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Molecular study of *Varicella Zoster* Virus Infection among individuals in Najaf province/Iraq

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Abstract : Varicella-zoster virus (VZV), double strand, recrudesces to the α-herpes virus family similarly identified as human herpes virus 3 (HHV3). Hereditarily, it is constant and is bifurcates into numerous genotypes founded upon the hereditary differences. The genotypes of herpes zoster virus (HZV) are not studied in Iraq.
HZV infection is a sorer international illness(55)adult patients with herpes zoster were confirmed for the existence of herpes-zoster virus(HZV) antibodies. All samples were assayed by real-time polymerase chain reaction(RT-PCR) for the exist of (HZV) DNA. The presence of anti -HZV antibody were detected by enzyme linked immunosorbent assay (ELISA) in 4 acute (7%) and22 recover (40%) zoster sera. VZV DNA was detected by PCR in 23 (41.8 %) acute zostersera and was shorter detectable in the recover patients.
Key word : Herpes zoster, *DNA* demodulation, RT-PCR, Sequence.

Introduction

Varicella-zoster virus (VZV)**,** similarly identified as human herpesvirus 3 (HHV3) (recrudesces to the herpesvirus family (Herpesviridae). This regulationis created depend on the morphological features of the virus and its physical andchemical characteristics. VZV which causes herpes zosterisdistributed locally asa pruritic vesicular exanthema in a dermatome in grown-up patient.Signstypicallyarise with achesideways the affected dermatome, subordinate in 2 to 3 days in a vesicular erosion that is normallyindicativeafter the primary infection of vzv, the causative agent gets latent in the ganglia of sensory nerve. This virus canimpartshingles (zoster) byThe next following reactivation .The maindangercause for shingles is increases with age,, inwhites thanblacks, the danger is advanced in females than males andwith a family historyindividuals of herpes zoster[1,2].Herpes Zoster(HZ) may sometimes cause lastingnervousdamagesinvolvingpost herpetic neuralgia, cranial nerve palsies, and ocularweaknesses[3].The infection become latent and recognized in individuals following*Varicella* whichmay be stimulated due to a variety of reasons involving elderly, and immunosuppression andproductshingles [4].In overall, difficulties and death of HZV disease are further usually noticed in immuno-deficient patients[5,6].The yearly occurrence of herpes zoster that have been record in population-basedstudies that have been publication in some countriesvarieties from 1.2 to 4.8 infectedcase per 1000 people/year[7].Genotypic information show that HZV is exact to topographicalsituation[8-10] and can be categorizedto four genotypes theAsian, African, Europa and Japanese. Furtherlately, the intermixed strains have been recognized in high number[11-13]. The genotypes of VZV nomenclature has been established indiverse genotyping approaches, lately a newfound worldwideterminology has been presented, (1–5) genotypes arefive major clades in addition to couplet new clades, VI and VII [14,15]

The prevalence rate of natural infection with HZ which determine in current study by ELISA and molecular assay amongpatients with different age groups in Al-Najaf province, Iraq.

Materials and Methods

Samples collection:

This cross sectional study was execute to detect herpes zoster via clinical characteristics , ELISA(Enzyme Linked ImmunoSorbent Assay) test kit and than confirmed by molecular assay of 55 shingles patients who are admitted in Al-Sadder medical city Hospital in Najaf province with one- year pursuing .Five ml of vinous blood were collected from each patient 2 ml of blood kept in (EDTA) tube as anticoagulant, and 3 ml left in the room temperature at 30 minutes. Then serum was separated by centrifuge at the 4000 rpm for 5 minutes. Both of the blood samples and sera stored at -20 °c in deep freeze [16] until used for serological and molecular assays.

Viral DNA extraction:

Viral DNA was extracted by utilizing (Genomic DNA Mini Kit, Geneaid. USA). The demodulation was achievedin accordance with company ordainingby thedemodulationProceduretechnique by used Proteinase K. Afterward, the removed DNA was testedby spectrophotometerNanodrop, then reservedat -20 °C at refrigerator tillsecondhand toperform Real-Time PCR assay.

RT-PCR (Real time- PCR)

RT- PCR technique was achieves torecognition Varicella-zoster virus by using SYBR Green I dyeqPCR amplification kit based on glycoprotein B gene (*gpB gene*).The primes were designed in this study by using NCBI-GenBank recorded sequence for Human herpesvirus 3 glycoprotein B gene, complete cds (GenBank: AY253673.1) and primer3 plus design online. The primers were supply through (Bioneer Company. Korea) as show in the following table 1.

Table (1):Varicella-zoster virus glycoprotein B gene(*gpB gene*) primers

Primer	Sequence (5'-3')		Amplicon
gpBgene	F	CGTTACGTCCGTGAAATCGC	234bp
	R	AATGGCCGTTCCGCTATCAT	

The RT- PCR amplification reaction was attained by using (AccuPower™ 2X Green star qPCR master mix kit, Bioneer. Korea) and qPCR master mix were equippedfor all samplesin agreement by company ordaining as following table (2):

Table (2):Samples DNA qPCR master mix

qPCR master mix	Volume
DNA template	2.5µL
2X Green star master mix	25µL
gpB Forward primer (10pmol)	1µL
gpB Reverse primer (10pmol)	1µL
DEPC water	20.5µL
Total volume	50µL

These qPCR master mix reaction ingredients that listed in table was placed in sterile white qPCR strip tubes and transferred into Exispin vortex centrifuge for 3minutes, the place in MiniOpticonRT- PCR method than applied the following thermocycler provisos as the following table(3):

Table (3): Real-Time PCR Thermo-cycler condition forVaricella-zoster virus

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 minute	1
Denaturation	95 °C	10 sec	45
Annealing\ Extension	60 °C	30 sec	
Detection(scan)			
Melting	65-95°C	0.5 sec	1

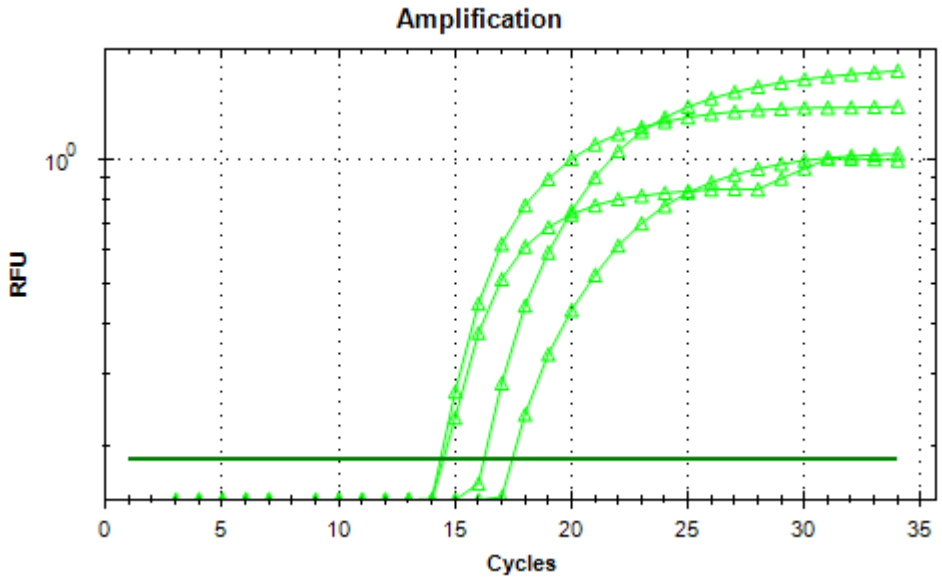
DNA Sequencer Method:

DNA sequencing technique was achieved for certaincharacterization ofVZV by the Phylogenetic tree testthat based on glycoprotein B gene. (PCR)polymerase chain reaction was done byusage same Real-Time PCR primers of glycoprotein B gene. DNA sequencing technique was done by Phylogenetic tree analysis usingUnweight Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version).

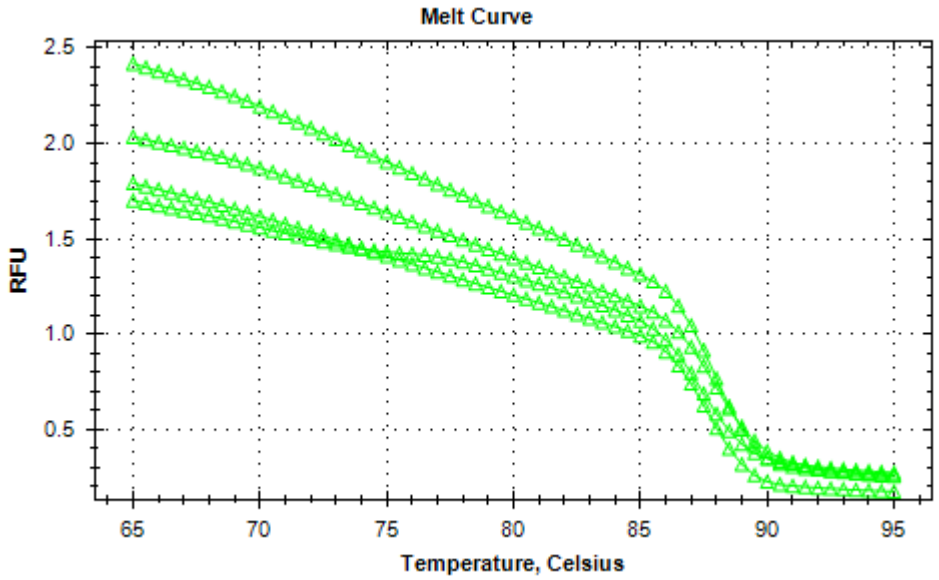
Results

Real-Time PCR

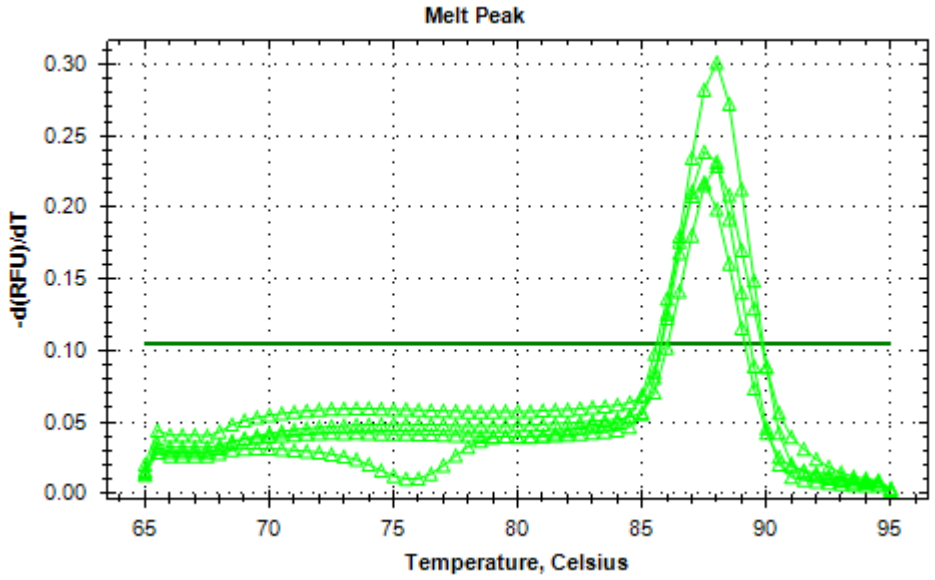
Real-Time PCR technique using SYBR Green I dye for detection of Varicella-zoster was show (4 positive samples) out of samples gave a positive result by amplification of gpB gene. (Figure-1)



(Fig. 1): Real-Time PCR amplification plots for glycoprotein B gene (*gpB gene*)Varicella-zoster virus positive samples.



(Fig. 2): Real-Time PCR melt curve of glycoprotein B gene (gpB gene) Varicella-zoster virus positive samples



(Fig. 3): Real-Time PCR melt peak of glycoprotein B gene (*gpB gene*)Varicella-zoster virus positive samples that show primers specifity at approximately 88C melt peak.

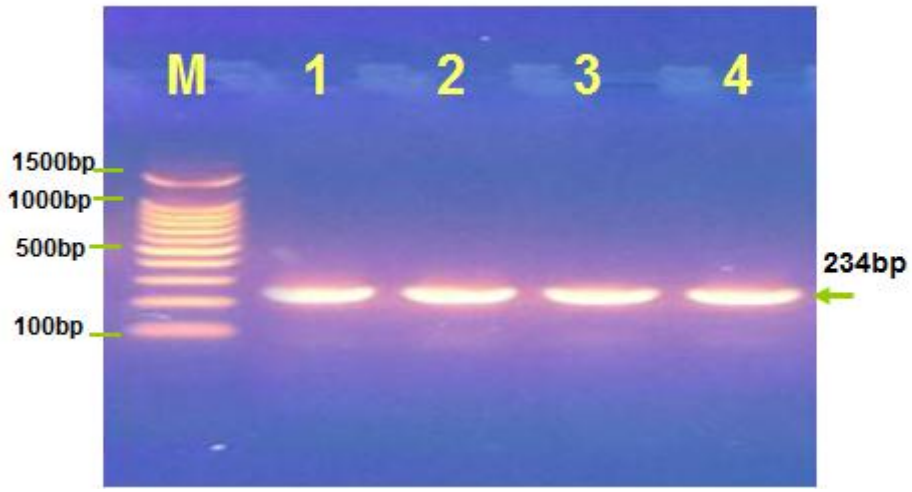


Figure 1: Agarosegel electrophoresis of PCR product analysis to glycoprotein B gene of Human herpesvirus 3 (Varicella-zoster virus) positive isolates. Where M: marker (100 -1500bp), lane (1-4) positive Varicella-zoster virus at (234bp) PCR product size.

DNA Sequencer Results:

1- Homology sequence identity of Human herpesvirus3 (VR Virus) IQ-S1 to NCBI-Blast Human herpesvirus 3 isolate.

Human herpesvirus 3 isolate V8VZV glycoprotein B gene (AY253703.1)

Sequence ID: Query_83189 **Length:** 2607 **Number of Matches:** 1

Range 1: 1807 to 2032 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
408 bits(452)	5e-118	226/226(100%)	0/226(0%)	Plus/Plus

```
Query  1     CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1807  CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  1866

Query  61    AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1867  AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  1926

Query  121   CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1927  CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  1986

Query  181   CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAA  226
             ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1987  CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAA  2032
```

2- Homology sequence identity of Human herpesvirus3 (VR Virus) IQ-S2 to NCBI-Blast Human herpesvirus 3 isolate.

Human herpesvirus 3 isolate V8VZV glycoprotein B gene (AY253703.1)

Sequence ID: Query_83189 **Length:** 2607 **Number of Matches:** 1

Range 1: 1807 to 2040 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
423 bits(468)	2e-122	234/234(100%)	0/234(0%)	Plus/Plus

```
Query  1     CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1807  CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  1866

Query  61    AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  120
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Sbjct  1867  AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  1926

Query  121   CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1927  CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  1986

Query  181   CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAACGGCCATT  234
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1987  CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAACGGCCATT  2040
```

3- Homology sequence identity of Human herpesvirus 3 (VR Virus) IQ-S3 to NCBI-Blast Human herpesvirus 3 isolate.

Human herpesvirus 3 isolate V8VZV glycoprotein B gene (AY253703.1)

Sequence ID: Query_83189 **Length:** 2607 **Number of Matches:** 1

Range 1: 1807 to 2032 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
408 bits(452)	5e-118	226/226(100%)	0/226(0%)	Plus/Plus

```
Query  1     CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1807  CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  1866

Query  61    AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  120
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Sbjct  1867  AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  1926

Query  121   CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1927  CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  1986

Query  181   CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAA  226
             ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1987  CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAA  2032
```

4- Homology sequence identity of Human herpesvirus3 (VR Virus) IQ-S4 to NCBI-Blast Human herpesvirus 3 isolate.

Human herpesvirus 3 isolate V8VZV glycoprotein B gene (AY253703.1)

Sequence ID: Query_83189 **Length:** 2607 **Number of Matches:** 1

Range 1: 1807 to 2040 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
423 bits(468)	2e-122	234/234(100%)	0/234(0%)	Plus/Plus

```
Query  1     CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  60
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Sbjct  1807  CGTTACGTCCGTGAAATCGCAGTCCATGATGTGGGAATGATTAGCACTTACGTAGATTTA  1866

Query  61    AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1867  AACTTAACACTTCTTAAAGATAGAGAGTTTATGCCGCTGCAAGTATATACAAGAGACGAG  1926

Query  121   CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1927  CTGCGGGATACAGGATTACTAGACTACAGTGAAATTCAACGCCGAAATCAAATGCATTCG  1986

Query  181   CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAACGGCCATT  234
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1987  CTGCGTTTTTATGACATAGACAAGGTTGTGCAATATGATAGCGGAACGGCCATT  2040
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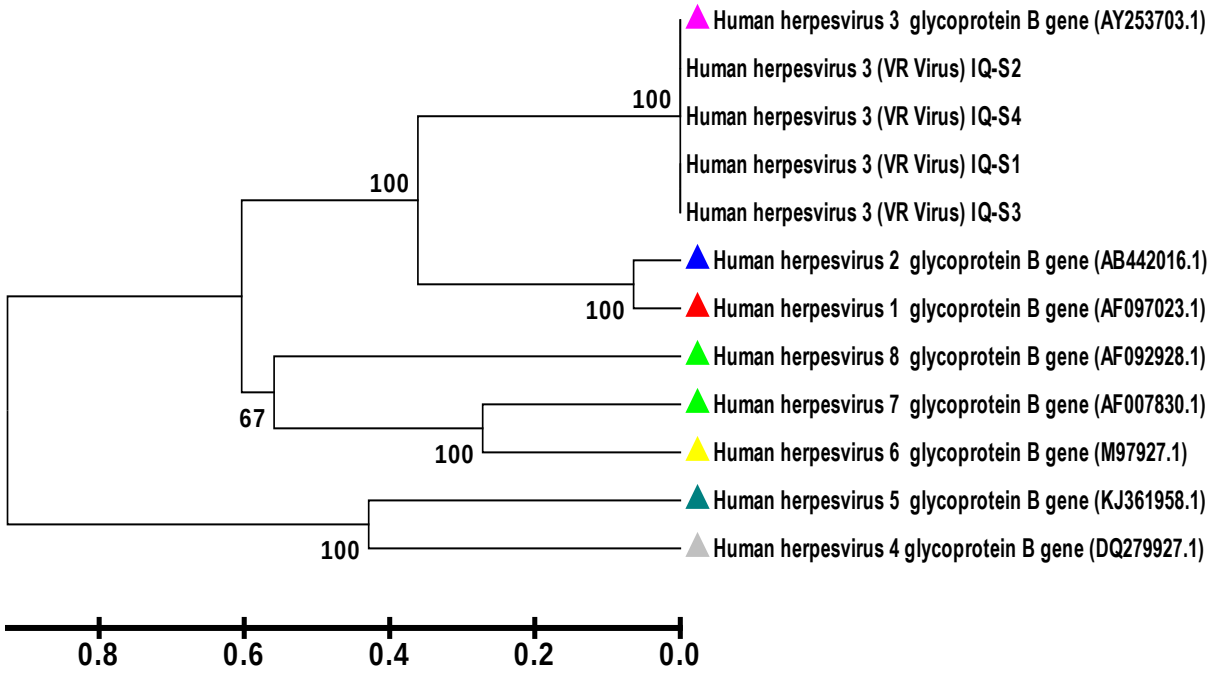

Figure (1): Phylogenetic tree analysis depend on the glycoprotein B gene partial sequence which used for Human herpesvirus 3 (Varicella-zoster virus) by using Unweight Pair Group method with Arithmetic Mean (UPGMA) based on software of (MEGA 6.0 version). The homemade Human herpesvirus 3 (VZ virus) (IQS1-IQS4) isolates were show closed related to NCBI-Blast Human herpesvirus 3 isolate (AY253703.1) .Whereas, other NCBI-Blast Human herpesvirus types were shown different and out of tree.

Discussion

The Varicella zoster virus (VZV) (**Human herpesvirus 3)**is reasonsVaricella (chicken pox),herpes zoster (shingles) and (PHN). Varicella, typicallyfound in children is anestablishedappearanceprimary and is very contagious touching the large number of population**.** Afterwardmelioration from varicella, the virus has noticeablecapacitydue to dissimulatingin human bodyasinactive (dormant) configurationatconfinedgroups of neuronsto existencein the host. Nevertheless, afterenvironments are appropriate the virus could berevival, than the zoster occurs. The infection always recordedamongelderpersons, which isdescribedin arestrictedoverspill and it is verysorer [17].There is significantcare in theillness due to epidemiological differences between geographic situation, particularlybetween temperate and tropicalregions of thebiosphere[18]. In our study confirmed that herpes zoster virusidentifiedin PCR in blood and not in vesicle liquid only and this result accord with *Marinko, et al* 2008 [19].In a precedingresearches, we confirmed the usefuleffectto theupper plasma volumes used for herpes zoster virus DNA extractionon the sensitivity of the PCR assess[20].The genotypes ofherpes zoster in Iraq are not confirmed. This current researchhas been done a primary detection on theVZV genotypes that isolates grouped at Najaf region of Iraq. The genotypes B for herpes zoster virus are detection from isolated strain in Najaf province and there is unimportant linkedwas revealed between VZV genotype withlife time of the peoples.The epidemiology of Herpes zoster infection is unlike in moderateenvironments21.In our study, 4 isolates wereglycoprotein B gene e, which was consistent with a previous study showing that isolates that were B gene were frequently found in Asia [21,22].Molecular study of vzvequips thebasis for additionalresearchesare requisite to find out the VZV occurrence in Iraq: epidemics, prevalenceof the virus, thepossibility for reactivation, and phenotypic featuresto thevaried strains.

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