for IgM antibodies were tested using polymerase chain reaction (RT PCR). **Results:** Phylogenetic tree: Human betaherpesvirus 5 (HCMV) UL7 and UL8 gene isolates from Iraq showed 100% similarity to Iranian isolates. This indicates a close genetic relationship between HCMV strains found in Iraq and those in Iran, especially in the UL7 and UL8 gene regions. This high level of similarity (100%) could have important implications for understanding HCMV transmission dynamics in the region. This suggests common transmission pathways between the two countries, Iraq and Iran. The genetic similarity between the Ul7 and Ul8 genes of the Iraqi isolates compared to various global strains appears to be high. The reported high similarity percentages indicate that these Iraqi isolates share significant genetic heritage with European, African, and Asian isolates, suggesting potential evolutionary links or common origin. The Iraqi genomes showed 98.03% to 97.54% similarity to European, African, and Asian isolates. The similarity percentage was high (98.03%) with Belgian isolates, indicating a close genetic relationship. It also showed a similarity of 97.78% with isolates from Germany, the Netherlands, China, and Zambia, and 97.54% with isolates from the United States and the United Kingdom.

KEYWORDS: HCMV, Pregnant women, Elisa PCR, Iraq.

INTRODUCTION

HCMV has been shown to be highly polymorphic both among and within individuals.[1, 2] Previous studies have used data on divergence between hosts or between species to map the evolutionary path of HCMV.[3, 4] The evolutionary history of human cytomegalovirus (HCMV) is characterized by high genetic diversity and complex patterns of variation. Studies have identified 74 variable regions in the HCMV genome; some of these regions show geographic population structure, while others lack such differentiation, suggesting two distinct evolutionary paths.[5] Furthermore, the adaptation of HCMV to the human host involves positive selection on viral core genes, such as the UL70 primase gene, which favors mutations that lead to reduced viral replication in human fibroblasts; this indicates a tendency for the virus to be less invasive. Also, phylogeographic analysis of HCMV suggests that the virus has dispersed from East Africa several times and that the global lineage probably originated from this region about 90–120 thousand years ago, which reinforces the hypothesis of successive migrations and the formation of global virus diversity.[6]

Genomic Structure

• Genome: HCMV has a large, linear genome of double-stranded DNA that is approximately 235 kilobases (kbp) long and encodes over 200 proteins.
• Long-specific (UL) and short-specific (US) regions: The genome is divided into two specific regions, UL and US, flanked by inverted repeat sequences.
• Genes: The virus encodes genes that are involved in viral replication, immune evasion, and regulation of host cell functions. HCMV enters human cells via direct integration or the intracellular route. Viruses infect via interactions between viral glycoproteins (e.g. gB and gH) and specific surface receptors (e.g. platelet-derived growth factor), which proceed with the fusion of the viral envelope with the cell membrane, allowing the entry of the neocapsids into the cytoplasm.

These neocapsids are transported to the nucleus, where the viral DNA is released. This process initiates the expression of the IE-1/IE-2 genes.

Viral replication and maturation proceed with the simultaneous stimulation and accumulation of viral synthetic activities. In this process, the recombinant viral DNA is packaged into a capsid, which is then transported from the nucleus to the cytoplasm. In the endoplasmic reticulum-Golgi intermediate compartment, the cytoplasm forms the second layer. This phase is followed by two complex steps of unfolding and subsequent release of the virion via extracellular translocation to the plasma membrane.[7]

HCMV phylogeny in Iraq

[7]the gB1 genotype is generally dominant, whereas the gB3 and gB4 genotypes are often observed in mixed infections. The specific distribution of gB genotypes may be associated with clinical outcomes, such as chronic HCMV infections and graft-versus-host disease. The function of the gN gene in HCMV is to help evade the neutralizing antibody response and facilitate reinfection in individuals who have previously been serologically positive.[8] The predominant genotype in infants with symptomatic congenital/perinatal infection is gB3, followed by gB1 and gB2.[9, 10]

The different gB genes of human cytomegalovirus (HCMV) include gB1, gB3, and gB4.[11] In addition, there are different genotypes of gB, such as gB1, gB2, gB3, and gB4, which are classified based on genetic variation in the coding region of the gB precursor protein.[12] The gB genotypes can be identified using multiplex real-time PCR. The distribution of gB genotypes may vary among populations and geographical regions.[13] The gN polymorphism, which results in the four major genotypes (gN1–4), can affect virus replication, gN expression, and gN–gM complex formation, but this effect is minor. However, viruses expressing the gN4 genotype are more effectively neutralized by antibodies (compared to viruses expressing other genotypes).[9] The gN protein is part of the gH/gL/gO complex, which is important for entry, expansion, and neutralization by antibodies.[12] The gO isoforms, which vary due to natural interspecies variation, can differentially protect the virus from neutralizing antibodies.[14] The gN protein also plays a critical role in HCMV secondary envelope and viral replication, and palmitoylation of gN is essential for its function in secondary envelope and replication.[15]

Phylogeny of HCMV in Iran

Human cytomegalovirus (HCMV) has been studied in Iran to determine its phylogenetic characteristics. Several papers have investigated the presence and genotypes of HCMV in different regions of Iran. One study showed that the gB3 genotype was the most prevalent genotype among kidney transplant recipients, followed by the gB4 and gB1 genotypes, while gB2 was not detected.[13]

Another study examined CMV isolates from tomato and cucurbit plants in northwestern Iran and showed that Iranian isolates formed two distinct phylogenetic groups that are similar to European and East Asian CBPVs.[16] In addition, a study of CBPV in honeybees in Iran identified two distinct phylogenetic groups among Iranian isolates.[17] Finally, a study on HCMV in breast cancer patients in Iran found a significant presence of HCMV genome and anti-CMV IgG antibodies in patients, confirming the role of HCMV in breast cancer.[18]

The main objective of the study

The present study aims to survey the epidemiological and diagnosis of HCMV and its association with miscarriage because few studies address the epidemiological survey of cytomegalovirus in Iraq.

MATERIALS AND METHODS

HCMV detection from blood samples of pregnant women in Diwaniyah Governorate by the following methods.

Study design, **duration, and population**. This descriptive cross-sectional study was conducted at the Maternity and Children's Hospital in Diwaniyah, Iraq, and in specialized clinics during the period from December 2021 to June 2022, The study included pregnant women aged 18 to 45 years who consented to participate in this study, whether or not they had signs and symptoms of CMV infection.

Ethics Statement: The Ethics Committee of Shahid Beheshti University, Tehran, Iran, reviewed and approved the present study protocol (IR. SBU.RE C.1403.030). Informed consent was obtained from all participants in our study.

Sample collection Six hundred blood samples were collected from the pregnant woman (5 ml) were placed in gel tubes and the blood samples were centrifuged for 5 minutes at 4000 (rpm) and the serum was collected in eppendrof for ELISA testing and 2 ml EDTA container the samples were kept in a deep freezer -20 until analysis was performed.

Detection of (HCMV) serological analysis

Rapid Spectrum HCMV IgM/IgG(CTK Biotech, catalog R0224c/USA) and Enzyme-Linked Immunosorbent Assay (ELISA), (Pishtaz Teb diagnostics, catalogue No.PT-HCMV-96/Iran) were tested for both IgG and IgM. The serum was tested for the presence of antibodies to HCMV according to the instructions of the laboratory kit manufacturer.

DNA Isolation from Whole Blood for CMV

Store kit contents at room temperature (RT). Store enzyme at -20°C. Tubes and bottles should be tightly closed.

Sample Preparation

Whole blood is collected in tubes containing EDTA (1 mg/ml) to prevent blood clotting. Heparinized blood is not recommended. Typically, 200 microliters of fresh blood is used for DNA extraction. Blood can be stored for up to two weeks at 2–8°C. For long-term storage, samples are divided into 200-microliter aliquots and stored at -20°C. To inhibit nuclease activity, samples are kept frozen until DNA extraction.

Estimating the Concentration and Purity of Extracted DNA

A Thermo NANODROP 2000C device was used to estimate the concentration and purity of the extracted DNA.

Molecular Identification of Human Cytomegalovirus Test Procedure

1. The Go Taq® Master Mix was thawed at room temperature. The mixture was then vortexed and briefly spun in a microcentrifuge.
2. The reaction mixture was prepared by mixing the following components: The required amount of each component was added to the mixture in the following order.

Table 1: Supplied materials for use in preparing the mixture and preparing it for the first stage of RT-PCR, directed to the HCMV molecular chain.

Components	Volume	Concentration
Go Taq® Green Master Mix	12.5 µl	**1x**
Forward primer	0.5 µl	**0.2 µM**
Reverse primer	0.5 µl	**0.2 µM**
DNA template	5 µl	**< 250 µg**
Nuclease free water	6.5 µl	**-**
Final volume	**25 µl**	

PCR amplification using gB primers The primers and probes used for molecular detection of HCMV (gB gene) in this study were selected from several primers and probes that have been used in previous papers for CMV detection using real-time PCR. Table 2.5 shows the sequences of the primers and probes used for molecular detection of CMV gB gene using real-time PCR.

Table 2: Forward and reverse primer sequences for RT-PCR reaction for molecular identification of HCMV (gB gene).[18]

Virus/genotypes Target genes	Primers and probes [5′ → 3′]
CMV-gB gene	F: TTT GGA GAA AAC GCC GAC R: CGC GCG GCA ATC GGT TTG TTG TA

The PCR machine was programmed for the following cycling protocol.

Table 3: First-step RT-PCR reaction program for molecular detection of HCMV.

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	**95°C for 3 min.**
Denaturation	30cycle	**95°C for 30 sec.**
Annealing		**64°C for 30 sec.**
Extension		**72°C for 30 sec.**
Final Extension	**1 cycle**	**72°C for 10 min.**

Procedure

1-- TaqMan Universal Master Mix II 2x), template DNA, and primer and probe solution 20x were thawed on ice

2- The reaction mixture was prepared according to the table.

Table 4: Reaction used to prepare the real-time PCR test primer mix.

Reaction Component	Volume(µl) per sample	Final Concentration
TaqMan Universal PCR Master Mix II	25	1x
20 X Primer\ Probe Mix1	2.5	0.4µM ST-Frt primer 0.4µM ST-Rrtprimer 0.15µM ST-P Probe
Nuclease free water	19.5	------
DNA	3	------
Total volume	50	------

The reaction mixture was thoroughly mixed and dispensed into the required number of PCR tubes or PCR plate wells (47 µL).

4. A 3 µL aliquot of template DNA was added to the wells.

5. The plate was covered with Micro Amp® 96-well optical adhesive film.

6. The plate was centrifuged briefly to transfer the contents to the bottom of the tube and to remove air bubbles from the solutions.

7. The Biorad CFX96 real-time PCR instrument was prepared for programming.

8. The PCR plate was placed in the real-time cycler and the cycling program was started.

9. Data analysis was performed.

Cycling conditions

Table 5: Temperature and time conditions in the instrument according to the kit instructions for real-time PCR testing.

Thermal cycler protocol	No. of cycle	Temperature –time
Enzyme Activation	1 cycle	95°C for 15 min.
Denaturation	45cycle	95°C for 30 sec.
Annealing		64°C for 30 sec.
Extension		72°C for 30 sec.
Denaturation		95°C for 30 sec.

Product Evaluation and DNA Sequencing

Before sequencing, the DNA concentration of the PCR product was measured using NanoDrop. The PCR products were sent to the sequencing center. This process was performed at the National Environmental Management Instrumentation Center (NICEM) using an ABI3730XL Applied Biosystems, USA. The results were directly compared with healthy Iraqi controls using Mega 6 software.

DNA sequencing. Sequencing was performed from an in-house produced polymerase chain reaction (PCR) product (406 base pairs). DNA sequencing was also performed from the CMV gB gene PCR product. Macrogen (Seoul, Korea) performed DNA purification and standard sequencing of the two gB gene strands.

DNA sequence similarity and alignment. Similarity in the nucleotide sequences of the two combined CMV gB gene strands was searched using BLAST.

Phylogenetic study

The CMV-positive RT-PCR samples were sent to Macrogen in South Korea for sequencing, and a phylogenetic tree was constructed using the maximum likelihood method. Statistical Analysis.

Statistical analyses of differences were conducted using SPSS version 27. Differences between means were assessed using analysis of variance (ANOVA). The acceptable significance level was $P \leq 0.05$.

RESULTS

Molecular results

Real-time PCR results.

RT-PCR results showed that this technique is specific and rapid for the detection of HCMV in CMV IgM-infected pregnant women. RT-PCR methods were performed on 25 blood samples from CMV-infected women, 11 women whose CMV IgM results were positive by ELISA, and 14 blood samples from women infected with CMV IgG. The results of CMV virus amplification by color-based Real-Time PCR of the samples showed different positive reaction cycles (CT). The results of 6 positive cases from CMV IgM-infected pregnant women by RT PCR were clear and pure.

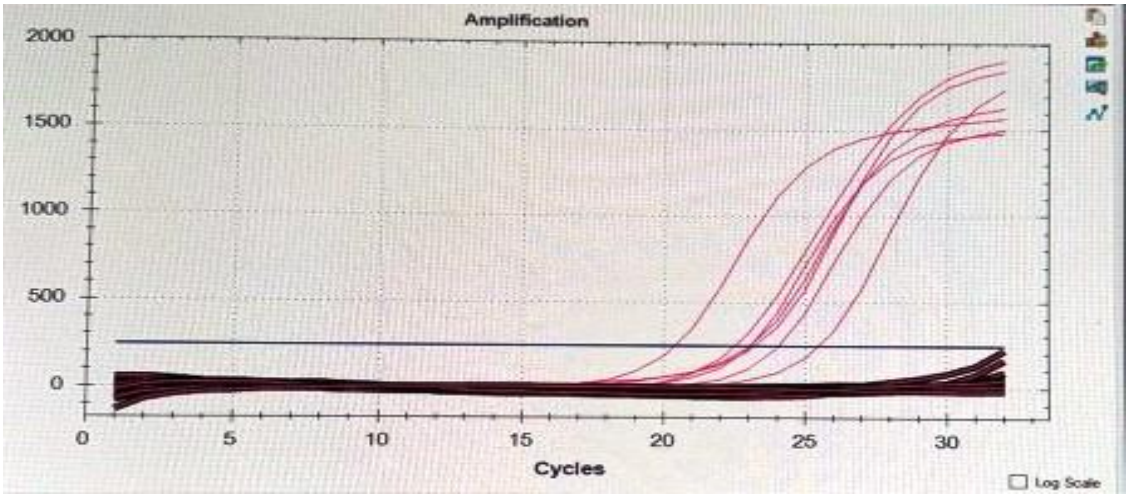


Figure 1: The results of 6 positive cases of pregnant women infected with CMV IgM by RT PCR method were clear and pure.

Sequencing Results

Nucleotide Sequencing Groups Six samples of purified PCR products were sent to Macrogen Sequencing Company in South Korea to obtain nucleotide groups for CMV (406 bp) isolated from pregnant women in Diwaniyah Province. They were sequenced in the form of peaks and nucleotide groups for each strain with a symbol for each individual representing the country, host, province and region. The sequences of the two isolates were imported after sending the reverse PCR products from Macrogen Sequencing Company in South Korea. The sequence length of the two CMV isolates obtained was 406 bp.

Table (6). Sequencing of local and global isolates from the study bank.

Name	Sequence ID	Organism	Date	Country	Host	Source	Identity	Coverage	Mismatches
Human herpesvirus 5 from Iran US7 ...	AY327403.1	Human betaherpesvirus 5		Iran			100	100	0
Human herpesvirus 5 strain BE/13/20...	KP745645.1	Human betaherpesvirus 5	06-May-...	Belgium	Homo sa...	urine	98.03	100	8
Human herpesvirus 5 strain HAN11, ...	KJ361950.1	Human betaherpesvirus 5	2007	Germany	Homo sa...	broncho...	97.78	100	9
Human betaherpesvirus 5 strain LUS...	MK290743.1	Human betaherpesvirus 5	2002	Zambia	Homo sa...	milk	97.78	100	9
Human herpesvirus 5 strain BE/2/201...	KP745710.1	Human betaherpesvirus 5	09-Jan-2...	Belgium	Homo sa...	urine	97.78	100	9
Human herpesvirus 5 strain BE/45/20...	KP745633.1	Human betaherpesvirus 5	27-Jan-2...	Belgium	Homo sa...	nasopha...	97.78	100	9
Human herpesvirus 5 strain BE/17/20...	KP745727.1	Human betaherpesvirus 5	14-Jun-2...	Belgium	Homo sa...	urine	97.79	100	8
Human herpesvirus 5 strain BE/13/20...	KP745657.1	Human betaherpesvirus 5	10-May-...	Belgium	Homo sa...	urine	97.78	100	9
Human betaherpesvirus 5 strain NL/R...	KT726941.2	Human betaherpesvirus 5	08-Feb-2...	Netherla...	Homo sa...	urine	97.79	100	8
Human herpesvirus 5 isolate HAN, co...	KJ426589.1	Human betaherpesvirus 5	10-Jan-2...	China	Homo sa...		97.78	99.75	8
Mutant Human betaherpesvirus 5 clo...	MN920393.1	Human betaherpesvirus 5			Homo sa...		97.54	100	9
Human herpesvirus 5 strain BE/26/20...	KP745703.1	Human betaherpesvirus 5	23-Aug-...	Belgium	Homo sa...	urine	97.54	100	9
Human herpesvirus 5 strain BE/24/20...	KP745711.1	Human betaherpesvirus 5	04-Aug-...	Belgium	Homo sa...	urine	97.54	100	9
Human herpesvirus 5 strain BE/15/20...	KP745693.1	Human betaherpesvirus 5	24-May-...	Belgium	Homo sa...	urine	97.54	100	9
Human herpesvirus 5 strain BE/19/20...	KP745712.1	Human betaherpesvirus 5	06-Jul-2...	Belgium	Homo sa...	urine	97.54	100	10
Human cytomegalovirus strain AD16...	X17403.1	Human betaherpesvirus 5					97.54	100	9
TPA_inf: Human herpesvirus 5 strain ...	BK000394.5	Human betaherpesvirus 5	1956	USA	Homo sa...	adenoids...	97.54	100	9
Human herpesvirus 5 strain BE/10/20...	KP745649.1	Human betaherpesvirus 5	02-Apr-2...	Belgium	Homo sa...	urine	97.54	100	9
Human betaherpesvirus 5 strain HAN...	KY123650.1	Human betaherpesvirus 5	22-Aug-...	Germany	Homo sa...	blood fro...	97.54	100	10
Human betaherpesvirus 5 isolate BP2...	PP412593.1	Human betaherpesvirus 5	02-Feb-2...	Belgium	Homo sa...	blood	97.54	100	9
Human betaherpesvirus 5 isolate S2_...	OV100763.1	Human betaherpesvirus 5					97.54	100	10
Human betaherpesvirus 5 strain UK/L...	KT726953.2	Human betaherpesvirus 5	23-Sep-2...	United Ki...	Homo sa...	urine	97.54	100	9
Human herpesvirus 5 strain BE/3/201...	KP745659.1	Human betaherpesvirus 5	14-Jan-2...	Belgium	Homo sa...	urine	97.54	100	10

Results of screening and validation of received nucleotide combinations

The results of nucleotide sets in this study were checked and validated using the main tool of NCBI (BLAST) local nucleotide alignment search (BLAST analysis) - search the nucleotide database using the online nucleotide query. Sequence alignment should be performed using references, information from the databases of cytomegalovirus sequences registered in GenBank and external collections to determine the degree of identity and similarity between the registered viruses and their comparison with cytomegalovirus in pregnant women in Al-Diwaniyah province. The results showed a high identity/identity with other global (100%) and to varying degrees with (98.03-97.54%). Table (6).

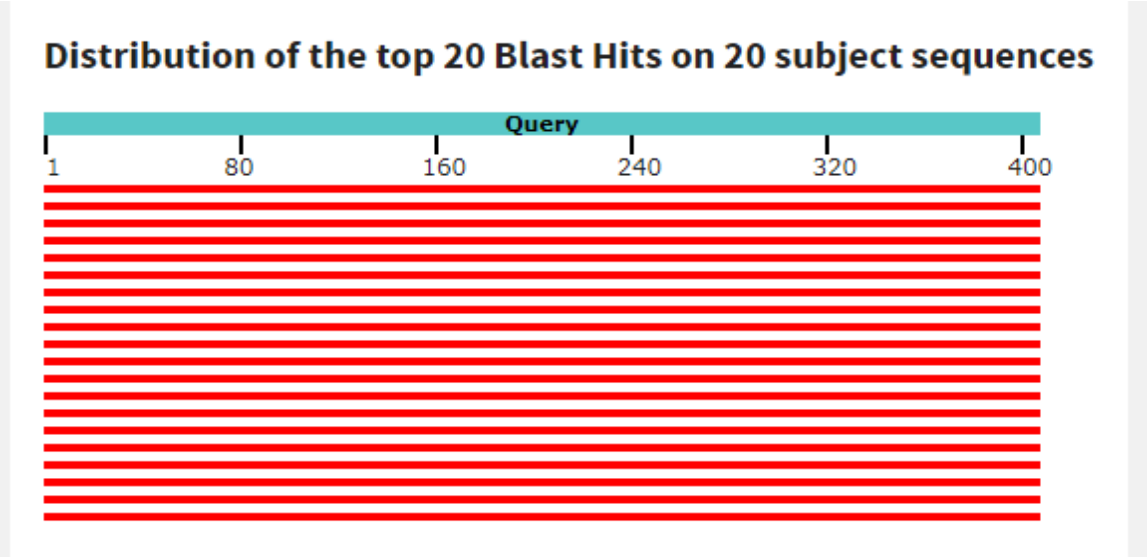


Figure 2: Distribution of the top 20 explosive topic sequences.

The forward and reverse nucleotide sequences of each sample in this study ranged from 97% to 100% in comparison with global virus strains.

Table (3-13) shows the degree of concordance between Iraqi isolates and international isolates, as Iranian isolates are 100% similar to the Iraqi isolate, and the similarity between Iraqi and European isolates was close, as the percentage of similarity is approximately 98% with Belgian isolates and (97.78, 97.54, 97.78), with German, American and Chinese isolates, respectively.

Phylogenetic Analysis Results

Cytomegalovirus DNA Sequencing Results.

DNA sequencing results showed the presence of two sequences.

Subsequently, HCMV-infected pregnant women were submitted to the NCBI-GenBank database to obtain accession number codes for the first time in Iraq. (Figure 5). DNA sequencing was analyzed for phylogenetic confirmation to identify CMV virus.

Genetic analysis and construction of a phylogenetic tree for the two Iraqi isolates from this study included (PP828639,PP828640).

Results of cytomegalovirus strain registration in pregnant women in Al-Diwaniyah province.

Two copies of the cytomegalovirus nucleocapsid gene were discovered and submitted to GenBnk - Bankit was submitted to register these copies in the US GenBank, and submission IDs were received for both submitted sequences. All previous transcription results for the nucleocapsid gene were analyzed and screened by GenBank professional staff, entered into the databases, and assigned accession numbers starting from Fasta options (PP828639.1 -PP828640.1) (Figure 4).

These results have been registered and published in the International Database for Sequence Collaboration (INSDC) on this site, which includes the National Center for Biotechnology Information (NCBI), GenBank, the European Nucleotide Archive (ENA), and the DNA Data Bank of Japan (DDBJ).

Description	Score	E value	Accession
Human betaherpesvirus 5 [viruses]	▼Next	▲Previous	◀◀First
Human herpesvirus 5 from Iran US7 and US8 genes, partial cds	745	0.0	AY327403
Human herpesvirus 5 strain BE/13/2010, complete genome	701	0.0	KP745645
Human herpesvirus 5 strain BE/13/2011, complete genome	695	0.0	KP745657
Human herpesvirus 5 strain BE/17/2010, complete genome	695	0.0	KP745727
Human herpesvirus 5 strain BE/2/2012, complete genome	695	0.0	KP745710
Human herpesvirus 5 strain BE/45/2011, complete genome	695	0.0	KP745633
Human betaherpesvirus 5 strain LUS248, complete genome	695	0.0	MK290743
Human betaherpesvirus 5 strain NL/Rot2/Urine/2012, partial genome	695	0.0	KT726941
Human herpesvirus 5 strain HAN11, complete genome	695	0.0	KJ361950
Human herpesvirus 5 isolate HAN, complete genome	693	0.0	KJ426589
Human herpesvirus 5 strain JHC, complete genome	689	0.0	HQ380895
Human herpesvirus 5 strain BE/16/2012, complete genome	689	0.0	KP745665
Human herpesvirus 5 strain AD169, complete genome	689	0.0	FJ527563
Human betaherpesvirus 5 strain HANSCTR8, complete genome	689	0.0	KY490082
Human betaherpesvirus 5 strain UK/Lon8/Urine/2012, complete genome	689	0.0	KT726951
Human betaherpesvirus 5 strain UK/Lon9/Urine/2012, partial genome	689	0.0	KT726953
Human herpesvirus 5 strain BE/26/2010, complete genome	689	0.0	KP745719
man herpesvirus 5 strain BE/11/2012, complete genome	689	0.0	KP745680

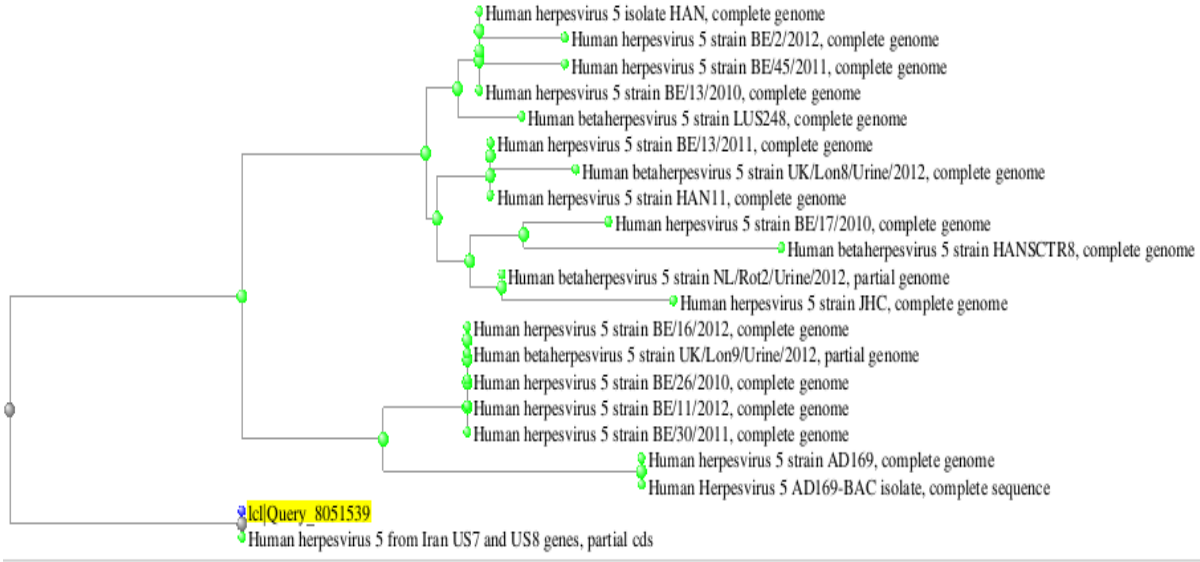
Figure (3) GenBank message with accession number.

Results of Phylogenetic Tree Construction

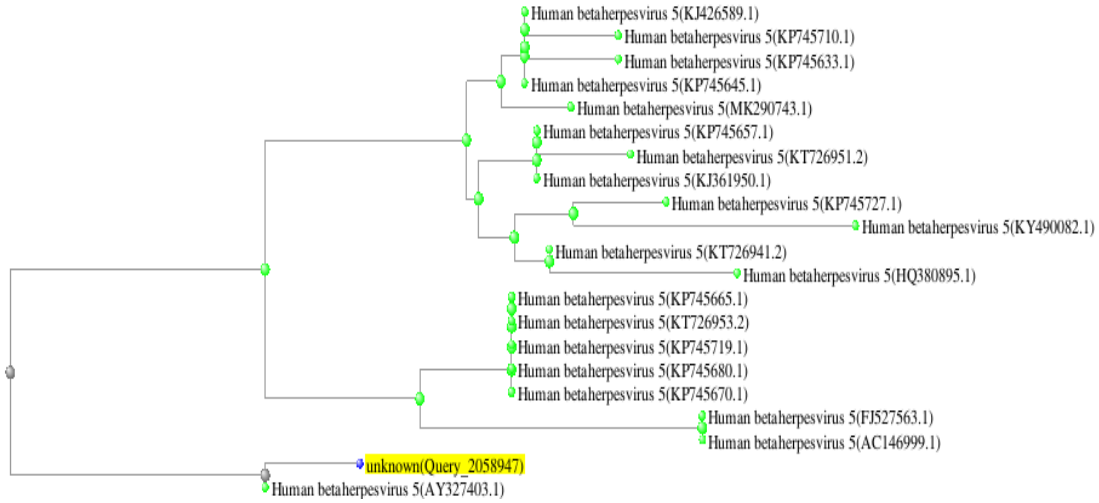
Phylogenetic tree construction was performed based on BLAST-NCBI-GenBank databases with Tree Distance, and sequence analysis of the HCMV nucleocapsid coding gene was performed using global resources for the dissemination of unclassified cytomegalovirus strains of the Herpesviridae family. The degree of identity between our clones and global CMV reference strains ranged from 97% to 100% compared to other members of the Herpesviridae family.

Results of phylogenetic studies for cytomegalovirus

(CMV) Evolutionary studies (PP828639,PP828640) on two CMV positive samples showed the presence of genotypes US8 and US7 in the studied samples. Figure 5. Phylogenetic tree showing partial nucleotide sequences of genes encoding viral structural proteins of cytomegaloviruses isolated from blood samples of pregnant women in Diwaniyah city.



Figer 4: Phylogenetic analysis of partially sequenced HCMV gL genes from 2 clinical samples from pregnant women.



Figer 5: Phylogenetic analysis of partially sequenced HCMV gL genes from 2 clinical samples from pregnant women.

DISCUSSION

Phylogenetic Tree

The UL8 gene of human cytomegalovirus (HCMV) is one of the genes that plays a critical role in viral replication and evolution. The UL7 gene of this virus is also an important part of viral biology, especially in the field of viral replication and aggregation. Understanding the evolutionary relationships between UL7, UL8, and similar genes in other viruses can provide valuable information about the evolutionary history and functional significance of these genes. Studies have shown that UL7 and UL8 genes have homologous sequences to the UL8 gene in other herpesviruses.[19, 20]

Phylogenetic tree analysis showed that the UL7 and UL8 genes of human beta-herpesvirus type 5 (HCMV) isolates in Iraq share 100% similarity with Iranian genetic isolates. This indicates a very close genetic relationship between HCMV strains in Iraq and Iran, especially in the UL7 and UL8 gene regions. This high level of similarity (100%) could have important implications for understanding the dynamics of HCMV transmission in the region and suggests that there are common transmission routes of this virus between Iraq and Iran.

The genetic similarity of the UL7 and UL8 genes in Iraqi isolates has been reported to be very high compared to different global strains. The high similarity ratios indicate that these Iraqi isolates have significant genetic inheritance with European, African, and Asian isolates, suggesting a possible evolutionary relationship or common origin. The Iraqi genes showed 98.03% to 97.54% similarity with European, African and Asian isolates. The similarity ratio with Belgian isolates was reported to be 98.03%, indicating a close genetic relationship. Also, 97.78% similarity was observed with isolates from Germany, the Netherlands, China and Zambia, and 97.54% with isolates from the United States and the United Kingdom.

In a study conducted by Ahmed et al. in Iraq in 2022, samples were analyzed for amplification of the UL122 gene of human beta-herpesvirus type 5 (CMV), which had 99% sequence similarity to European strains. Also, the sequence of the TRM3 gene from HHV-6 (subunit 3) showed 100% similarity to African and European sources. Finally, amplification of the DNA polymerase B gene, which encodes the UL15 subunit protein of human gamma-herpesvirus type 4 (EBV), had 100% similarity to the American strain of this virus.[21]

A study by S. Pignatelli et al. of 223 clinical CMV samples identified four main regions of gN gene prevalence according to geographic origin: Europe, China, Australia, and North America. It was found that the different gN variants were widely and similarly distributed in these regions (without significant differences).[22]

A study by Suarez et al. (2019) showed that multi-allelic regions have a different distribution of alleles depending on geographical location, but the majority of alleles are evenly distributed globally.[23]

In Charles 2023's PhD thesis at the University of London, it was shown that the allelic distribution is similar between continents and that this distribution is mainly observed in genes associated with the interaction of CMV with the host immune system.[24]

In a study by Sijamoun et al.it was found that Asian strains in the genetic analysis network are in the neighborhood of European and North American strains, but there is no separate clustering for them.[25]

In another study by Charles et al. at least two main groups of human social cytomegalia (CMV) were identified responsible for geographical differences. These differences are mainly expressed in the protected areas of the genome, although the time of separation and the expansion between the two groups is still unclear.[5]

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