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RESEARCH ARTICLE

Genotyping of Human Cytomegalovirus Envelop Glycoprotein B in Iraqi Immunocopromised and immunosupresser Patients

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Abstract

Envelope glycoprotein B is a surface protein of CMV which plays an important role in the pathogenesis of the virus, polymorphism in the gB gene may interfere with transmission of infection. This study was aimed to investigate the HCMV glycoprotein B genotypes in immunosupressed and immunocompromised Iraqi patients. A total of 362 Iraqi patients were collected from immunosupressed (pregnant women and infants) and immunocompromised (renal transplant and malignancies patients) that have been tested for the presence of acute infection of HCMV, during the period from November 2014 to February 2015. While another 20 individuals with negative serum IgM/IgG were included as negative control. The blood samples were positive for CMV-IgM and both IgM /IgG by ELISA and ELFA were found to be present in 85 (23.48 %) out of 362 blood samples. Five PCR confirmed isolates were chosen randomly for DNA sequences and similarity. Searches were carried out with the Basic Local Alignment Search Tool (BLAST) in National Center of Biotechnology Information (NCBI). The data showed that the glycoprotein B type 2 was the most common genotypes among the studied immunocoprimised and immunosupressed Iraqi patients. The nucleotide sequences of the glycoprotein B UL55 region from HCMV in this study have been deposited in the Gen Bank sequence database of HCMV under the following accession numbers: M17209.1, KP745721.1 and X17403.1.

Introduction

Human Cytomegalovirus (HCMV) is widely distributed among humans. Like other viruses of the Herpesviridae family, it causes a primary infection and then remains latent in the body. Despite causing a usually harmless primary infection, CMV can be life-threatening for immune compromised patients and can cause serious fatal damages. Hence, infection in pregnant women assumes high importance [1]. The HCMV particles exhibit a highly complex structure. They have approximately 250 nm in diameter and are composed of four morphologically defined structures; the DNA genome and the capsid, together are called nucleocapsid, the tegument layer and the surrounding envelope [2].

This virus encodes a number of surface glycoproteins that are critically involved in its lifecycle [3]. Viral envelope glycoproteins play an important role in most stages of viral replication and infectivity as they mediate the viral attachment and fusion to neighboring cells as well as uncoating of the virus within the cell [4]. Glycoprotein B is the most immunogenic of all glycoproteins and a variety of evidence indicates that glycoprotein B has an essential function particularly in cell entry, transmission, viral attachment, targeting of progeny virus to the apical membrane for release from polarized cells, and fusion to the host cell membrane [5, 6].In order to improve and enhance diagnosis, management and treatment of such infection, the investigation of the molecular sequence of glycoprotein B genotyping from HCMV that invades the imunocompromised and immunosupresser may improve our understanding of HCMV epidemiology, and pathogenesis.

Depending on our best knowledge, no study has been performed in Iraq for identifying HCMV glycoprotein B genotyping and sub typing and its distribution among Iraqi patients. Hence, the aim of the present study was to Screen the blood samples of immunocompromised and immunosupresser Iraqi patients by using ELISA and ELFA techniques to detect the infection of HCMV by detecting IgM and IgG. Also determine HCMV glycoprotein B genotypes found in blood samples of immunocompromised and immunosupresser patients by using specific multiplex nested PCR assay for each glycoprotein. As well as to study the prevalence of HCMV glycoprotein B

genotyping in Iraqi patients and sequence analysis.

Material and Methods

This cross sectional prospective study was conducted from first November 2014 to end February 2015 at Baghdad medical city hospital and the practical part of the study was accomplished in Baghdad Central Public Health Laboratory (CPHL) / Molecular biology department.

Study Groups

A total of 362(245 female and 117 male) patients (immunosupresser patients that are listed in the table (1)) were included in this study. Anti-CMV IgM/ IgG antibodies were detected in samples from 362 patients, while the others 20 individuals were chosen as a control group where anti CMV-IgM and IgG antibodies were not detected in their samples.

A sample of 5 ml blood was drawn from each patient by venipuncture. Investigations included: anti-CMV antibodies by (Enzyme-linked Immunosorbent Assay (ELISA), Enzyme Linked Fluorescent Assay (ELFA) in the serum, while the extraction of CMV DNA in the plasma.

Table 1: Group and number of immunocoprimised & immunosupresser patients

NO	Type of the patients	Number of the patients
1	pregnant women	100
2	Infants	45
3	Child with acute leukemia and malignant tumor	131
4	Adult with acute leukemia and malignant tumor	39
5	Renal transplant patients	47
Total		362

Glycoprotein B Genotypes by Multiplex Nested PCR Assay

Viral DNA was extracted from plasma samples by ExiprepTMPlus Viral DNA/RNA Kit (Bioneer, Korea) and the genomic DNA concentration and purity was determined by using the nanodrop. The oligonucleotides primers are described by Tarrago ET al.2003 [7] listed in table 2 and were supplied by Biosynthesis Company. HCMV gB genotype was performed by multiplex nested PCR using a mixture of specific primers to each of gB types. For detection of different gB genotypes, nested multiplex PCR was performed with two external primers and five upstream inner primers specific for each gB genotype (gB-1, gB-2, gB-3, gb-4, and gB-5) and a one downstream primer.

The first round of the nested multiplex PCR was carried out in a 50 µl reaction volume using 1 µl of each external upstream and downstream primers(10 pmol/ µl), 5 µl of purified DNA, 25µl GoTaq green Master Mix 2X (promega, Germany) and 18 µl of nuclease free water. The PCR thermal profile started with an initial denaturation 94°C for 5min, followed by 35 cycles at 94°C for 45s ,60°C for 1min ,and 72°C for 45 s, followed by terminal extension at 72°C for10 min. The second round of PCR was performed using 5 µl of the first amplified products as DNA template and a mixture of (10 pmol/ µl) of each inner primer in a 50 µl total volume. Reaction was carried out under conditions identical to those used in the first round, but the annealing temperature was 58°C instead of 60°C.

Table 2: Primer sequences of multiplex nested PCR for HCMV glycoprotein B (UL55) gene

Primers	Primer sequences	Amplicon length
First round:	5' TTT GGA GAA AAC GCC GAC3' 5'CGC GCG GCA ATC GGT TTG TTG TA3'	**751 bp**
Forward CMV Q1+ Reverse CMV Q1-		
Second round: Forward primers		
CMV GT1+ (gB1)	5' ATG ACC GCC ACT TTC TTA TC 3'	**420 bp**
CMV GT2+ (gB2)	5' TTC CGA CTT TGGA AGA CCC AAC 3'	**613 bp**
CMV GT3+ (gB3)	5'TAG CTC CGG TGT GAA CTC C 3'	**190 bp**
CMV GT4+ (gB4)	5' ACC ATT CGT TCC GAA GCC GAG GAG TCA 3'	**465 bp**
CMV GT5+ (gB5)	5' TAC CCT ATC GCT GGA GAA C 3'	**139 bp**
Common reverse Primer CMV Q2-	5' GTT GAT CCA CAC ACC AGG C 3'	

Agarose Gel Electrophoresis of PCR Products

Ten µl of amplified PCR products were analysed on 2 % agarose gels (Bio-Rad/ USA) stained with ethidium bromide and viewed

under UV transilluminator. The amplified products size was determined by comparing with the reference DNA molecular weight marker (Ladder), in this study 100-1000 bp Ladder was used

DNA Sequencing of MPCR Products

To confirm the results of HCMV genotyping, the entire gB gene was sequenced. Eight PCR confirmed isolates were chosen randomly for DNA sequences and similarity. PCR products were commercially sequenced at Macrogen Company (Korea) for the DNA sequence analysis. Sequencing was performed on each sample using the same upstream and downstream primers used in the amplification of the entire gB. Searches were submitted with the Basic Local Alignment Search Tool (BLAST) in National Center of Biotechnology Information (NCBI).

Statistical Analysis

The Statistical Analysis System- SAS [8] was used to study the effect of different factors in studied parameters. Chi-square test was used to compare between means and in this study.

Results

A total of 362 serum specimens were tested for the presence of CMV IgM and IgG using ELISA and ELFA techniques suspected hematological malignancy patients (Childs and adult), renal patients, pregnant women and infants shown in Table (3) (from previous our study [9,10].

Table 3: Distribution of samples study by ELISA and ELFA according to patients group

Patients	Age group	Sex	IgG	IgM	IgG+IgM	Negative	Total	P value
Renal patients	17-65 years	F	8 (4.88%)	2(8.00%)	4(6.67%)	3 (2.66%)	17(4.70%)	NS
		M	19(11.59%)	1(4.00%)	3(5.00%)	7 (6.20%)	30(8.29%)	NS
malignancy Adults	17-65 years	F	12 (7.32%)	0 0.00%)	2(3.33%)	6 (5.31%)	20(5.53%)	NS
		M	5 (3.05%)	2(8.00%)	3(5.00%)	9 (7.97%)	19(5.24%)	NS
Malignancy Children	10 m-14years	F	39(23.78%)	4(16.0%)	9(15.0%)	30 (2.65%)	82(22.65%)	S
		M	26(15.85%)	3(12.0%)	8(13.33%)	12(10.62%)	49(13.54%)	NS
Infants	32d-8m	F	6 (3.66%)	5(20.0%)	6(10.00%)	9 (7.97%)	26 (7.18%)	S
		M	4 (2.44%)	2(8.00%)	3 (5.00%)	10 (8.85%)	19 (5.25%)	NS
Preg.	20-35 years	F	45(27.44%)	6(24.0%)	22(36.67%)	27(23.89%)	100(27.62%)	S
Total	--	--	164	25	60	113	362	-
Control group	Different age and sex		-	-	-	20	20	-
P-value	--	--	S **	S **	S **	S **	S **	-
* (P<0.05) Significant, ** (P<0.01) Highly significant, NS: Non-significant.								-

Genotyping of Glycoprotein B by Multiplex Nested PCR

From previous our study [9, 10] the results showed that HCMV glycoprotein B type 2 was highest prevalent among immunosupresser Iraqi patients (infants and pregnancy) and who have received chemotherapy (leukemia & tumor) patients (children and adults). The distribution of gp2 was as follow (In renal transplant patients gp 2 was in 2 patients out of 7 patients; in malignancies patients there was 8 patients out of 12 patients; in infants there was 5 patients out of 9 patients; and in pregnant women there was 7 patients out of 10 patients). So the total distribution of gp2 in all patients was 22 out of 38 patients. From these 22 patients only 5 samples were randomly chosen for sequencing.

Sequence Analysis

Nucleic acid sequencing was performed on PCR products to confirm their specificity and provide the ultimate means to identify and

characterize the virus. Nucleic acid sequencing of selected genomic region (glycoprotein B UL55 region) of HCMV has been used to determine the genetic relatedness of isolates. Nucleotide sequencing of nested multiplex PCR products for glycoprotein B type 2 (613bp) were carried out for five chosen randomly isolates using the applied biosystem (ABI) capillary system (Macrogen Research, Seoul, Korea). PCR products were subjected to direct sequencing, forward strand of PCR products were sequenced with an automatic sequencer. DNA sequences were analyzed and similarity searches were carried out with the Basic Local Alignment Search Tool (BLAST) in National Center of Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of the glycoprotein B UL55 region from HCMV in the present study have been deposited in the GenBank sequence database under the following accession numbers (M17209.1, KP745721.1 and X17403.1). The sequence analysis of five HCMV isolates that produced identity in nucleotides sequence of the glycoprotein B region type 2 ranged to100.0% were documented in the present study, these isolates under the accession numbers (M17209.1 , KP745721.1 and X17403.1) and no deletion and insertion of nucleotide were seen in these isolates (samples No. 1,6,9,15 and 24) Figures 1 to 5.

Sequence of HCMV glycoprotein B type 2(Sample No. 1)

TAGGTTGGTGGCTTTTCTCGACGTGCCGACTCGGTGATCTCTTGGGATATACAGGACGAGA
AGAATGTCACCTGCCAGCTCACCTTCTGGGAAGCCTCGGAACGTACTATCCGTTCCGAAGC
CGAAGACTCGTACCACTTTTCTTCTGCCAAAATGACTGCAACTTTTCTGTCTAAGAAACAAG
AAGTGAACATGTCCGACTCCGCGCTGGACTGCGTACGTGATGAGGCTATAAATAAGTTACA
GCAGATTTTCAATACTTCATACAATCAAACATATGAAAAATACGGAAACGTGTCCGTCTTC
GAAACCAGCGGCGGTCTGGTGGTGTTCTGGCAAGGCATCAAGCAAAAATCTTTGGTGGAA
TTGGAACGTTTGGCCAATCGATCCAGTCTGAATATCACTCATAGGACCAGAAGAAGTACGA
GTGACAATAATACAACTCAATTGTCCAGCATGGAATCGGTGCACAATCTGGGTCTACGCCC
AGCTGCAGTTCACCTATGACACGGTTTGCGCGGGTTACATCAACCGGGGCGCTGGCGCAA
ATCGCAGAAACCCTGGGTGTGTGG

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CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGTA
TTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGCCAA
ACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCGCCG
CTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATTGAA
AATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGACATG
TTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGTACG
AGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGCAGG
TGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAAAGC
CACC

Standard Gene Sequence from GenBank

CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGTA
TTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGCCAA
ACGTTCCAATTCCACCAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCGCCGCT
GGTTTCGAAGACGGACAAACGTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATTGAAA
ATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGACATGT
TCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGTACGA
GTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGCAGGT
GACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAAAGCC
ACC

Human cytomegaloviruses F fragment DNA encoding DNA polymerase and glycoprotein B, complete cds. Sequence ID: gb|M17209.1|HS5VF Length: 20349Number of Matches: 1Related Information GEO Profiles-microarray expression data Range 1: 17458 to 18010 GenBank Graphics Next Match Previous Match First Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1022 bits(553)	0.0 (0)	553/553(100%)	0/553(0%)	Plus/Plus

Features:

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Sbjct 17458
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17517

Query 61
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGT
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Sbjct 17518
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGT
17577

Query 121
ATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
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Sbjct 17578
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17637

Query 181
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240
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Sbjct 17638
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17697

Query 241
GCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGT
300
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Sbjct 17698
GCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGT
17757

Query 301
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360
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Sbjct 17758
ATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTC
17817

Query 361
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420
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Sbjct 17818
GGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAA
17877

Query 421
GTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAG
480
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Sbjct 17878
GTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAG
17937

Query 481
CTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTC
540
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Sbjct 17938
CTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTC
17997

Query 541 GAGAAAAGCCACC 553
||||||||||||
Sbjct 17998 GAGAAAAGCCACC 18010

Figure 1: sequences of HCMV isolate (Sample No.1) with glycoprotein B type2 genotype

Sequence of HCMV glycoprotein B type 2 (Sample No. 6).

GTGGCTTTTCTCGAACGTGCCGACTCGGTGATCTCTTGGGATATACAGGACGAGAAGAATG
TCACCTGCCAGCTCACCTTCTGGGAAGCCTCGGAACGTACTATCCGTTCCGAAGCCGAAGA
CTCGTACCACTTTTCTTCTGCCAAAATGACTGCAACTTTTCTGTCTAAGAAACAAGAAGTGA
ACATGTCCGACTCCGCGCTGGACTGCGTACGTGATGAGGCTATAAATAAGTTACAGCAGAT
TTTCAATACTTCATACAATCAAACATATGAAAAATACGGAAACGTGTCCGTCTTCGAAACCA
GCGGCGGTCTGGTGGTGTTCTGGCAAGGCATCAAGCAAAAATCTTTGGTGGAATTGGAAC
GTTTGGCCAATCGATCCAGTCTGAATATCACTCATAGGACCAGAAGAAGTACGAGTGACAA
TAATACAACTCATTTGTCCAGCATGGAATCGGTGCACAATCTGGTCTACGCCCAGCTGCAG
TTCACCTATGACACGTTGCGCGGTTACATCAACCGGGCGCTGGCGCAAATCGCAGAAGCCT
GGTG

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CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG
GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT
GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
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CCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATT
GAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGAC
ATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGT
ACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGC
AGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAA
AGCCACC

Standard Gene Sequence from GenBank

CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG
GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT
GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
CAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCG
CCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATT
GAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGAC
ATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGT
ACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGC
AGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAA
AGCCAC

Human cytomegalovirus F fragment DNA encoding DNA polymerase and glycoprotein B, complete cds Sequence ID: gb|M17209.1|HS5VF Length: 20349Number of Matches: 1Related Information GEO Profiles-microarray expression data Range 1: 17456 to 18010GenBankGraphics Next Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
1002 bits(1110)	**0.0**	**555/555(100%)**	**0/555(0%)**	**Plus/Minus**

Query 1
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Sbjct 17456
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17515

Query 61
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Sbjct 17516
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17575

Query 121
GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTG
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Sbjct 17576
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17635

Query 181
GCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGA
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Sbjct 17636
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17695

Query 241
CCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAA
300

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Sbjct 17696
CCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAA
17755

Query 301
GTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAG
360

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Sbjct 17756
GTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAG
17815

Query 361
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Sbjct 17816
TCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAA
17875

Query 421
AAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTG
480

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Sbjct 17876
AAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTG
17935

Query 481
AGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGT
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Sbjct 17936
AGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGT
17995

Query 541 TCGAGAAAAGCCACC 555

|||||||||||||||

Sbjct 17996 TCGAGAAAAGCCACC 18010

Figure 2: sequences of HCMV isolate (Sample No. 6) with glycoprotein B type 2 genotype

Sequence of HCMV glycoprotein (Sample No. 9)

BGGTGGCTTTTCTCGAACGTGCCGACTCGGTGATCTCTTGGGATATACAGGACGAGAAGAA
TGTCACCTGCCAGCTCACCTTCTGGGAAGCCTCGGAACGTACTATCCGTTCCGAAGCCGAA
GACTCGTACCACTTTTCTTCTGCCAAAATGACTGCAACTTTTCTGTCTAAGAAACAAGAAGT
GAACATGTCCGACTCCGCGCTGGACTGCGTACGTGATGAGGCTATAAATAAGTTACAGCAG
ATTTTCAATACTTCATACAATCAAACATATGAAAAATACGGAAACGTGTCCGTCTTCGAAAC
CAGCGGCGGTCTGGTGGTGTTCTGGCAAGGCATCAAGCAAAAATCTTTGGTGGAATTGGA
ACGTTTGGCCAATCGATCCAGTCTGAATATCACTCATAGGACCAGAAGAAGTACGAGTGAC
AATAATACAACTCATTTGTCCAGCATGGAATCGGTGCACAATCTGGTCTACGCCCAGCTGC
AGTTCACCTATGACACGTTGCGCGGTTACATCAACCGGGCGCTGGCGCAAATCGCAGAAG
CCTGG

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CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGTA
TTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGCCAA
ACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCGCCG
CTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATTGAA
AATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGACATG
TTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGTACG
AGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGCAGG
TGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAAAGC
CACC

Standard Gene Sequence from GenBank

CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGTA
TTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGCCAA
ACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCGCCG
CTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGTATTGAA
AATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTCGGACATG
TTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGTACG
AGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAGCTGGCAGG
TGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAAAGC
CAC

Human cytomegalovirus F fragment DNA encoding DNA polymerase and glycoprotein B, complete cds Sequence ID: gb|M17209.1|HS5VF Length: 20349Number of Matches: 1 Related Information GEO Profiles-microarray expression data Range 1: 17458 to 18010GenBankGraphics Next Match Previous Match

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
998 bits(1106)	**0.0**	**553/553(100%)**	**0/553(0%)**	**Plus/Minus**

Query 1
CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
60

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Sbjct 17458
CCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAGGT
17517

Query 61
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGT
120

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Sbjct 17518
GAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTTGT
17577

Query 121
ATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
180

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Sbjct 17578
ATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
17637

Query 181
CAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACC
240

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Sbjct 17638
CAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACC
17697

Query 241
GCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGT
300

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Sbjct 17698
GCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTGTATGAAGT
17757

Query 301
ATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTC
360

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Sbjct 17758
ATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCCAGCGCGGAGTC
17817

Query 361
GGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAA
420

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Sbjct 17818
GGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAA
17877

Query 421
GTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAG
480

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Sbjct 17878
GTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGCTTCCCAGAAGGTGAG
17937

Query 481
CTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTC
540

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Sbjct 17938
CTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTC
17997

Query 541 GAGAAAAGCCACC 553

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Sbjct 17998 GAGAAAAGCCACC 18010

Figure 3: sequences of HCMV isolate (Sample No. 9) with glycoprotein B type2 genotype

HCMV glycoprotein B type 2 (Sample No. 15)

GTGGCTTTTCTCGAACGTGCCGACTCGGTGATCTCTTGGGATATACAGGACGAGAAGAATG
TCACCTGCCAGCTCACCTTCTGGGAAGCCTCGGAACGCACTATCCGTTCCGAAGCCGAAGA
TTCGTACCACTTTTCTTCTGCCAAAATGACTGCAACTTTTCTGTCTAAGAAACAAGAAGTGA
ACATGTCCGACTCCGCGCTAGACTGCGTACGTGATGAGGCTATAAATAAGTTACAGCAGAT
TTTCAATACTTCATATAATCAAACATATGAAAAATACGGAAACGTGTCCGTCTTCGAAACCA
GCGGCGGTCTGGTGGTGTTCTGGCAAGGCATCAAGCAAAAATCTTTGGTGGAATTGGAAC
GTTTGGCCAATCGATCCAGTCTGAATATCACTCATAGGACCAGAAGAAGTACGAGTGACAA
TAATACAACTCATTTGTCCAGCATGGAATCGGTGCACAATCTGGTCTACGCCCAGCTGCAG
TTCACCTATGACACGTTGCGCGGTTACATCAACCGGGCGCTGGCGCAAATCGCAGAAGCCT
GGTGT

Minus → **plus**

CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG
GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT
GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC
CAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCG
CCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTATATGAAGTATT
GAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCTAGCGCGGAGTCGGAC
ATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGT
ACGAATCTTCGGCTTCGGAACGGATAGTGCGTTCCGAGGCTTCCCAGAAGGTGAGCTGGC
AGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAA
AGCCAC

Standard gene sequences from GenBank

CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG
GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT
GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTGGC

CAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGACCG CCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTATATGAAGTATT GAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCTAGCGCGGAGTCGGAC ATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAAAAGTGGT ACGAATCTTCGGCTTCGGAACGGATAGTGCGTTCCGAGGCTTCCCAGAAGGTGAGCTGGC AGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGTTCGAGAAA AGCCA

Human Herpes virus 5 strain BE/14/2010, complete genome Sequence ID : gb|KP745721.1| Length: 234537Number of Matches: 1 Related Information Range 1: 82678 to 83231GenBankGraphics

Query 1 CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG 60

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Sbjct 82678 CACCAGGCTTCTGCGATTTGCGCCAGCGCCCGGTTGATGTAACCGCGCAACGTGTCATAG 82737

Query 61 GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT 120

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Sbjct 82738 GTGAACTGCAGCTGGGCGTAGACCAGATTGTGCACCGATTCCATGCTGGACAAATGAGTT 82797

Query 121 GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTG 180

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Sbjct 82798 GTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGATATTCAGACTGGATCGATTG 82857

Query 181 GCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGA 240

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Sbjct 82858 GCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTTGCCAGAACACCACCAGA 82917

Query 241 CCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTATATGAA 300

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Sbjct 82918
CCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATATGTTTGATTATATGAA
82977

Query 301
GTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCTAGCGCGGAG
360

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Sbjct 82978
GTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGCAGTCTAGCGCGGAG
83037

Query 361
TCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAA
420

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Sbjct 83038
TCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCATTTTGGCAGAAGAA
83097

Query 421
AAGTGGTACGAATCTTCGGCTTCGGAACGGATAGTGCGTTCCGAGGCTTCCCAGAAGGTG
480

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Sbjct 83098
AAGTGGTACGAATCTTCGGCTTCGGAACGGATAGTGCGTTCCGAGGCTTCCCAGAAGGTG
83157

Query 481
AGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGT
540

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Sbjct 83158
AGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACCGAGTCGGCACGT
83217

Query 541 TCGAGAAAAGCCAC 554

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Sbjct 83218 TCGAGAAAAGCCAC 83231

Figure 4: sequences of HCMV isolate (Sample No. 15) with glycoprotein B type2 genotype

Sequence of HCMV glycoprotein B type 2 (Sample No. 24)

ACGTGCCGACTCGGTGATCTCTTGGGATATACAGGACGAGAAGAATGTCACCTGCCAGCTC
ACCTTCTGGGAAGCCTCGGAACGTACTATCCGTTCCGAAGCCGAAGACTCGTACCACTTTT
CTTCTGCCAAAATGACTGCAACTTTTCTGTCTAAGAAACAAGAAGTGAACATGTCCGACTC
CGCGCTGGACTGCGTACGTGATGAGGCTATAAATAAGTTACAGCAGATTTTCAATACTTCA
TACAATCAAACATATGAAAAATACGGAAACGTGTCCGTCTTCGAAACCAGCGGCGGTCTGG
TGGTGTTCTGGCAAGGCATCAAGCAAAAATCTTTGGTGGAATTGGAACGTTTGGCCAATCG
ATCCAGTCTGAATATCACTCATAGGACCAGAAGAAGTACGAGTGACAATAATACAACTCAT
TTGTCCAGCATGGA

Plus – minus ⟹ **plus –plus**

TCCATGCTGGACAAATGAGTTGTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGAT
ATTCAGACTGGATCGATTGGCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTT
GCCAGAACACCACCAGACCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATA
TGTTTGATTGTATGAAGTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGC
AGTCCAGCGCGGAGTCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCAT
TTTGGCAGAAGAAAAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGC
TTCCCAGAAGGTGAGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACC
GAGTCGGCACGT

Standard gene sequence from GenBank

TCCATGCTGGACAAATGAGTTGTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTGAT
ATTCAGACTGGATCGATTGGCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATGCCTT
GCCAGAACACCACCAGACCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTTTCATA
TGTTTGATTGTATGAAGTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCACGTACGC
AGTCCAGCGCGGAGTCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTTGCAGTCAT
TTTGGCAGAAGAAAAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGTTCCGAGGC
TTCCCAGAAGGTGAGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAAGAGATCACC
GAGTCGGCACGT

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
815 bits(441)	**0.0**	**441/441(100%)**	**0/441(0%)**	**Plus/Plus**

Human cytomegalovirus strain AD169 complete genome Sequence ID :emb |X17403.1| Length: 229354Number of Matches: 1 Related Information Range 1: 82075 to 82515 Gen Bank Graphics

Query 1
TCCATGCTGGACAAATGAGTTGTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTG
60

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Sbjct 82075
TCCATGCTGGACAAATGAGTTGTATTATTGTCACTCGTACTTCTTCTGGTCCTATGAGTG
82134

Query 61
ATATTCAGACTGGATCGATTGGCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATG
120

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Sbjct 82135
ATATTCAGACTGGATCGATTGGCCAAACGTTCCAATTCCACCAAAGATTTTTGCTTGATG
82194

Query 121
CCTTGCCAGAACACCACCAGACCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTT
180

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Sbjct 82195
CCTTGCCAGAACACCACCAGACCGCCGCTGGTTTCGAAGACGGACACGTTTCCGTATTTT
82254

Query 181
TCATATGTTTGATTGTATGAAGTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCA
240

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Sbjct 82255
TCATATGTTTGATTGTATGAAGTATTGAAAATCTGCTGTAACTTATTTATAGCCTCATCA
82314

Query 241
CGTACGCAGTCCAGCGCGGAGTCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTT
300

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Sbjct 82315
CGTACGCAGTCCAGCGCGGAGTCGGACATGTTCACTTCTTGTTTCTTAGACAGAAAAGTT
82374

Query 301
GCAGTCATTTTGGCAGAAGAAAAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGT
360

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Sbjct 82375
GCAGTCATTTTGGCAGAAGAAAAGTGGTACGAGTCTTCGGCTTCGGAACGGATAGTACGT
82434

Query 361
TCCGAGGCTTCCCAGAAGGTGAGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAA
420

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Sbjct 82435
TCCGAGGCTTCCCAGAAGGTGAGCTGGCAGGTGACATTCTTCTCGTCCTGTATATCCCAA
82494

Query 421 GAGATCACCGAGTCGGCACGT 441

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Sbjct 82495 GAGATCACCGAGTCGGCACGT 82515

Figure 5: sequences of HCMV isolate (Sample No. 24) with glycoprotein B type2 genotype

Discussion

From the 362 samples, 113 serum samples were negative both IgM and IgG. Twenty negative control individuals with negative serum from both IgM and IgG were included. These ELISA tests were not differed on age or gender [11, 13]. Finding in the present study indicate high prevalence of HCMV 249 (68.78%) among Iraqi patients were included in this study and the present results agreement with previous investigation reported in Iraq at 57.2 % [14] and another study in Iraq by Al-azzawi(2012) showed both IgG and IgM sero positive was 50 (31.1%) [15]. The study of Bareq, *et.al*, 2018 showed Anti-HCMV IgG antibody was presented in 9/10 (90%) of normal Iraqi women, benign breast tumor patients 19/20 (95%) and malignant breast tumor patients 60/60 (100%) while anti-HCMV IgM antibody was only detected in breast cancer Iraqi patients 5/60 (8.3%) [16].In Iran at 72.1% [17] and in Nigeria at 87% [18]. The results from the present study were higher to those obtained

in developed countries for example in France 46.8 % and in Australia 56.9% [19]. The low percent in developed counties are probably due to inclusion of routine HCMV screening and improved hygienic standard [20]. The highest rates were probably associated with lack of proper hygienic practices and seroprevalence of HCMV vary geographically [21]. In the presented study, all IgG samples and seronegative samples were excluded, only 85(23.48%) samples out of 362 samples were found to be positive for IgM and mixed IgM/IgG by ELISA technique and the same results were found by ELFA technique. Samples were positive for IgM and both IgM/IgG were selected for multiplex nested PCR study.

Implementation of PCR for the detection of viral DNA in clinical samples has resulted in considerable improvement in diagnosis. The development of a multiplex PCR assay permit the amplification of multiple target sequences, for a rapid and accurate detection and typing of Cytomegalovirus (CMV) genotypes is very important for clinical diagnosis allow the delivery of therapy as early as possible [22; 23]. The present study used a multiplex PCR for the detection of HCMV glycoprotein B genotypes in both immunocompromised and immunosupressed patients. A multiplex nested PCR a range of primer pairs specific for a number of nucleotide sequences, careful design of the primer pairs used is critical to effective mPCR. Ideally the sets of primers should all have similar Tm scores and amplify sequences of a similar length.

This simplifies the optimization of the reaction. It may also be necessary to provide an increased concentration of both the polymerase enzyme and nucleotides to facilitate the amplification of multiple target sequences. Multiple PCR can be more prone to the production of non-specific amplified DNA molecules and so a nested format is used with a high annealing temperature. Use of high annealing temperatures favours specific primer target binding.

It may also be necessary to increase the number of cycles used in both rounds of amplification to maintain the sensitivity of the assay in comparison to any single PCR. Although the numbers of the results are small the data are in agreement with the numbers of studies through the literature [24, 26] indicating the glycoprotein B type 2 has been identified as the highest genotype in congenital infection. A study was carried out in Poland by Rycel *et al.,* (2014) who found that gB type 2 was the most common genotype samples of premature babies with HCMV congenital asymptomatic infection and 50% of women [26]. The findings of the present results were different from some other studies reported the gB type 2 less frequently than gB1 and gB3 for example, a study carried out in South Hungary by Lukacsi *et al.,* in 2001[27].

HCMV is considered to be most important opportunistic pathogen in organ and bone marrow transplant patients, because it is known to cause febrile illness in the post transplant period and may lead to tissue invasive disease [28]. Despite treatment with anti viral agent, the morbidity rate remains high, several studies have investigated the glycoprotein B of HCMV plays an important role in virus infectivity and correlation of gB type distribution in HCMV disease among immunocoprimised patients, but these studies found no significant association among glycoprotein B types in immunocoprimised patients with HCMV infection [29, 30].

Leukemia and tumor (hematological malignancies patients) are in immunodeficiency state as a result of treating with immuno- chemotherapy; therefore, the risk of HCMV infection increases in these patients and this virus can worsen, especially in those with mechanical ventilation, leukocytosis, and lack of appropriate early treatment [31, 32].

Conclusions

Multiplex nested PCR assay is useful to amplify a multiple target sequences for less effort and in a shorter time. PCR amplification of different HCMV glycoproteins from different regions of the local HCMV genome and sequencing proved and observed sequence variability in these fragment comparing with the published wild and lab strains sequences.

Also HCMV glycoprotein B type 2 was highest prevalent among immunosupresser Iraqi patients (infants and pregnancy) and who have received chemotherapy (leukemia & tumor) patients (children and adults).

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