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RESEARCH ARTICLE

Comparative studies of Partial Sequence Analysis of the Varicella zoster Virus ORF 63 Isolated from Chickenpox and Herpes zoster patients from Chennai, India

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Abstract

ORF 4, 21, 29,62,63,66 of VZV is the genes expressed during latency. Out of which ORF 63 is found to be expressed first and abundant gene to be expressed. It is present in two copies (ORF63/70) and codes with 278 amino acids. ORF 63 is expressed in the neurons during latency. Amplified and sequenced ORF 63 of Varicella zoster from chickenpox and herpes zoster DNA successfully. The partial sequence of both was published in NCBI. The expression of IE 63 was carried out in BL21pLys cells. Bioinformatics analysis was done to compare the partial sequence of protein and nucleotide sequence of ORF 63 of Varicella zoster and Herpes zoster with the ones in NCBI, from which it was inferred that the sequence of ORF 63 of clinical isolates, Chennai was different of a sequence of NCBI. The authors are happy to communicate the fact that this is the first work on sequencing of Varicella zoster ORF 63 and Herpes zoster ORF 63 from samples collected from vesicles sample of chickenpox and herpes zoster in Chennai, India. Thus the authors claim that different isolates of Varicella zoster and herpes zoster prevail in Chennai, India.

Introduction

The smallest human neurotropic Alpha herpesvirus (150-200 nm) is Varicella zoster virus which is closely related to the equine herpesvirus and Pseudo rabies virus that infect horses and pigs respectively. Varicella zoster virus causes chickenpox in children which becomes latent in cranial nerve, dorsal root and autonomic ganglia along the entire neuraxis and its cause herpes zoster (herpes zoster) among elderly or immunocomprised patients.

A long unique region (U_L) -10Kbp , short unique region (U_S) - 5.2Kbp with a pair of repeat elements in opposite directions (R_L- 88bp and R_S - 7.3bp) with terminal (T) and internal (I) copies of the repeats from the genome of Varicella zoster virus . The complete DNA molecule has the arrangement TR_L-U_L- IR_L- IR_S - U_S - TR_S. When DNA replication occurs, the U_S region inverts, resulting in the formation of two isomeric forms of VZV DNA. More than thirty structural proteins including six to seven glycoproteins are present in the VZV virions. The size of messenger RNA (mRNA) ranges from 0.8 to 6.5 Kb (Ruzena Stranska et al., 2004). The G+C contents of the U_L and U_s, TR_s and IRs, TR_s and IR_L are 43- 44%, 59% and 68% respectively. Fifty - six out of the sixty- two genes present within the U_L region of VZV have HSV homologs while all four of the VZV genes located in the US region of the VZV have HSV homologs.

A condition in which the virus can remain dormant for years is termed latency. When an individual loses his immunity, he is susceptible to viral infection. Varicella zoster virus which has mannose-6-phosphate on its surface is found to enter our body through the nerves having receptors for mannose-6-phosphate. The receptors and the phosphate complement each other and bind together to enable the virus to enter the nerve cell. The genes that are expressed during the early stages of infections are the ones expressed in the nerve cells, and not the ones articulated later. From this, it is understood that the Varicella zoster virus is very constrained with the production of protein in the nerve cells. As a result, disorientation in normal cellular function is not seen when the virus is inside these cells (Chen et al., 2004).

The important site for viral latency is neuronal nuclei of sensory ganglia. It is also found that some genes are expressed during latency in the satellite cells that surrounds the neurons. In Varicella zoster virus ORF 4, 21, 29, 62, 63 and 66 are the genes that are expressed during latency, its genome remains circular and the antigens available for presentation are limited during latency. VZV gene 29 encodes a major DNA-binding protein, and immediate early genes 62 and 63 encode regulators of virus gene transcription. It is also established that VZV ORF 63 is one of the most abundant transcripts expressed during VZV latency in humans. The potential contributors to persisting T cell responses are at least some VZV encoded proteins present in the cytoplasm of neurons during latency (Cohrs et al., 1998).

Varicella zoster virus open reading frame 63 (ORF63) is located between nucleotide 110581 and 111417 (834bp) in the internal repeat region (IR_s) that encodes a nuclear phosphoprotein which is homologous to herpes simplex virus type I (HSV-1) ICP 22 and is duplicated in the terminal repeat region as ORF 70 (Perera, 1992). IE 63 is expressed in the cytoplasm of neurons during VZV latency and in both the cytoplasm and the nucleus during productive infection. However, the mechanism involved in the IE 63 nuclear import and retention has remained unclear. Analysis of a series of IE 63 truncation and substitution mutant indicates that amino acids 186-195 are required for antibody binding (Mueller et al.,2009; Kennedy et al.,2000).

VZV ORF 63 is one of the most abundant transcripts expressed during latency in experimentally infected rodents and human ganglia. VZV ORF 63 protein is located predominantly in the nucleus during lytic replication in *in vitro* and in cells transfected with a plasmid expressing a protein. During latency, however the protein is present in the cytoplasm of neurons.

VZV IE 63 amino acids 171 to 208 and putative phosphorylation sites of IE63, both of which are critical for virus replication and latency in rodents, are important for the interaction of IE 63 with ASF1 (Anti-silencing factor). Finally, IE63 increased the binding of ASF 1 to histone H3.1 and H3.3 which helps in regulating the levels of histones in virus-infected cells. Since, ASF1 mediates eviction and deposition of histones during transcription, the interaction of VZV IE 63 with ASF1 may help with regulated transcription of viral or cellular genes during lytic and latent infection (Ambagala *et al.,* 2009).

On the basis of the VZV DNA sequence, it has been shown that IE 63 exhibits hydrophilic and acidic properties suggesting that this protein could play a regulatory role during the infectious cycle. Co-transfection experiments demonstrate that the VZV gene 63 protein strongly represses, in a dose-dependent manner, the expression of VZV gene 62, promotes the activation of the thymidine kinase gene but cannot affect the expression of the genes encoding glycoproteins I and II. VZV gene 63 protein could play a pivotal role in the repression of IE gene expression as well as in the activation of early gene expression (Jackers *et al.,* 1992).

Using a rat model of VZV latency, it is shown that IE 63 is highly expressed essentially in neurons during latency. IE 63 can also be detected in the skin of patients showing early herpes zoster symptoms (Debrus *et al.,* 1995).

As of July 2008, there were 23 complete genomic sequences of VZV strains in NCBI. The authors partially sequenced the ORF 63 of Varicella zoster (VZ) for chicken pox and ORF 63 of viral isolates from patients with herpes zoster (HZ) in Chennai. Also, the expression of the gene of both IE 63 of Varicella zoster from chicken pox and viral isolates of Herpes zoster were checked by SDS-PAGE. A comparative analysis using Bioinformatics tools was performed to check for similarities/ difference if any between the different isolates with our partially sequenced ORF63.

Materials and methods

Isolation and amplification of ORF 63 of VZ and ORF 63 of viral isolates of HZ

DNA from the viral isolates from chickenpox and herpes zoster was obtained using Calbiotech Isolation kit by adhering to the kit protocol. The isolated DNA was stored at -20°C until further use. 3µg of total VZ DNA was applied to the mixture [50µl] with DEPC - treated water and the PCR reaction was set up with the following reaction: 0.1µl XT-5A polymerase enzyme, 1µl [2mM] dNTPs, 0.5µl template, 1µ l [20pMol] ORF 63 forward primer, 1µl [20pMol] ORF 63 reverse primer, 1µl XT-5A polymerase buffer and 5.4µl of distilled water in order to obtain a final volume of 10µl reaction.

The PCR condition was set at : 95°C/5mins [Initial denature], 95°C [denaturate], 45°C [anneal], 72°C/50sec [extension] [go to step 2, 5 times], 95°C/15sec [denature] and 60°C/30 sec [final anneal] for 25 times. The amplified products were checked by running on 0.8% agarose gel. The forward primer: GCCGCTAGCCATATGATGTTTTGCACCTCACCGGCTA and reserve primer : GCCGGATCCCTACACGCCATCGGGGGGGCGGTATAT

Restriction digestion, ligation and transformation

The pRSETc vector using the primer for PvuII site was incubated with PvuII at 37°C for 3.5 hour. The PvuII-digested pRSETc vector was electrophorsed on 0.8% agarose gel in buffer at 100V for 30 mins and was visualized by UV illumination; 1Kb DNA ladder was run in parallel as a size marker. The purified PCR product was ligated into a digested PvuII- pRSETc vector by mixing with DNA ligase enzyme and respective buffer by incubating it overnight at 16°C. BL21pLysS competent cells were prepared by $CaCl_2$ method.

The ligated product was transformed into 1µl of BL21pLysS competent cells, plated on LB agar with ampicillin (50µg/ml) and incubated at 37°C for 12 hrs. A single colony was transferred into 2ml LB broth with appropriate antibiotics and incubated at 37°C for 12hrs. The culture was centrifuged at 13000 rpm for two mins and the supernatant was discarded. Plasmid preparation (minipreparation) of both clones was done using Alkaline lysis method [17.]

To check the clone by restriction digestion

The prepared clone was checked by performing a restriction digestion with BamHI for the correct orientation. Restriction digestion was performed as mentioned earlier. After that, the clone was run on a 0.8% agarose gel and the band was checked under UV illumination.

To check the expression of VZ IE63 and HZ IE 63 protein on SDS-PAGE

Two µl of the plasmid was transferred into 50 µl of BL21pLyS, Origami, Rill and C43 competent cells as described before and incubated at 37°C for 12 hrs. After 12 hours of incubation, a single colony was inoculated into 2ml of LB broth with ampicillin and chloramphenicol (170µl/ml) and incubated at 37°C till its O.D reaches the value of 0.6. Then, a different concentration of 2 µl Isopropyl β-D-1-thiogalactopyranoside [IPTG] [0.3mM, 0.5mM and 1.0mM] was induced into the cultures and it was growing at 37°C for 3-3.5hrs. The culture was centrifuged at 10,000rpm for 2 mins at 4°C. The supernatant was discarded and the pellet was resuspended in 50µl Tris-Nacl buffer. The sample [20µl of suspension + 10µl SDS loading dye] was heated at 95°C for 5 mins and loaded onto an SDS-PAGE gel. The gel was run at 125 volts for 2 hrs.

Restriction Fragment of Linear Polymorphism (RFLP) of VZ ORF 63 and HZ ORF 63

Restriction digestion of VZ and HZ ORF 63 was carried out using EcoRI and XhoI. The digested product gel was extracted and the column was purified using the GeneiPure™ gel extraction kit. *In silico* analysis was carried out. Based on the analysis, two restriction enzymes were chosen for RFLP, namely Taq I and Nde I. The RFLP patterns for both Taq I and Nde I were analyzed.

Both clones of VZ ORF 63 and HZ ORF 63 were sent for sequencing and submitted to NCBI for publication.

Subcloning of pRSETc – VZV ORF 63 and pRSETc –HZ ORF 63 into pET21b VECTOR

Midipreparation of pET21b vector and PCR amplification of pRSETc-VZV ORF63 and pRSETc –HZ ORF63

Primary inoculums was prepared and transferred into 200ml LB broth with an appropriate antibiotic and incubated at 37°C for 12 hrs and the following steps remained the same as in the minipreparation of plasmid explained earlier. Oligonucleotide primers specific for pRSETc- VZVORF63 and pRSETc-HZ ORF63 was used. In this amplification, about 1µg of pRSETc-VZV ORF63 and pRSETc-HZ ORF 63 was applied to the mixture [20µl] containing 20pmol each of forward and reverse primers, 10mM of deoxynucleotide triphosphate, DMSO, 1.5U of Phusion enzyme and buffer with autoclaved distilled water. After an initial denaturation of the template DNA at 98°C for 2mins, 30 cycles of amplification were carried out for 1mins at 98°C, 1mins at 54°C and 30 sec at 72°C followed by a final extension of 10mins at 72°C. Then the amplified products were electrophorsed on 0.8% agarose gel. The PCR products were purified using NUCLEOSPIN EXTRACT II- MACHEREY_NAGEL purification kit.

Restriction digestion of PCR product and pET21b

The purified PCR product [pRSETc- VZV ORf63 and pRSETc-HZ ORF63] and pET21b were incubated with ECORI and XhoI site at 37°C for 3hrs in the buffer recommended by the supplier [Fermentas]. It was electrophorsed on 0.8% agarose gel and visualized under UV illumination. The gel was cut into pieces and stored in a sterile 1.7ml eppendrof tube. The gel containing digested pET21b was purified using NUCLEOSPIN EXTRACT II-MACHEREY_NAGEL purification kit.

Ligation and transformation

The purified digested PCR product was ligated into digested EcoRI-XhoI – pET21b vector by mixing it with DNA ligase enzyme and respective buffer by incubating overnight at 16°C. BL21pLysS competent cells were prepared by $CaCl_2$ method.

The ligated product was transformed into 1µl of BL21pLysS competent cells which was plated into LB agar with ampicillin and incubated at 37°C for 12 hrs. A single colony was transferred into 2ml LB broth with appropriate antibiotics and incubated at 37°C for 12hrs. The culture was centrifuged at 13000rpm for 2 mins and the supernatant was discarded. The preparation of plasmid of both the viruses was carried by the Alkaline lysis method.

Screening of clones by PCR amplification and to check the expression of VZ and HZ ORF 63

Two separate reactions were set up for varicella zoster virus ORF 63 and Herpes zoster ORF 63 for amplification. The PCR reaction was set up with the following reaction: 2µl Taq polymerase enzyme, 0.4µl (10mM) dNTPs, 0.4µ l (20pMol) ORF 63 forward primer (new), 0.4µl (20pMol) ORF 63 reverse primer (new), 2µl Taq polymerase buffer, and 16.2µl of distilled water to obtain a final volume of 20µl reaction. 0.4 µl plasmid preparations of both clones were added in the respective tubes and the reaction was set: 94°C/2mins (initial denature), 94°C/2mins (denature), 54°C/1 mins, 72°C/1 mins (30 cycles), 72°C/10mins (final extension) and 20°C/1mins (hold).

The sample was prepared and run on 12% SDS-PAGE to check the expression of proteins.

Analysis of Bioinformatics tools

Pairwise alignments and sequence analyses of Varicella zoster and herpes zoster ORF 63

Nucleotide and amino acid sequence alignments were generated using LALIGN & FFAS03 server respectively. The analysis of nucleotide and amino acid was deduced. Multiple sequence alignment of nucleotide and protein was performed in the partial sequence of chickenpox (JQ287497) and chickenpox MAV06 strain (JF306641). Also, the multiple sequence alignments of nucleotide and protein were carried out for the partial sequence of herpes zoster (JN315707) and herpes zoster strain (DQ479956).

Results

PCR amplification of Varicella zoster ORF 63 and herpes zoster ORF 63 and Restriction digestion of pRSETc vector

Viral RNA of herpes zoster and viral DNA of Varicella zoster were isolated from the stock. Both the viral RNA and viral DNA were confirmed by Polymerase chain reaction as respective virus based on ORF 63 gene size (834bp). The amplified products were allowed to run on 0.8% gel and the result was observed. Blunt – end cloning: The vector was digested with PvuII enzyme and observed by running on 0.8% gel .

Plasmid preparation of Varicella zoster and herpes zoster and screening of clones by restriction digestion

The digested vector was ligated with the purified DNA samples and transformed into DH5α cells. The transformed cells were inoculated into LB plate containing ampicillin and incubated at 37ºC overnight. Colonies were observed in LB plate after overnight incubation.

A single colony was picked and inoculated into culture vials containing LB broth with ampicillin and incubated for 12 hrs. The plasmid was prepared from the incubated culture containing ORF 63 of chickenpox and herpes zoster and was checked by running on a gel. The clones prepared from ORF 63 of chickenpox and herpes zoster was further confirmed and screened by digesting it with BamHI enzyme. The results were checked by running on 08. % gel.

To check the expression of Varicella zoster IE 63 and herpes zoster IE 63

The expressions of Varicella zoster IE63 and herpes zoster IE63 were checked on BL21pLyS, Origami, Rill and C43 competent cells .

Subcloning of pRSETc vector-VZ ORF 63 and pRSETc vector –HZ ORF 63 into Pet 21b vector

PCR amplification of pRSETc vector- VZ ORF63 and PSRETC vector-HZ ORF63 with new sets of primers

Both the pRSETc- VZ ORF 63 and pRSETc- HZ ORF 63 was amplified with new sets of primers (Table 3) by Polymerase chain reaction. The bands were observed in 0.8% gel (Figure 6). The confirmed PCR product and pET 21b vector were purified to remove primer-dimer and other impurities.

Plasmid midiprep and double digestion of pet21b

Plasmid midiprep of pet 21 b was allowed to run on 0.8% agarose gel and the result were recorded .The vector was digested with 4 sets of restriction enzymes. The 4 sets of enzymes were EcoRI- XhoI, EcoRI – NotI, NheI- NotI and NheI- XhoI, observed by running on 0.8% gel. The EcoRI- XhoI digested the vector successfully .

Ligation of purified digested PCR products with digested pET21b vector

The digested pET 21b vector was ligated with the purified pRSETc- VZ ORF 63 and pRSETc-HZ ORF 63. . Later it was transformed into DH5α cells. The transformed cells were inoculated into LB plate containing ampicillin and incubated at 37ºC overnight. Colonies were observed in LB plate after overnight incubation.

A single colony was picked and inoculated into culture vials containing LB broth with ampicillin, and incubated for 12 hrs. The plasmid was prepared from the incubated culture of VZ IE 63 and HZORF 63 was checked by running on a gel.

Screening of clones by PCR amplification and check the expression of Varicella zoster IE 63 and herpes zoster IE 63

Both the clones of VZ and HZ were screened by PCR amplification using Taq-polymerase enzyme. Both the clones were successfully amplified by PCR and seen as a band on the gel.The plasmid was transformed into BL21pLysS cells and the expression of Varicella zoster IE 63 and herpes zoster IE 63 was checked by running on SDS-PAGE .

Restriction Fragment of Linear Polymorphism (RFLP) of VZ ORF 63 and HZ ORF 63

RFLP analysis for HZ ORF 63 showed an 800 bp release after the plasmid was digested with EcoRI and XhoI (Figure 1a). Taq I did not give proper RFLP pattern due to very less base pair difference, while with Nde I digestion, two fragments were obtained - 262bp and 575bp (Figure 1b). But, the RFLP analysis of Varicella zoster ORF 63 was not properly obtained. So, the clones were sent for sequencing.

Varicella zoster ORF 63 partial sequences published in NCBI- accession number: JQ287497

The clone containing pRSETc- VZ ORF 63 transformed into pET21b was sent for sequencing and the partial sequence was published in NCBI accession number JQ287497 (Figure 2a).

Herpes zoster ORF 63 partial sequences published in NCBI – accession number: JN315707

The clone containing pRSETc- HZ ORF 63 transformed into pET21b was sent for sequencing and the partial sequence was published in NCBI ac

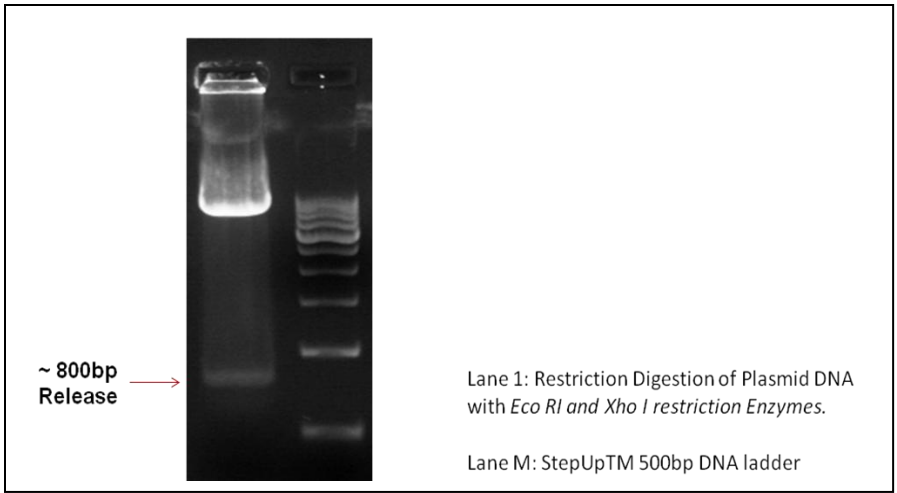

Figure 1: Restriction Fragment of Linear Polymorphism (RFLP) of Herpes zoster ORF 63

Figure1a

Figure 1a : Restriction digestion with EcoRI and XhoI

Figure 1b

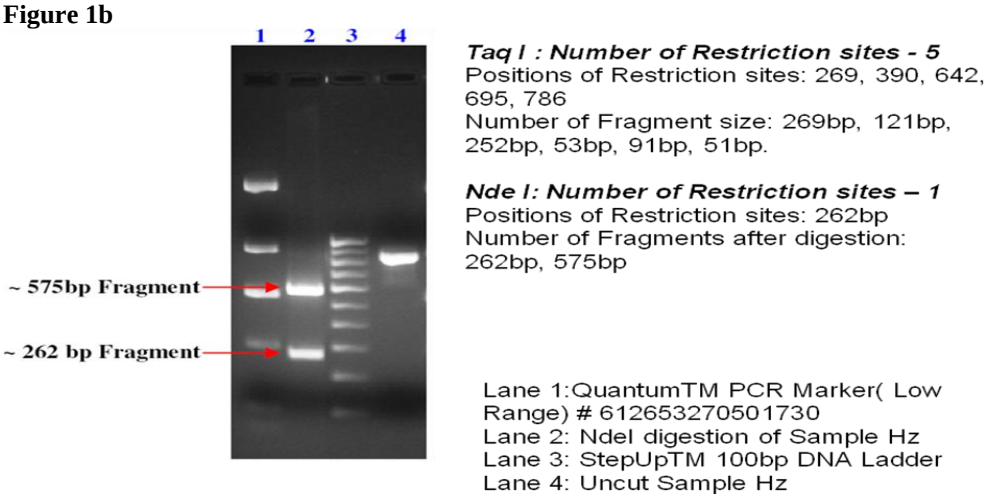

Figure 1b: Restriction Fragment of Linear Polymorphism (RFLP) pattern of herpes zoster ORF 63

Figure 2a: Partial sequence of ORF63 amplified from Chicken pox DNA sample published in NCBI- accession number: JQ287497 (size-494bp)

Nucleotide sequence
>gi|380005450|gb|JQ287497.1| Human herpesvirus 3 strain Indian immediate early protein 63 gene, partial cds
TTTATCTAATGGGTCGCACCAGACAGCGTCCCACGTTAGCGTGCTGGGAGGAATTGTTACAGCT
TCAACCCACCCAGACGCAGTGCTTACGCGCTACTTTAATGGAAGTGTCCCATCGACCCCCTCGG
GGGGAAGACGGGTTCATTGAGGCGCCGAATGTTCCTTTGCATAGGAGCGCACTGGAATGTGACG
TATCTGATGATGGTGGTGAAGACGATAGCGACGATGATGGGTCTACGCCATCGGATGTAATTGA
ATTTCGGGATTCCGACGCGGAATCATCGGACGGGGAAGACTTTATAGTGGAAGAAGAATCAGAG
GAGAGCACCGATTCTTGTGAACCAGACGGGGTACCCGGCGATTGTTATCGAGACGGGGATGGG
TGCAACACCCCGTCCCCAAAGAGACCCCAGCGTGCCACCGAGCGATACGCGGGTGCAGAAACC
GCGGAATATACAGCCGCGAAAGCGCTTACCGCGTTGGCGAGGGGGGTG

Protein sequence >gi|380005451|gb|AFD29182.1| immediate early protein 63, partial [Human herpesvirus 3]
YLMGRTRQRPTLACWEELLQLQPTQTQCLRATLMEVSHRPPRGEDGFIEAPNVPLHRSALECDVSD
DGGEDDSDDDGSTPSDVIEFRDSDAESSDGEDFIVEEESEESTDSCEPDGVPGDCYRDGDGCNTPSPKR
PQRATERYAGAETAEYTAAKALTALARGV

Figure 2b: Herpes zoster ORF 63 partial sequence published in NCBI – accession number: JN315707 (size-756bp)

Nucleotide sequence>gi|369961337|gb|JN315707.3| Human herpesvirus 3 immediate early protein IE63 gene, partial cds
ATGTTTTGCACCTCACCGGCTACGCGGGGCGACTCGTCCGAGTCAAAACCCGGGGCATCGGTTG
ATGTTAACGGAAAGATGGAATATGGATCTGCACCAGGACCCCTGAACGGCCGGGATACGTCGCG

GGGCCCCGGCGCGTTTTGTACTCCGGGTTGGGAGATCCACCCGGCCAGGCTCGTTGAGGACATC
AACCGTGTTTTTTTATGTATTGCACAGTCGTCGGGACGCGTCACGCGAGATTCACGAAGATTGC
GGCGCATATGCCTCGACTTTTATCTAATGGGTCGCACCAGACAGCGTCCCACGTTAGCGTGCTG
GGAGGAATTGTTACAGCTTCAACCCACCCAGACGCAGTGCTTACGCGCTACTTTAATGGAAGTG
TCCCATCGACCCCCTCGGGGGGAAGACGGGTTCATTGAGGCGCCGAATGTTCCTTTGCATAGGA
GCGCACTGGAATGTGACGTATCTGATGATGGTGGTGAAGACGATAGCGACGATGATGGGTCTAC
GCCATCGGATGTAATTGAATTTCGGGATTCCGACGCGGAATCATCGGACGGGGAAGACTTTATA
GTGGAAGAAGAATCAGAGGAGAGCACCGATTCTTGTGAACCAGACGGGGTACCCGGCGATTGTT
ATCGAGACGGGGATGGGTGCAACACCCCGTCCCCAAAGAGACCCCAGCGTGCCACCGAGCGAT
ACGCGGGTGCAGAAACCGCGGAATATACAGCCGCGAAAGCGCTTACCGCGTTG

Protein sequence>gi|369961338|gb|AEO13238.3| immediate early protein IE63, partial[Human herpesvirus 3]
MFCTSPATRGDSSESKPGASVDVNGKMEYGSAPGPLNGRDTSRGPGAFCTPGWEIHPARLVEDINRV
FLCIAQSSGRVTRDSRRLRRICLDFYLMGRTRQRPTLACWEELLQLQPTQTQCLRATLMEVSHRPPR
GEDGFIEAPNVPLHRSALECDVSDDGGEDDSDDDGSTPSDVIEFRDSDAESSDGEDFIVEEESEESTDSC
EPDGVPGDCYRDGDGCNTPSPKRPQRATERYAGAETAEYTAAKALTAL

Figure 3: Comparison of ORF 63 (nucleotide sequences) of chicken pox(JF306641) retrieved from NCBI with the partial sequence of chicken pox obtained from this work and herpes zoster(JN704703) retrieved from NCBI with the partial sequence of herpes zoster obtained from this work

Figure 3a Figure 3b

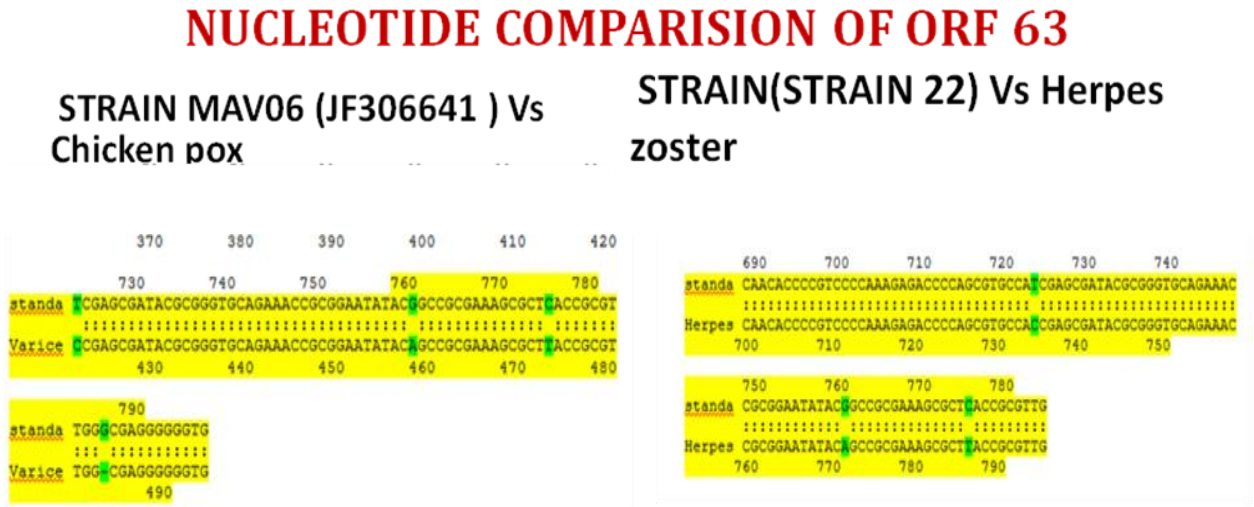

Figure 4: Comparison of ORF 63(protein sequences) of chicken pox(JF306641) retrieved from NCBI with the partial sequence of chicken pox obtained from this work and herpes zoster(JN704703) retrieved from NCBI with the partial sequence of herpes zoster obtained from this work

Figure 4a Figure 4b

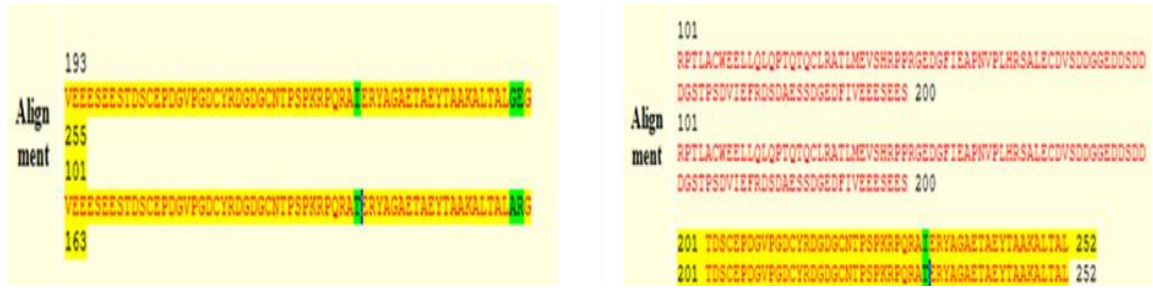

Figure 5: Difference between chicken pox strain (JN704702) and herpes zoster(JN704703) sequences at nucleotide level retrieved from NCBI.

cession number JN312707 (Figure 2b).

Analysis by Bioinformatics tools

Multiple alignments and sequence analyses

ORF 63: Nucleotide Strain (MAV06) Vs Varicella zoster

Pairwise comparison was performed using LALIGN between the nucleotide sequence of ORF63 VZ strain (MAV06) retrieved from NCBI and the partial sequence of the isolate sample collected from the chickenpox patients in our study (JQ287497) (Figure 3a).

Strain (Strain 22) Vs Herpes zoster

Pairwise comparison was performed using LALIGN between the nucleotide sequence of ORF63 HZ Strain (Strain 22) retrieved from NCBI and the partial sequence of the isolate sample collected from herpes zoster patients in our study (JN315707) (Figure 3b).

ORF 63: Protein

Standard with Varicella zoster

Pairwise comparison was performed using FFAS03 server between the protein sequence of ORF63 VZ strain (MAV06) retrieved from NCBI and the partial sequence of the isolate sample collected from chickenpox patients in our study (AFD29182) (Figure 4a).

Standard with herpes zoster

Pairwise comparison was performed using FFAS03 server between the protein sequence of ORF63 HZ strain (strain 22) retrieved from NCBI and the partial sequence of the isolate sample collected from herpes zoster patients in our study (AEO13238) (Figure 4b).

Discussion

In conform to the objective of the present work to explore the differences, if any,in the highly conserved and highly expressed gene, the ORF63 during dormant period in the ganglia of chickenpox and herpes zoster. The DNA from viral isolates of chickenpox as well as viral isolates of herpes zoster was isolated successfully. Attempts were made to express the clone product (pRSETc- VZV ORF 63 and pRSETc- HZ ORF 63) in different competent cells.

However owing to non – expression of protein IE 63, subcloning was done on pET21b vector that resulted in expected amplification and successful expression of IE protein . When the same gene was subcloned in pET21b vector, the protein was expressed .

Only a few of the previous researchers had mentioned the source of sample used for their work. But, till date, no work has been carried out comparing ORF 63 based on the expression of protein and sequencing. In the absence of a comparative study on ORF 63 of chickenpox and zoster, the present researcher presumes that most of the early researchers had studied on ORF 63 of VZV amplified from chickenpox sample only. It is not surprising that specific mention of the source of sample was not underlying them because of the common assumption that the causative agents of the both diseases are one and the same. The present researcher on the other hand, envised keen interest in finding the possibility of differences between ORF 63 of DNA isolate of chickenpox and DNA isolate of herpes zoster. To the best of the knowledge of the investigator, the comparative observation of ORF 63 after amplification and expression of ORF 63 seems to be first of its kind.

The expression of protein from both source of ORF 63 was eventually successful on SDS-PAGE by sub cloning in pET 21b vector (Figure 9). It is interesting to note that the expressed protein from both sources was of similar molecular weight. It is inferred that IE 63 amplified from chickenpox and IE 63 amplified from herpes zoster seems to be same. This comparative information on expressed protein of ORF 63 from two sources seems to be new to the literature. Previous work was carried out on the repeated detection of open reading frame (ORF) 63 transcripts and protein in latently infected human ganglia (Cohrs *et al.*, 1996; Mahalingam *et al.*, 1996; Lungu *et al.*, 1998; Cohrs *et al.*, 2000; Kennedy *et al.*, 2000; Grinfeld and Kennedy, 2004; Gary *et al.*, 2006; Cohrs and Gilden, 2007), ganglia from VZV-infected guinea pigs (Chen *et al.*, 2003) and cotton rats (Cohen *et al.*, 2004), as well as in the simian model of VZV latency (Messaoudi *et al.*, 2009) which suggests that this gene and its product are important for the maintenance of virus latency.

As IE 63 is also abundantly expressed during lytic VZV replication (Debrus et al 1995, Kinchington *et al.,* 1995, Stevenson *et al.,* 1996); a component of the VZV tegument (Kinchington *et al.,* 1995) and represses the activity of a number of VZV and heterologous viral and cellular promoters (Bontems *et al.,* 2002, Di Valentin *et al.,* 2005), also required to overcome the host innate response mediated by alpha interferon (Ambagala and Cohen, 2007); to inhibit apoptosis in primary human neuronal cells infected with VZV in culture (Hood *et al.,* 2006); IE63 binds to RNA polymerase II and VZV IE62, the major viral transactivator, and enhances the activity of the VZV gI promoter (Lynch *et al.*,2002); substitution of single or multiple sites plays a key role in regulating ORF63p cellular localization (Bontems *et al.,* 2002; Habran *et al.,* 2005; Walters *et al.,* 2008), protein–protein interactions (Ambagala *et al.,* 2009), regulation of transcription (Ambagala and Cohen, 2007; Bontems *et al.,* 2002; DiValentin *et al.,* 2005; Jackers *et al.,* 1992; Kost *et al.,* 1995; Lynch *et al.,* 2002) and VZV ganglionic infection in cotton rats (Cohen *et al.,* 2005).

Further research on ORF 63 from two different sources using RFLP was carried out in order to study the similarity as well as difference. Though, the present study can be considered as the first attempt on adoption of RFLP technique to study the ORF 63, fragmentation of DNA by NdeI restriction enzyme yielded better result in the separation of two fragments (575 bp and 262 bp) than TaqI (269 bp, 121 bp, 252 bp, 53 bp, 91 bp, 51 bp) compared to the uncut herpes zoster sample . As many restriction sites for the restriction enzymes (Nde I and Taq I) were present within the gene of interest in ORF 63 of chickenpox, the fragments of expected size for ORF 63 from chickenpox were not obtained. As a result, RFLP analysis of ORF 63 from chickenpox was not reported here. This is the first work to compare the ORF 63 from clinical samples collected from chickenpox and herpes zoster in Chennai. Previous work on genotyping studies was carried out on samples collected either from chickenpox or from zoster on genes 12 to 16, 38 to 43, 54 to 60 in Japan (Takayama *et al.*,1996) gene 1, 21, 22, 54 in China (Liu *et al.*,2009) ORF 38, 54, 62 in Australia (Campsall *et al.,* 2004) ORF 38, 54 in Kuwait (Qasem *et al.,* 2012) ORF 22, 38, 54 in South Korea (Kim *et al.,* 2011).

The clone containing ORF 63 amplified from chickenpox and herpes zoster patients' samples was sent for sequencing. The partial sequence of ORF 63 of chickenpox (494bp) and herpes zoster (756bp) was obtained and published in NCBI This is the first work in which the ORF 63 from amplified products of chickenpox and herpes zoster samples collected from Chennai was sequenced and published in NCBI. The previous studies on sequences of ORF 63 were carried out on ORF 63 amplified from chickenpox samples only and till date no study has been done to sequence of ORF 63 amplified from chickenpox and herpes zoster from India. The sequencing of ORF 63 present in latently infected human trigeminal ganglia (Cohrs *et al.*,1994, Cohrs *et al.*,1996, Cohrs *et al.*,2007), primary human neurons(Hood *et al.,* 2006) human sensory ganglia (Zerboni *et al.*,2010) was done from amplified ORF 63 of chickenpox.

In the present study, the complete genome of chickenpox and herpes zoster was first listed according to the differences in the clades as mentioned in the earlier study (Zell *et al.,* 2012). The comparative analysis of partial sequence of ORF 63 of chickenpox obtained from our study was compared with the ORF 63 of chickenpox sequence retrieved from NCBI, and the same was repeated in the case of herpes zoster sequence. Difference was found in between ORF 63 gene sequence of chickenpox and herpes zoster retrieved from NCBI submitted by other countries and chickenpox and herpes zoster sequence from the sequences submitted by us.

Conclusion

In the present study, the complete genome of chickenpox and herpes zoster was first listed according to the differences in the clades as mentioned in the earlier study (Zell *et al.,* 2012). The comparative analysis of partial sequence of ORF 63 of chickenpox obtained from our study was compared with the ORF 63 of chickenpox sequence retrieved from NCBI, and the same was repeated in the case of herpes zoster sequence. Difference was found in between ORF 63 gene sequence of chickenpox and herpes zoster retrieved from NCBI submitted by other countries and chickenpox and herpes zoster sequence from the sequences submitted by us (Figure 3,4). However, no remarkable differences were observed between the partial sequence of chickenpox and herpes zoster obtained in this study. The complete sequence comparison will provide valid difference or similarities between chickenpox and herpes zoster.

Many studies have been carried out on the highly expressed genes during the dormant period in the ganglion. The genes studied were ORF-4, ORF-21, ORF-29, ORF-62, and ORF-63, ORF 66 as well as their

expression. However, comparative studies between the ORF-63 and any of the above - mentioned gens of virus of chickenpox and of zoster have not been carried out.

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