Molecular Analysis of *Varicella Zoster Virus* Among School Age Children in Al-Diwaniyah Governorate

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Abstract

The aim of the present study was to figure out the genetic strains of *Varicella Zoster Virus* (VZV) that are common in the Middle district region of Iraq. A total of 43 Samples selected randomly for molecular diagnosis and identifying genetic strains in laboratory, by conventional PCR, showed that all the 43 children were positive for VZV.

Three single nucleotide polymorphism (SNP) alleles were estimated and these were ORF34, ORF54 and ORF62. All the cases were positive for ORF34 (100.0 %) and the number of cases that were positive for ORF54 were 31 out 43 (72.1 %) whereas, none of the cases positive for ORF62 (0.0%). Accordingly virus genotype strains were identified. Cases which were positive for ORF34 and ORF54 SNPs were considered genotype B whereas cases which were positive for ORF34 only were labeled as genotype A. However, no case was labeled as genotype C (ORF62). Therefore, the most prevalent VZV genotype in Diwanyiah region was the B (ORF34 & ORF54) genetic subtype (wild type) accounting for 31 out of 43 (72.1 %) whereas, the A genetic subtype accounted for 12 out of 43 (27.9 %). Genotypes A and B were also neither correlated significantly to age of children nor gender of children.

Keywords: *Varicella-Zoster virus*; School Age Children;genotyping,Middle Eupharates;IRAQ.

Introduction

Varicella-Zoster virus (VZV) is one of the human herpes viruses (HHVs); it's official name known as (*Human Herpes Virus -3*) HHV-3, a member of DNA-containing *Herpesviridae* family. VZV causes varicella (chickenpox) as a primary infection and herpes zoster (HZ) after the reactivation of a latent VZV. Varicella is a childhood illness with highest incidence between 1 and 9 years of age, characterized by fever and a generalized pruritic vesicular rash and Varicella is a worldwide infection, is more prevalent in temperate climates than tropical ones, and often occurs in late winter and spring seasons; furthermore, varicella transmitted by the respiratory aerosols from infected individuals and by direct contact with skin lesions of individuals affected by VZV.

The VZV genome was originally reported to encode 65 unique viral genes, three of which are located in the duplicated IRS/TRS region (1). Four additional VZV genes have since been identified including ORF0 (2), ORF9A (3), ORF33.5 (4), and the newly discovered VZV latency-associated transcript (VLT) (1). An underappreciated feature of VZV is that transcription of several genes, including ORF0, ORF42/45, ORF50, and VLT, require the host-splicing machinery to remove introns from pre-mRNA and have also shown evidence of alternative splicing, resulting in the synthesis of alternative proteins (5). It thus seems likely that the full transcriptional potential of VZV has yet to be revealed, and we predict that the latest technological advances (e.g., full length sequencing of native RNA) will yield further novel discoveries. It is also worth noting that the encoding of additional RNA types, including microRNAs and small non-coding RNAs, is still an area of active study with contrasting results (6).The aim of present study was to Confirmative identification of major capsid protein gene of *Varicella-Zoster virus* by using conventional polymerase chain reaction (PCR) and Genotyping of

Varicella Zoster Virus of ORF34, ORF54 and ORF62 in Chickenpox Patients using Restriction Fragment Length Polymorphism (RFLP) Technique.

Materials and Methods

Out of the many primary schools in AL-Diwaniyah governorate , a randomly selected 10 schools including rural and urban areas. A total of 4545 children were enrolled with an age range of 6 to 10 years 800 were diagnosed clinically to have VZV infection, then information about age, gender and residency about each child were introduced into an excel spread sheet. After that 43 children were selected for genetic assessment of the VZV.Skin crusted lesion was taken for purpose of analysis. The samples of crusted obtained by crust lever then transferred into a tube (Texwipe's Absorbond® Swab) which is made of polyester (hydroentangled) nonwoven material. Then samples transferred into freeze at -20 C.

- Polymerase Chain Reaction (PCR)

PCR technique was performed for direct detection of *Varicella Zoster Virus* (VZV) based on amplification of major capsid protein(*Mcp*) gene **(table 1).**

Table (1): The PCR detection primers with their sequence and amplicon size

Primers	Sequence (5'-3')		Amplicon
Mcp	F	TGACAAATGCTAGGCGGGTT	520bp
	R	CGACGCAACGATTCGGTAAC	

Viral DNA was extracted from transport media of skin lesions scrap samples were extracted by using Genomic DNA mini kit extraction tissue, and done according to company instructions .

The extracted DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), that check and measurement the purity of DNA through reading the absorbance in at (260 /280 nm) . PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions .

After that, these PCR master mix components that mentioned above were placed in standard AccuPower PCR PreMix Kit that containing all other components which needed to PCR reaction. Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes, then placed in PCR Thermocycler (Mygene. Bioneer. Korea).

PCR thermocycler conditions were done by using convential PCR thermocycler system . The PCR products were analyzed by agarose gel electrophoresis and visualized by using UV Transilluminator.

-RFLP-PCR Genotyping Technique

RFLP-PCR technique was performed for genotyping of positive *Varicella Zoster Virus* (VZV) in direct PCR. The VZV genotyping depend on amplification of ORF54, ORF34, and ORF62(table 2) . RFLP-PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions .

Table (2): The RFLP-PCR genotyping primers with their sequence and amplicon size

Primers	Sequence (5'-3')		Amplicon
ORF54	F	CGTAATGCATAACAGGCCAACAC	-
	R	AAACCTGGCGTCAAACATTACA	
ORF38	F	AAGTTTCAGCCAACGTGCCAATAAA	-
	R	AGACGCGCTTAACGGAAGTAACG	
ORF62	F	TTCCCACCGCGGCACAAACA	-
	R	GGTTGCTGGTGTTGGACGCG	

After that, these PCR master mix component that mentioned in table above placed in standard AccuPower PCR PreMix Kit .Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea). PCR thermocycler conditions were done for each gene independent .

RFLP-PCR mix was prepared by using *Bgl*I, *Pst*I, and *Sma*I restriction enzymes for ORF54, ORF34, and ORF62 respectively , and this master mix done independent according to company instructions .

After that, this master mix placed in Exispin vortex centrifuge at 3000rpm for 2 minutes, then transferred into incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods that mention in PCR product analysis.

Statistical analysis

Data were summarized, analyzed and presented using two software programs; these were the statistical package of social sciences (SPSS version 23) and Microsoft Office Excel 2010.. The level of significance was considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results and discussion:

-Molecular and genetic analysis

Three single nucleotide polymorphism (SNP) alleles were estimated and these were ORF34, ORF54 and ORF62, as shown in figures (1,2,3).All the cases were positive for ORF34 (100.0 %). The number of cases that were positive for ORF54 were 31 out 43 (72.1 %) whereas, none of the cases were positive for ORF62 (0.0%), as shown in figure(4).

Accordingly virus genotype strains were identified. Cases which were positive for ORF34 and ORF54 SNPs were considered genotype B whereas cases which were positive for ORF34 only were labeled as genotype A, as shown in figure(4).

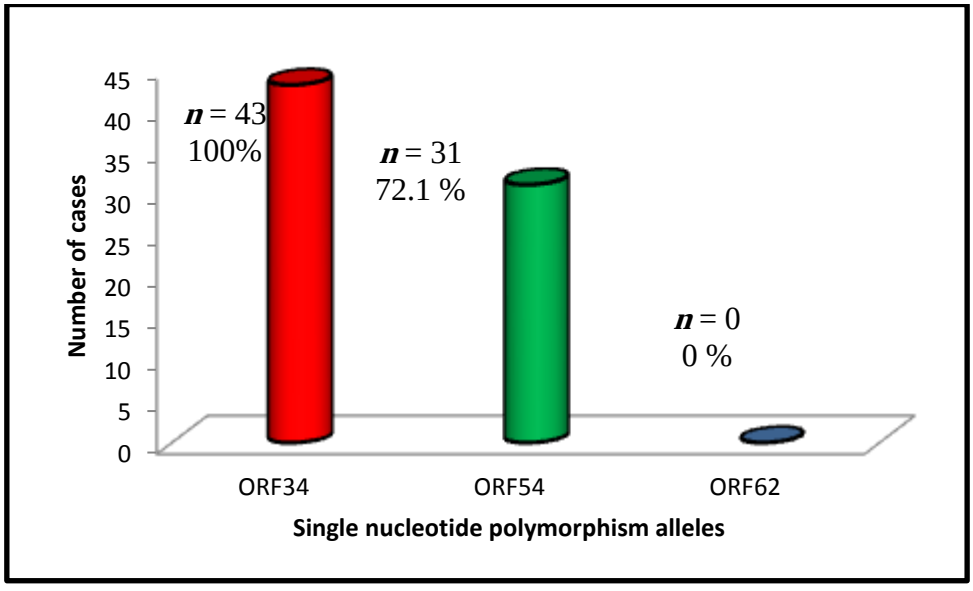

Figure 1: The number and percentage of cases according to single nucleotide polymorphism alleles (ORF43, ORF54 and ORF62)

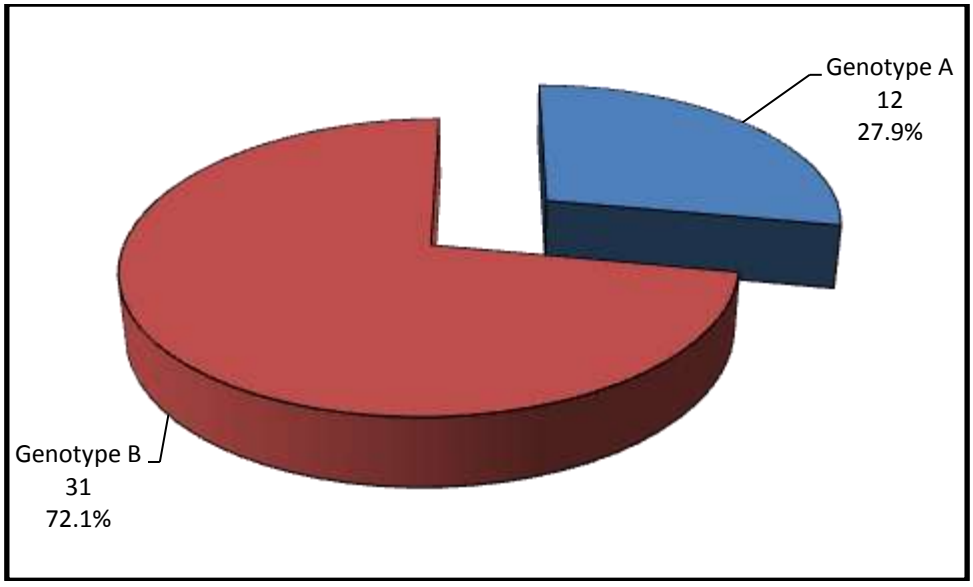

Figure 2: The number and percentage of cases according to genotype (A versus B)

The correlation between VZV gene SNP alleles and age and gender of children. These correlations were not possible for both ORF34 and ORF62 since ORF34 is positive in all case and ORF62 is negative in all cases. Therefore, we linked ORF34 only to age and gender of infected children, as shown in table 3, figure 3.

There was no significant association between age of child and ORF54 SNP allele frequency (P= 0.951), as shown in table 4 and figure 4. In addition, the association between ORF54 and gender of infected children was insignificant (P = 0.078), as shown in table 4.

Genotypes A and B were also neither correlated significantly to age of children nor gender of children, as shown in table(3) and figure(3).

Table 3: Comparison of mean age between cases with positive ORF54 and cases with negative ORF54

ORF54	*n*	Mean	SD	*P* *
Positive	31	8.45	1.83	0.951 NS
Negative	12	8.42	1.61	

n: number of cases; SD: standard deviation; *: independent samples t-test; NS: not significant at $P \leq 0.05$

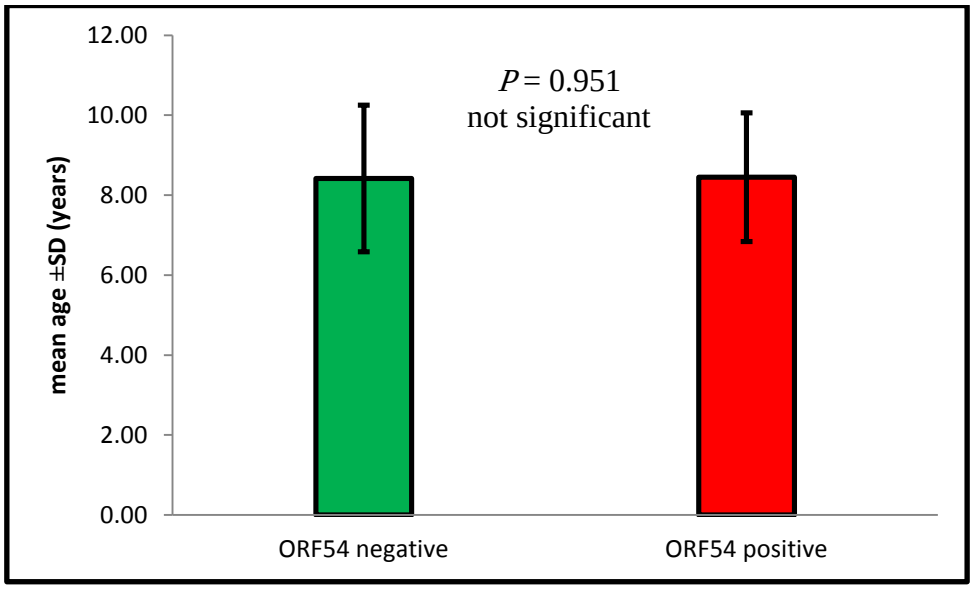

Figure 3: Comparison of mean age between cases with positive ORF54 and cases with negative ORF54

Table 4: Association between ORF54 SNP and gender of children

ORF54	Total *n* = 43	Male *n* = 23	Female *n* = 20	χ^2	*P*
Positive	31 (72.1 %)	14 (60.9 %)	17 (85.0 %)	3.096	0.078 NS
Negative	12 (27.9 %)	9 (39.1 %)	3 (15.0 %)		

Table 5: Comparison of mean age according to VZV genotype (A versus B)

Genotype	*n*	Mean	SD	*P* *
A	12	8.42	1.61	0.951 NS
B	31	8.45	1.83	

n: number of cases; SD: standard deviation; *: independent samples t-test; NS: not significant at $P \leq 0.05$

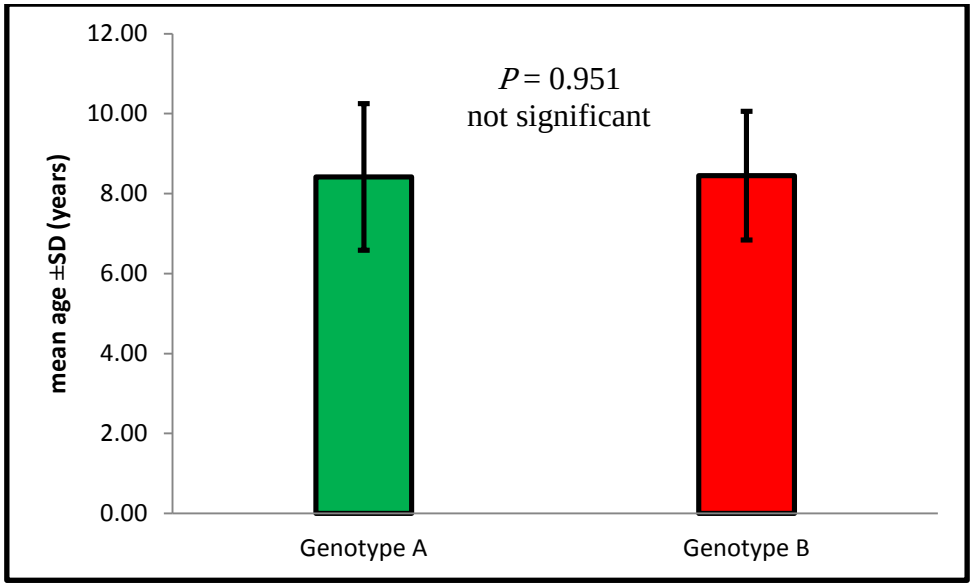

Figure 4: Comparison of mean age according to VZV genotype (A versus B)

PCR product analysis for major capsid protein *(mcp)* gene in positive varicella-zoster virus isolates at 520bp PCR product size**(figure 5).**

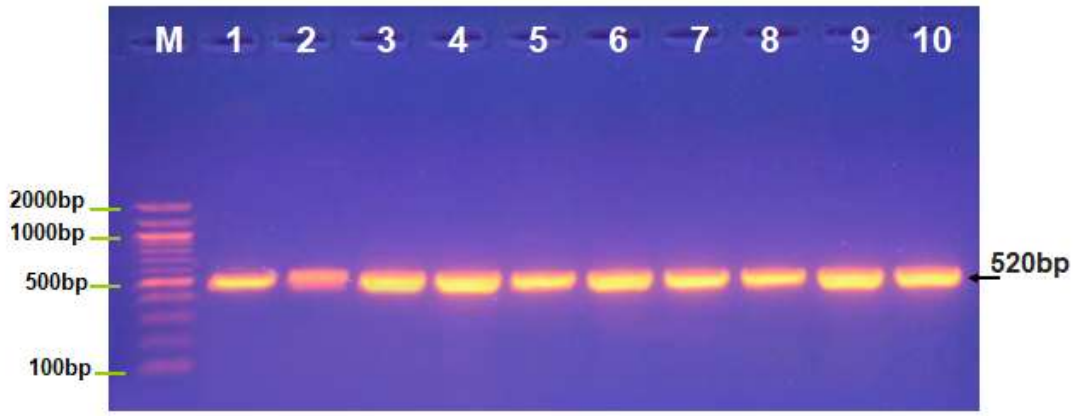

Figure 5: Agarose gel electrophoresis image that showed PCR product analysis for major capsid protein (mcp) gene in varicella-zoster virus. M (Marker ladder 2000-100bp). Lane (1-10) some positive varicella-zoster virus isolates at 520bp PCR product size.

-Genotyping of *Varicella-zoster virus* by RFLP-PCR:

Figure 6.shows the PCR product genotyping analysis for ORF54in *varicella-zoster virus* . The positive ORF54 varicella-zoster virus isolates gave 497bp PCR product size.

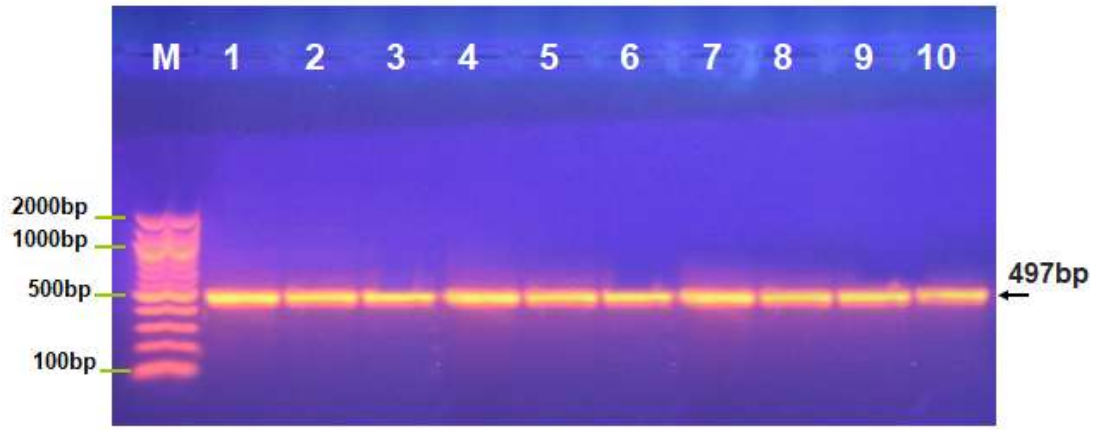

Figure 6: Agarose gel electrophoresis image that showed PCR product genotyping analysis for ORF54in varicella-zoster virus. M (Marker ladder 2000-100bp). Lane (1-10) some positive ORF54varicella-zoster virus isolates at 497bp PCR product size.

Figure 7.shows the RFLP-PCR product genotyping analysis for ORF54in *varicella-zoster virus* by using restriction enzyme (*BglI*).The positive digestion *BglI* restriction enzyme for some varicella-zoster virus isolates into overlapping 255bp product size.

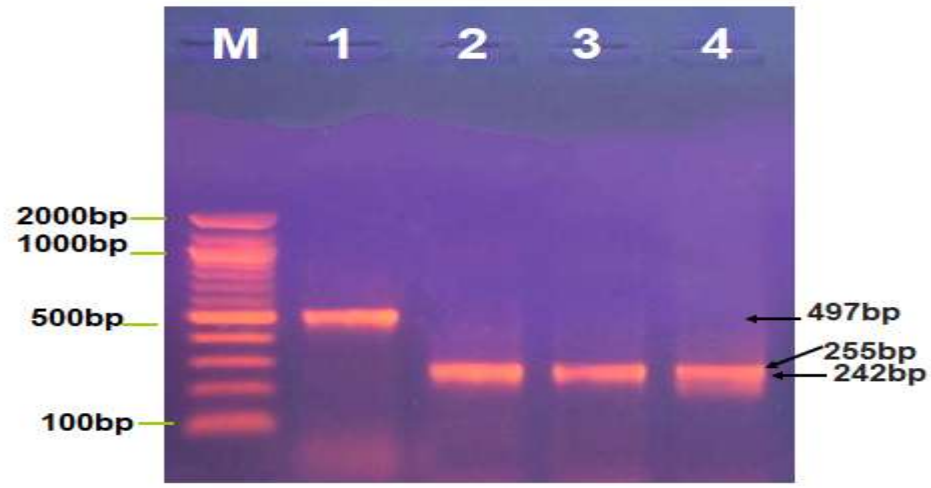

Figure :7 Agarose gel electrophoresis image that showed RFLP-PCR product genotyping analysis for ORF54in varicella-zoster virus by using restriction enzyme (*BglI*). M (Marker ladder 2000-100bp). Lane (1) positive control ORF54varicella-zoster virus isolate at 497bp PCR product size and lane (2-3) positive digestion *BglI* restriction enzyme for some varicella-zoster virus isolates into overlapping 255bp product size.

Figure 8.shows the RFLP-PCR product genotyping analysis for ORF34in varicella-zoster virus.The positive ORF34varicella-zoster virus isolates gave 647bp PCR product size.

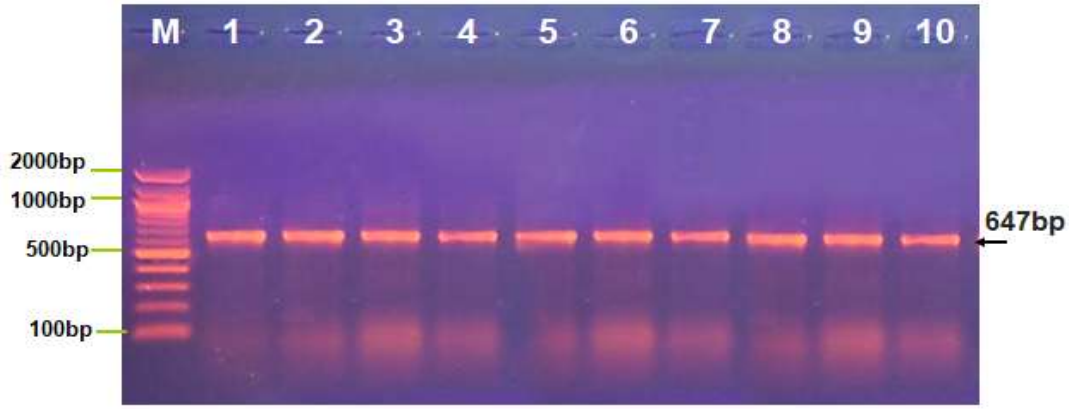

Figure 8: Agarose gel electrophoresis image that showed PCR product genotyping analysis for ORF34in varicella-zoster virus. M (Marker ladder 2000-100bp). Lane (1-10) some positive ORF34varicella-zoster virus isolates at 647bp PCR product size.

Figure 9.shows the positive digestion *PstI* restriction enzyme for varicella-zoster virus isolates gave 357bp and 290bp control ORF54varicella-zoster virus isolate at 647bp PCR product size **.**

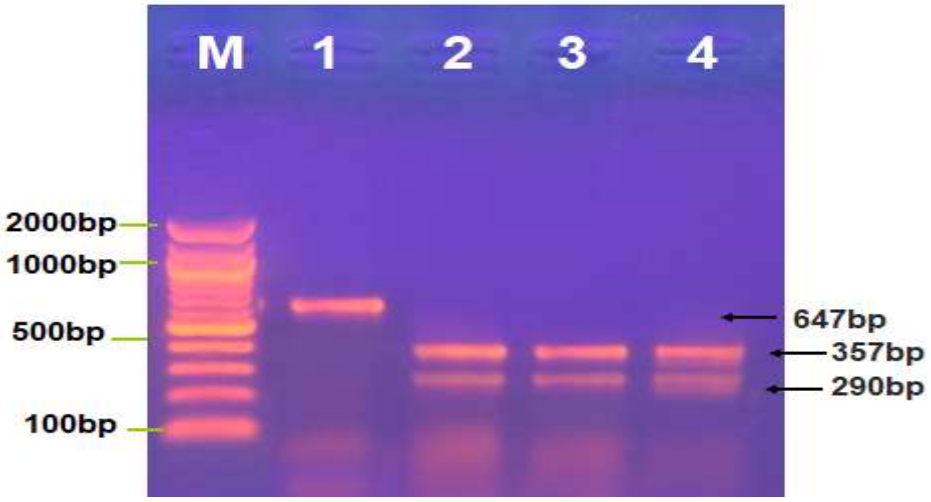

Figure 9: Agarose gel electrophoresis image that showed RFLP-PCR product genotyping analysis for ORF34in varicella-zoster virus by using restriction enzyme (*PstI*). M (Marker ladder 2000-100bp). Lane (1) positive control ORF54varicella-zoster virus isolate at 647bp PCR product size and lane (2-3) positive digestion *PstI* restriction enzyme for some varicella-zoster virus isolates into 357bp and 290bp product size.

Figure 10.shows product genotyping analysis for ORF62 in varicella-zoster virus positive ORF62 varicella-zoster virus isolates at 268bp PCR product size.

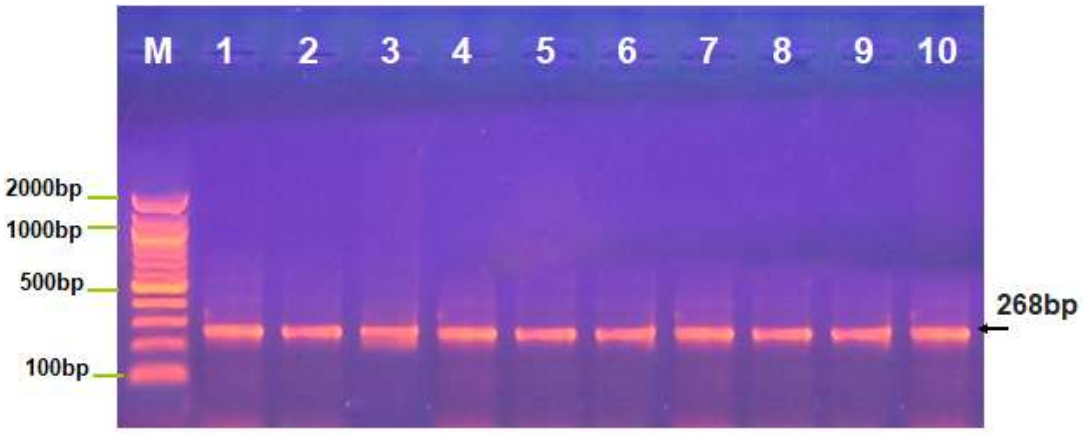

Figure 10: Agarose gel electrophoresis image that showed PCR product genotyping analysis for ORF62 in varicella-zoster virus. M (Marker ladder 2000-100bp). Lane (1-10) some positive ORF62 varicella-zoster virus isolates at 268bp PCR product size.

Figure 11.shows the RFLP-PCR product genotyping analysis for ORF62 in varicella-zoster virus by using restriction enzyme (*SmaI*). positive digestion *SmaI* restriction enzyme for some varicella-zoster virus isolates into 153bp and 79bp product size.

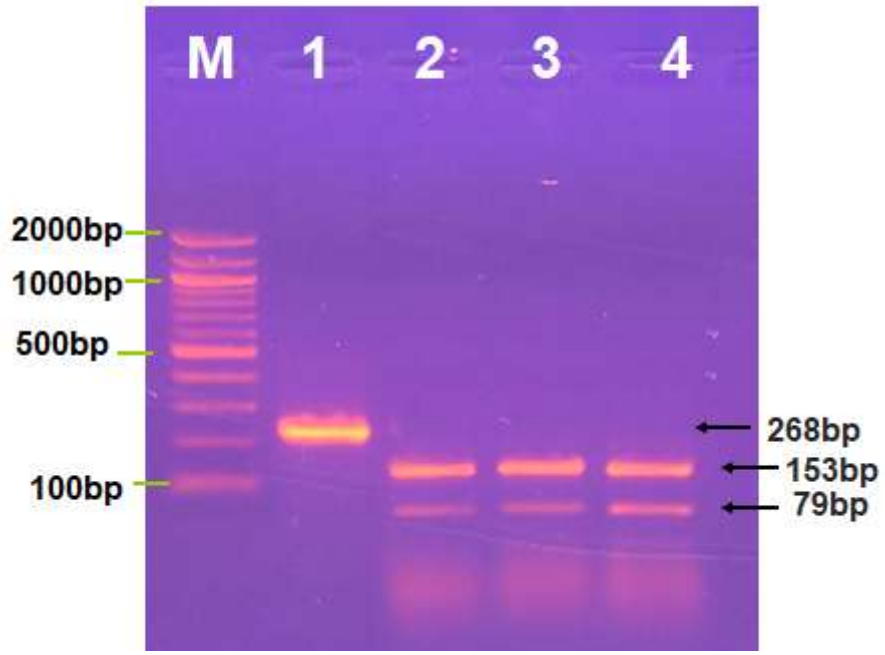

Figure11: Agarose gel electrophoresis image that showed RFLP-PCR product genotyping analysis for ORF62 in varicella-zoster virus by using

restriction enzyme (*SmaI*). M (Marker ladder 2000-100bp). Lane (1) positive control ORF54varicella-zoster virus isolate at 647bp PCR product size and lane (2-3) positive digestion *SmaI* restriction enzyme for some varicella-zoster virus isolates into 153bp and 79bp product size.

VZV encodes at least five transcriptional regulatory proteins specified by four putative IE genes, ORF4, ORF61, ORF62 and ORF63, and one L gene, ORF10. All except the ORF61 protein, IE61, are part of the VZV virion (7). Our understanding of the transcriptional regulation of VZV genes remains incomplete, in part due to the high cell-associated nature of VZV that precludes synchronized infections using cell-free viruses. The dominant transcriptional regulator and possibly only true immediate-early protein encoded by Varicellovirus is homologous to VZV IE62 (8,9). Consistent with this idea, the VZV IE62 major viral transactivator protein can activate all three kinetic classes of VZV genes in the absence of other viral proteins, including all IE genes, ORF4, ORF61, ORF62, and ORF63, while IE4, IE61 and IE63 either do not or minimally stimulate the ORF61 promoter (10,11). Host transcription factors, either by themselves or through interactions with viral transcriptional regulatory proteins, also contribute to viral gene expression (11). VZV virion proteins delivered into newly infected cells upon entry are not absolutely required to initiate VZV gene expression, as evidenced by the resulting VZV replication upon transfection of cells with viral DNA (12). Notably, near identical VZV transcriptomes are detected during productive infection of diverse cell types, including neurons, suggesting a prominent role for either commonly expressed cellular transcription factors or viral proteins in coordinating VZV gene expression (13,14,15).

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