Iranian Journal of Microbiology
Volume 16 Number 4 (August 2024) 524-535

ORIGINAL ARTICLE

Sequence analysis of isolated strains of herpes zoster virus among patients with shingles

Mohammed Jasim Mohammed Shallal[1*], Hind Ali Nasser[1], Alaa Abdul Hassen Naif[2]

[1]Department of Microbiology, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq
[2]Department of Dermatology, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq

Received: February 2024**, Accepted:** July 2024

ABSTRACT

Background and Objectives: Herpes zoster, or shingles, is caused by the varicella-zoster virus (VZV), which initially presents as chickenpox in children. VZV is a global health concern, especially in winter and spring, affecting 10-20% of adults over 50 and posing a 30% risk for the general population. This study used PCR to detect VZV, confirming results with duplicated DNA samples and identifying 234 bp fragments by targeting the gpB gene.
Materials and Methods: This study examined 50 herpes zoster cases from October 2020 to April 2021, involving 30 males and 20 females aged 10 to 90, diagnosed by dermatologists. Data were collected via a questionnaire. PCR detected VZV by amplifying the gpB and MCP genes from skin lesion samples. Six positive 234-bp PCR products were sequenced at Macrogen Inc. in Seoul, South Korea.
Results: Six DNA samples with 234 bp amplicons were sequenced, showing 99-100% similarity to human alpha herpesvirus sequences in the gpB gene. NCBI BLAST matched these sequences to a reference (GenBank acc. MT370830.1), assigning accession numbers LC642111, LC642112, and LC642113. Eight nucleic acid substitutions caused amino acid changes in the gpB protein: isoleucine to threonine, serine to isoleucine, and threonine to Proline. These variants were deposited in NCBI GenBank as gpB3 samples.
Conclusion: The study found high sequence similarity to known VZV sequences, identifying six nucleic acid variations and eight SNPs. Notable amino acid changes in the gpB protein were deposited in NCBI GenBank as the gpB3 sample.

Keywords: Herpes zoster virus; Human herpesvirus 3 (HHV-3); Varicella zoster virus; Shingles; Major capsid protein (HSV-1 capsid protein ICP5); Herpesvirus 3 (alpha)

INTRODUCTION

Herpes zoster virus, stemming from the varicella zoster virus (VZV), is characterized as a recurring skin viral ailment. Due to latent infection, the virus can reactivate, typically resulting in symptoms and shedding of the virus, facilitating its transmission through direct contact among individuals. Considered a recurrent skin viral disease caused by the reactivation of latent varicella-zoster virus (VZV) infection. The virus can reactivate, causing symptoms and shedding of the virus, leading to transmission through direct contact (1).

Sequence analysis methods have been employed to identify HZV strains in shingles lesions. These methods include PCR-based techniques such as restriction fragment length polymorphism (RFLP) analysis and DNA sequencing, as well as serological methods like

*Corresponding author: Mohammed Jasim Mohammed Shallal, Ph.D, Department of Microbiology, College of Medicine, University of Thi-Qar, Thi-Qar, Iraq. Tel: +9647802406226 Email: mohammed-j@utq.edu.iq

enzyme-linked immunosorbent assay (ELISA) and immunoblotting. Combining these methods can offer a comprehensive analysis of the HZV strains present in shingles lesions (2).

Research has also investigated the association between HZV strains and clinical characteristics of shingles. For instance, Recent studies have shown that HZV strains with a specific genetic variant were correlated with more severe pain in shingles patients (3-4). Similarly, a study conducted in the United States observed that certain HZV strains were more prevalent in shingles cases involving the eye compared to other locations (5). These findings indicate that sequence analysis of HZV strains might provide valuable insights into the clinical course of shingles.

Furthermore, sequence analysis can guide antiviral therapy for shingles. Various studies related to the treatment of HZV suggested that patients with shingles caused by a specific HZV strain responded better to acyclovir treatment compared to those with other strains (6, 7). Another study in the United States revealed that certain genetic variants of HZV strains were associated with reduced susceptibility to specific antiviral drugs (8). Glycoprotein B is one of the most conserved envelope proteins in the herpesvirus family, which includes not only VZV but also other viruses such as HSV-1 and HSV-2. Its conservation facilitates the development of broadly applicable diagnostic tools that are reliable due to the stability of the target gene sequence across different strains and over time (9). Glycoprotein B plays a crucial role in mediating the entry of the virus into cells and subsequent cell-to-cell spread. This makes it a vital target for therapeutic interventions and a key marker for identifying active viral infection (10). The specificity of the gpB gene for VZV allows for the distinction of VZV from other herpesviruses, particularly in the clinical setting where symptoms may overlap with those caused by HSV. Molecular assays targeting gpB can accurately confirm VZV infection, essential for appropriate management and treatment (11). Sequencing the gpB gene helps in monitoring the genetic evolution of VZV, which is crucial for tracking the emergence of drug-resistant strains and understanding the genetic factors that contribute to varied clinical manifestations and severity of herpes zoster (12). Varicella-zoster virus (VZV) is known to exhibit strain variation, which can have clinical implications regarding the severity of disease, vaccine efficacy, and potential reactivation events leading to herpes zoster (shingles). VZV strains exhibit genetic diversity, which can influence various aspects of the virus, including virulence, pathogenesis, and antigenicity. Studies have suggested that different VZV strains may be associated with varying clinical manifestations of varicella and herpes zoster, including differences in rash severity, duration of illness, and risk of complications (13-15).

MATERIALS AND METHODS

Data collection from patients. This study focused on patients with dermatological diseases who were referred to dermatologists. Data was collected from various dermatology consultants, and outpatient clinics such as Al-Hussein Teaching Hospital and Al-Nassiriyah Teaching Hospital, from October 2020 to April 2021. A cohort of 50 cases of herpes zoster (HZ) was monitored and documented, encompassing 30 male and 20 female individuals aged between 10 and 90 years. These cases were clinically diagnosed by specialized dermatologists based on distinct clinical signs. To ascertain the occurrence of herpes zoster (HZ) or shingles among patients, specific data is gathered directly from individuals through a specialized questionnaire. This questionnaire encompasses details such as age, gender, residency, household size, and any prior occurrences of chickenpox or shingles within the individual or their family.

Inclusive criteria. Individuals aged above 10 years but less than 90 years, both male and female, were enrolled in this study either as patients with herpes zoster virus (HZV) or as part of the control group.

Exclusive criteria. Groups excluded from the study included ages less than 10 years and those older than 90 years. Other skin diseases, as well as those with chronic diseases, cases of all types of cancer, and cases that use chemotherapy and other various immune disorders were also excluded.

Collection of samples. Samples were obtained from the vesicular fluid of herpes-zoster-infected patients. Swabs were taken from the localized skin lesions on a specific side of the body, including cranial, thoracic, lumbar, sacral, and foot regions. The vesicle's base and its transparent fluid content were gathered to detect the presence of the virus. A ster-

ile, single-use blade was used to scrape and lift the lesion, and the vesicular fluid was collected using special cotton swabs. The specimens were preserved in sterile laboratory tubes that contained viral transport medium (VTM) with RPMI and frozen at -20°C. A control group of 50 healthy individuals are included in this study, and the same tests were performed on this group, except for collecting vesicle swabs.

Viral DNA extraction. DNA samples of the herpes zoster virus were extracted from both the vesicular fluid and the base of vesicles acquired from skin lesion scrap samples. This extraction procedure employs the Viral Nucleic Acid Extraction Kit III supplied by Geneaid, a USA-based company. Subsequently, the extracted DNA underwent evaluation for concentration and purity using a Nanodrop spectrophotometer from Avans, a Taiwan-based manufacturer. This spectrophotometer measures the absorbance of DNA at 260 nm and 280 nm to determine its purity.

To directly detect the varicella-zoster virus (VZV), a conventional PCR method was utilized. This technique amplified both the glycoprotein type B (gpB) and major capsid protein (MCP) genes present in VZV from skin lesion scrap samples. The PCR protocol adhered to the procedures outlined by Aubaid et al. (2020) (16) and involved the use of two sets of primers specified for the amplification of a specific gene of herpes zoster virus glycoprotein type B (gp B) gene within amplicon size of 234bp which included a forward primer (gpB-F) CGTTACGTCCGTGAAATCGC and a reverse primer (gpB-R): AATGGCCGTTCCGCTATCAT. This set of primers was used in the conventional PCR reaction within typical Thermocycler conditions including an initial denaturation step within 94°C for 5 min of one cycle, and a denaturation step within 94°C for 30 secs; annealing step of 58°C for 30 sec., an extension step within 72°C for 1 min, and a final extension step within 72°C for 5 min all these steps are repeated for 30 cycles. Subsequently, the PCR products were subjected to analysis and observation through agarose gel electrophoresis.

DNA sequencing of PCR amplicons and phylogenetic tree. Following the amplification, the 234-bp PCR viral DNA products were selected for sequencing. Six positive PCR products, each with a length of 234 bp, were chosen for sequencing. The sequencing was carried out commercially in both forward and reverse directions (Macrogen Inc., Seoul, South Korea).

To ensure the reliability of the results and exclude potential artifacts from the PCR or sequencing processes, only clear chromatographs from the ABI (Applied Biosystems) sequence files were taken into consideration. The identification of PCR fragments involved comparing the observed DNA sequences of the viral samples with those available in the viral database. The identified variants of VZV isolates in this study were compared to their neighboring homologous reference sequences using the NCBI-BLAST server.

To discern nucleic acid variations, the DNA chromatograms were carefully examined against deposited viral DNA sequences, using BioEdit version 7.1 by DNASTAR in Madison. Each variant within the human herpes zoster virus genes was annotated using Snap-Gene Viewer version 4.0.4, accessible at https://www.snapgene.com. Subsequently, an inclusive tree incorporating the observed variant was constructed using the neighbor-joining method and visualized utilizing the iTOL suite, which provides a traditional tool for branch construction.

Ethical considerations. Ethical considerations for this study involve obtaining formal approvals and ensuring direct supervision by a medical specialist in the sampling and handling of patients. The participation of dermatologists, who have approved the study, will be integral to its conduct within a hospital setting. Prior to registration, explicit informed consent will be obtained from all participants, and strict measures will be implemented to maintain confidentiality and anonymity throughout the entire study period.

RESULTS

Detection VZV by PCR assay. DNA samples were duplicated for confirmation of PCR results. A standard PCR protocol was employed to concurrently analyze the presence of the gpB gene. Additionally, the identification of the gpB gene MCP gene was confirmed by the presence of a 234 bp, as depicted in Fig. 1.

DNA sequencing of PCR amplicons. Within this locus, six samples were analyzed, revealing amplicons of approximately 234 bp in length. Sequencing reactions confirmed the identity of the amplified products through NCBI BLAST analysis. Regarding the 234

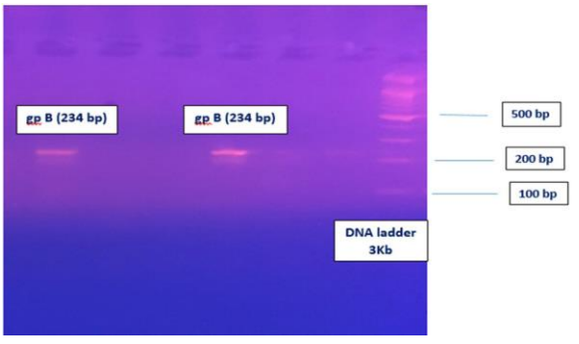

Fig. 1. The gel electrophoresis results show PCR products run on a 0.8% agarose gel. In the leftmost lane, it is observing the expected PCR products containing the glycoprotein type B (gpB) gene of the herpes zoster virus, with a band size of 234 base pairs (bp). The DNA ladder used in this gel spans the entire size range from 3000 bp.

bp PCR amplicons of the targeted gpB sequences, the NCBI BLAST engine demonstrated a significant sequence similarity between the sequenced samples and human alpha-herpesvirus sequences, indicating a homology of approximately 99 to 100%. This homology partially covered the coding region of the gpB gene sequences. By comparing the observed DNA sequences of the investigated samples with retrieved DNA sequences (GenBank acc. MT370830.1), precise positions and other details of the retrieved PCR fragments were identified (Fig. 1) (under the accession numbers LC642111, LC642112, LC642113). In this study, the identification and characterization of VZV isolate variations entailed discerning between synonymous and non-synonymous mutations. This approach aligns with established methods in virology research, where synonymous mutations do not result in changes to the encoded amino acid sequence, whereas non-synonymous mutations lead to alterations in the amino acid sequence of the viral protein (17).

Alignment results for the 234 bp samples revealed the detection of six nucleic acid variations when compared to the corresponding human alpha-herpesvirus reference sequences (Fig. 2). These sequences were generated by aligning our analyzed samples with the most pertinent sequences accessible in the NCBI database (GenBank accession no. MT370830.1) (Fig 3).

A highly interesting eight nucleic acid substitution SNPs and were detected in very high frequency in the investigated specimens, these sequences were shown according to their positions in the PCR amplicons (Table 1). The identified nucleic acid variations, including eight nucleic acid substitution SNPs, were found to occur frequently, deviating from the reference sequences. Upon amino acid alignment, modifications were observed in the encoded gpB protein, specifically involving isoleucine to threonine, serine to isoleucine, and threonine to proline substitutions. These variations were cataloged in the NCBI-bank database as the gpB3 sample.

The differences observed in the nucleic acid sequences of the analyzed samples were not present in the corresponding reference sequences. These observed mutations were further investigated to determine whether such substitutions might result in potential alterations in their designated positions within the encoded gpB protein (Fig. 4).

All nucleic acid sequences were translated into their respective amino acid sequences using the Bioedit translate suite. The total count of identified nucleic acid substitutions was four. Amino acid alignment of these sequences with their references revealed specific variations in the protein encoded within the amplified PCR product, namely isoleucine to threonine, serine to isoleucine, and threonine to proline.

Nevertheless, it is crucial to emphasize that these identified variations have been individually submitted to the NCBI-bankit database, specifically representing the gpB3 sample.

The evolutionary history was deduced using the Neighbor-Joining method (18, 19). The presented optimal tree has a sum of branch length equal to 0.02040816. Evolutionary distances were calculated using the p-distance method (20), expressed in units of the number of base differences per site as presented in Fig. 5.

The phylogenetic tree displays the proportion of sites with at least one clear base in at least one sequence for each descendant clade, denoted adjacent to internal nodes. The analysis encompassed six nucleotide sequences as presented in Figs. 6-8, including codon positions of 1st, 2nd, 3rd, and Noncoding. Ambiguous positions were omitted for each sequence pair, resulting in a final dataset of 197 positions. These evolutionary assessments were carried out using MEGA7 (21).

DISCUSSION

Studying shingles across a broad age range allows for a comprehensive assessment of the disease bur-

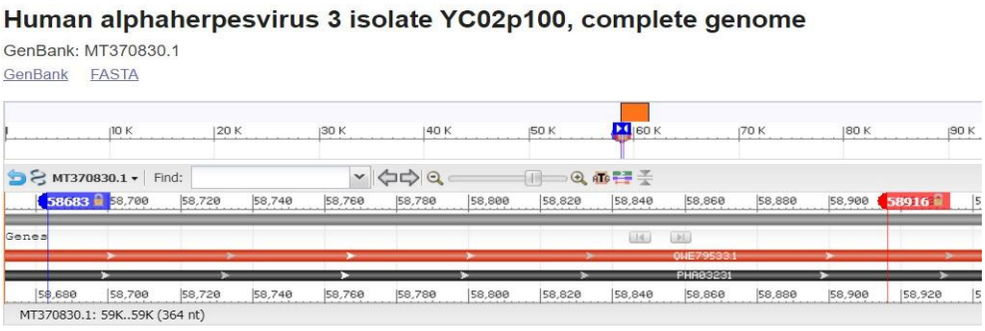

Fig. 2. The precise location of the 234 bp fragments, which cover a segment of the coding region of the gpB gene in the genomic sequences of human alpha-herpesvirus (GenBank acc. no. MT370830.1), is indicated in the figure, and showing both the starting and ending points of this amplicon.

Fig. 3. The alignment of DNA sequences for six samples was conducted with their respective reference sequences of the 234 bp amplicons of the gpB genetic DNA sequences. The symbol "MT37083" represents the NCBI reference sequence, while the labels "gpB1-gpB6" correspond to samples 1-6, respectively.

Table 1. Type and position of SNP

Sample	SNP	Position	Type
gpB1	C>A	191	Transversions
gpB2	C>A	191	Transversions
	T>C	5	Transitions
gpB3	G>T	8	Transversions
	C>A	9	Transversions
	A>C	10	Transversions
gpB4	C>A	191	Transversions
gpB5	C>A	191	Transversions
gpB6	None		

den in the population. While shingles is less common in younger individuals, understanding the incidence and severity of the disease across all age groups can provide valuable insights into its epidemiology and healthcare burden. Shingles can have long-term effects and complications, including postherpetic neuralgia, which can significantly impact quality of life. Studying individuals across a wide age range allows for the investigation of these long-term effects and the evaluation of interventions to mitigate them. Therefore, by including individuals from 10 to 90 years old in this study, it can be evaluating the vaccine's impact

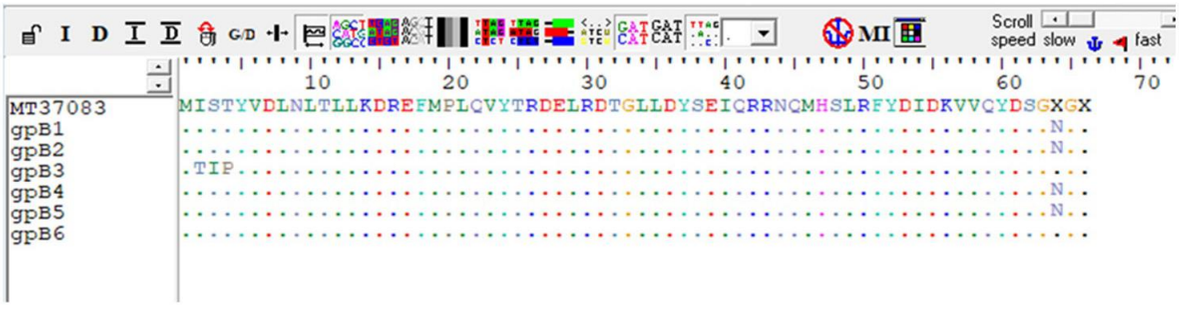

Fig. 4. displays the alignment of amino acid residues with detected variations in the gpB gene.

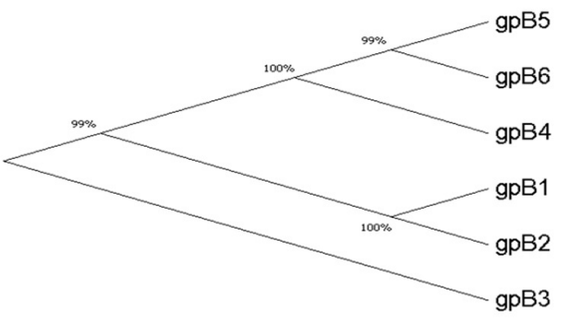

Fig. 5. Phylogenic tree of Human herpesvirus samples.

on both younger individuals who may be at risk due to immunocompromised conditions and older adults who are at higher risk of developing shingles. According to results of Yawn et al. 2009, prevalence of herpes zoster in family practice in some community populations provided insights into the age distribution of the disease and highlighting the importance of studying shingles across different age groups. This investigates the relationship between herpes zoster and immune status above and below 50 years of age, emphasizing the importance of considering age as a factor in studying the disease (22-24).

Various molecular methods have been employed to analyze the sequence of isolated HZV strains among patients with shingles. PCR-based assays, including restriction fragment length polymorphism (RFLP) analysis, DNA sequencing, and real-time PCR, are commonly used techniques. These methods allow for the amplification and analysis of specific regions of the HZV genome, such as the glycoprotein E (gE) gene or the DNA polymerase gene. Serological methods, such as enzyme-linked immunosorbent assay (ELISA) and immunoblotting, can also be utilized to detect specific antibodies to HZV antigens (25-27).

The relationship between HZV strain and clinical presentation: Numerous studies have explored the correlation between specific HZV strains and the clinical presentation of shingles. A study conducted in the United States revealed that HZV strains with a particular genetic variant were associated with more severe pain in patients with shingles (28). Similarly, a study carried out in the United States found that HZV strains with a distinct genetic variant were more frequently linked to shingles involving the eye, as compared to those affecting other locations (29, 30). These findings suggest that sequence analysis of HZV strains can yield valuable insights into the clinical course of shingles.

Conventional PCR is a widely used molecular biology technique for amplifying specific DNA sequences. In the context of VZV detection, conventional PCR can target specific genes, such as the gpB gene, which codes for glycoprotein B, an important surface antigen of the virus. The gpB gene, which codes for glycoprotein B, is an ideal target for VZV detection due to its specificity and abundance in the viral genome. The presence of the gpB gene can serve as a molecular marker for the presence of VZV in clinical samples. Varicella-zoster virus (VZV) is the causative agent of chickenpox and herpes zoster (shingles). VZV glycoprotein B (gB) is a critical component of the virus envelope and plays a crucial role in viral entry and fusion with host cells. VZV gB is involved in the initial stages of viral infection. It facilitates viral attachment to host cells and subsequent fusion of the viral envelope with the host cell membrane, allowing the virus to enter the cell. As such, gB is an attractive target for therapeutic intervention or vaccine development against VZV (31). There are several reasons why researchers might choose VZV gB as a target for study: Firstly, gpB is a highly conserved protein among herpesviruses, indicating its importance in viral biology and suggesting that targeting it could have broad implications for controlling VZV infection. Accordingly, the sequences of glycoprotein B from herpes simplex virus type 1

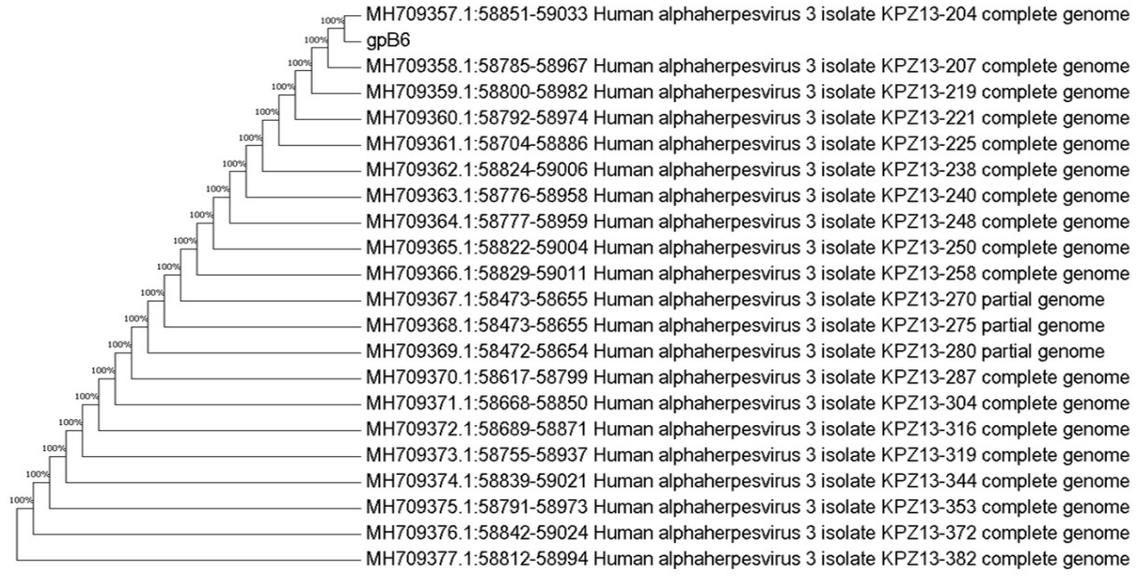

Fig. 6. Phylogenic tree of Human herpesvirus sample (gpB6) with NCBI.

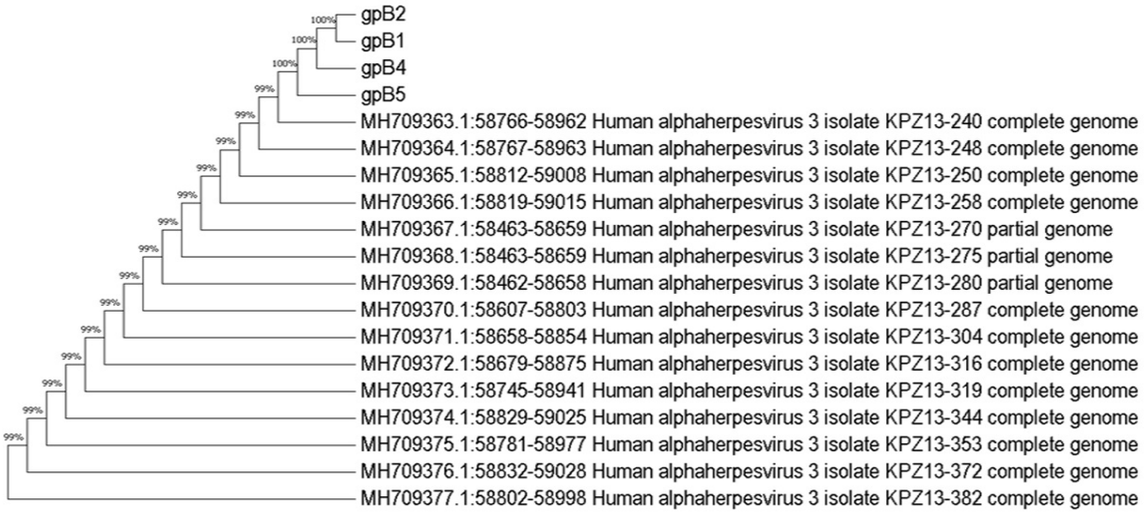

Fig. 7. Phylogenic tree of Human herpesvirus samples (gpB1,2,4,5) with NCBI.

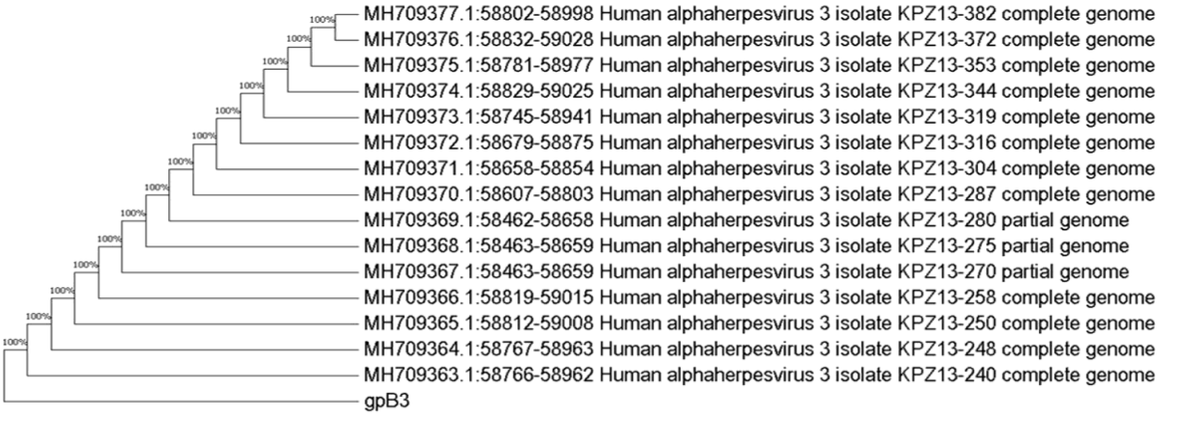

Fig. 8. Phylogenic tree of Human herpesvirus samples (gpB3) with NCBI.

(HSV-1) and varicella-zoster virus (VZV), highlighting the conservation of this protein between different herpesviruses (32).

Confirmation of the presence of the gpB gene by PCR amplification and detection of a specific amplicon size (e.g., 234 bp) further validates the specificity of the assay for VZV detection (33). Several studies have employed PCR-based methods for the detection of VZV, targeting various viral genes including gpB. For example, a study by Bai et al. (2000) utilized PCR targeting the gpB gene for the detection of herpesviruses, such as CMV and VZV in clinical specimens (34). The confirmation of the identity of the amplified products is essential to ensure that the PCR assay specifically amplifies the target gene (gpB) of VZV. The use of sequencing reactions followed by NCBI BLASTn analysis provides a reliable method for confirming the specificity of the PCR assay and the identity of the amplified products. The high sequence similarity (about 99 to 100%) between the sequenced samples and Human alphaherpesvirus sequences, particularly VZV, confirms the specificity of the PCR assay for detecting VZV. This finding is consistent with previous studies that have demonstrated the high sequence conservation of the gpB gene among different strains of VZV (35, 36). The comparison of the observed DNA sequences of the investigated samples with retrieved DNA sequences from GenBank (e.g., accession number MT370830.1) allows for the identification of exact positions and other details of the PCR fragments. This comparative analysis provides further validation of the specificity and accuracy of the PCR assay for detecting VZV. The accession numbers (LC642111, LC642112, LC642113) likely refer to the sequences deposited in a public database, such as GenBank, following sequencing

The present results showed different mutations in all samples except gpB6. In gpB1, gpB2, gpB4, and gpB5 were suited in a place different from that observed for gpB3; therefore, these four samples had identity varied with last sample. The reason for such differences was attributed to the observed 191C>A variant. This variant was behind this type of difference in the currently investigated viral samples. Thus, this variant has a possible evolutionary role in these pathogenic human alpha-herpesviruses family. As a result, the count of phylogenetic branches and clades showed an increase, indicating evidence of both ancient and inter-clade recombination, alongside contemporary recombination among strains of VZV (37). The amino acid transversion caused by this variant, in which the isoleucine was replaced with theronine, also serine was replaced with isoleucine and showed the threonine was replaced with proline, all variant was detected only in gpB3 sample. Despite the presence of SNP in all samples except gpB6, no detectable amino acid variant detected only in gpB3. In present results of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) that gpB1 sample had 100% identity with gpB2 sample; so there were found in same branch; and these samples had 100% identity with gpB4 and gpB5; while when compare these samples with other samples in NCBI showed had 99% identity with (MH709363.1 Humanherpesvirus3 isolate KPZ13-240; MH709364.1 Humanherpesvirus 3 isolate KPZ13-248, MH709365.1 Humanherpesvirus 3 isolate KPZ13-250, MH709366.1 Humanherpesvirus 3 isolate KPZ13-258 and MH709368.1 Humanherpesvirus 3 isolate KPZ13-275; and others samples) that isolated from USA (Fig. 6). The gpB6 sample was found in the separated branch, and it different from all studies samples, while the comparison this sample with samples in NCBI showed that the current sample (gpB6) had 100% identity with (MH709358.1 Humanherpesvirus3 isolate KPZ13-207, MH709359.1 Human herpesvirus-3 isolate KPZ13-219, MH709361.1 Humanherpesvirus3 isolate KPZ13-225, MH709375.1 Human herpesvirus3 isolate KPZ13-382) and other samples that maintain in phylogenetic tree (Fig. 5). Also the gpB3 was presented in separated branch from other studies samples and which had variation in nucleic acid, when comparison this sample globally with samples in NCBI showed 100% identity with many samples that isolated in US, as shown in (Fig. 7) such as: (MH709377.1 Humanherpesvirus3 isolate KPZ13-382, MH709376.1 Humanherpesvirus3 isolate KPZ13-372, MH709373.1 Humanherpesvirus3 isolate KPZ13-319, and MH709369.1 Humanherpesvirus3 isolate KPZ13-380). The presence of the observed nucleic acids variation in gpB3 sample may entail a potential role for the gpB gene fragment in the adaptation made by this sequence against the clinical sources from which there were obtained. Accordingly, the presently utilized gpB based amplicons can be useful in the resolution of genetic differences among the currently investigated gpB1, gpB2, gpB3, gpB4, gpB5 and gpB6 samples. The differences of gpB3 sample was credited from the observed

four variants in different position of this sample including: T>C in the position 5, G>T in the position 8, C>A in the position 9 and A>C in the position 10, this may also participate in inducing a direct effect on the mechanism of entry into host cells, membrane fusion, via a core complex of virally expressed envelope glycoproteins gB (38).

Furthermore, the aggregation of all investigated viral samples in these different positions may refer to the presence of different patterns of the phylogenetic distributions of these sequence. The recent observation of this tree (Fig. 7) had added another confirmation of sequencing reactions because it explained the actual neighbor joining-based positioning in such observed variations. Significantly, the inclusion of gpB gene sequences in this study has provided additional evidence supporting the accurate identification of the genotype of this viral organism.

Functional implications of such variations on protein function can be diverse and context-dependent. For instance, substitutions at crucial sites within the protein sequence may impact protein folding, stability, or post-translational modifications, consequently altering its biological activity (39). Moreover, changes in amino acid composition can influence protein-protein interactions, cellular localization, and immune recognition, potentially affecting viral pathogenesis and host immune response (40). Nucleic acid variations, such as single nucleotide polymorphisms (SNPs) or insertions/deletions, are crucial indicators of genetic diversity within viral populations. These variations can influence viral pathogenesis, virulence, and response to antiviral therapies (41). Human alpha-herpesviruses, including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), exhibit genetic variability due to factors like recombination, mutation rates, and selective pressures (42). This diversity contributes to differences in virulence, transmission dynamics, and disease outcomes. The alignment of the isolate samples with a reference sequence (MT370830.1) from the NCBI database is a standard practice in viral genomics research. It allows for the identification of genetic variations and the determination of their potential significance in relation to known viral strains. Comparative genomic analyses are essential for understanding the evolution and epidemiology of viral pathogens and also to identify novel variants, track their spread, and assess their impact on viral biology and clinical outcomes (43). six nucleic acid variations in the alignment of 234 bp samples with human alpha-herpesvirus reference sequences highlight the genetic diversity and evolutionary dynamics of these viral pathogens. Further investigations are needed to elucidate the functional significance of these variations and their implications for viral pathogenesis and public health. The investigation of these mutations to determine potential alterations in the designated positions within the encoded gpB protein is critical for understanding their functional consequences. The gpB protein is often involved in crucial aspects of viral biology, such as host cell entry, immune evasion, and virulence (44, 45). Even single amino acid substitutions can significantly impact protein structure and function, potentially altering viral phenotype and pathogenicity (46).

Translating nucleic acid sequences into amino acid sequences allows for a more comprehensive analysis of genetic variations at the protein level. Amino acid alignment with reference sequences facilitates the identification of specific variations in the encoded protein, providing insights into potential structural and functional changes (47, 48).

The submission of identified variations to the NCBI GenBank database ensures their availability to the scientific community for further analysis and reference. This contributes to the collective knowledge of viral genetic diversity and evolution, aiding in the development of diagnostic tools, therapeutic strategies, and epidemiological surveillance (49). The inclusion of codon positions (1st, 2nd, and 3rd) and noncoding regions in the analysis provides a comprehensive view of sequence evolution. Different codon positions may experience varying degrees of selective pressure, influencing the rate and pattern of nucleotide substitutions (50). Additionally, noncoding regions can contain important regulatory elements or phylogenetically informative sites. MEGA7 is a widely used software package for conducting molecular evolutionary analyses, including phylogenetic inference, sequence alignment, and evolutionary rate estimation (26).

CONCLUSION

Based on the findings derived from this investigation, the study concluded that the utilization of conventional PCR targeting the gpB gene proves invaluable in VZV detection, benefiting from its specificity

and abundance within the viral genome. Confirmation of amplified products' identity through sequencing and BLAST analysis assures assay specificity, corroborated by high sequence similarity to known VZV sequences, revealing six nucleic acid variations and eight nucleic acid substitution SNPs. Moreover, nucleic acid variations identified through comparative genomic analyses highlight the genetic diversity and evolutionary dynamics of VZV, potentially influencing pathogenesis and public health implications. Also, the amino acid alignment demonstrated changes in the encoded gpB protein, with notable substitutions such as Isoleucine to Threonine, Serine to Isoleucine, and Threonine to Proline. And the identified variations were deposited in the NCBI Gen-Bank database as the gpB3 sample, contributing to the genetic repository. Finally, the evolutionary analysis emphasized significant genetic diversity in the gpB gene of VZV, highlighting its dynamic nature.

Further research is needed on nucleic acid variations within the gpB gene of Varicella-Zoster Virus (VZV) is crucial for understanding their functional effects on viral pathogenesis, host interactions, and immune evasion. Investigating the impact of single amino acid substitutions on gpB protein structure and function is essential for deciphering their role in viral virulence and pathogenicity. Continuing to translate nucleic acid sequences into amino acid sequences allows for a comprehensive analysis of genetic variations' impact on viral proteins, shedding light on VZV pathogenesis.

ACKNOWLEDGEMENTS

Authors would like to express gratitude and appreciation to the College of Medicine, University of Thi-Qar, and all individuals within the Microbiology Department for their support and assistance in advancing this project. Additionally, special thanks are extended to the personnel of the Dermatology outpatient clinic at Al-Hussein Teaching Hospital and Al-Nassiriyah Teaching Hospital for their provision of facilities that aided in the progress of this project.

REFERENCES

1. Weidner-Glunde M, Kruminis-Kaszkiel E, Savanagouder M. Herpesviral latency-common themes. *Pathogens* 2020; 9: 125.
2. Własiuk A, Giannopoulos K. Herpes zoster infections: epidemiology, diagnostics, and prophylaxis in light of a growing clinical problem. *Hematol Clin Pract* 2023; 14: 69-79.
3. Johnson RW, Bouhassira D, Kassianos G, Leplège A, Schmader KE, Weinke T. The impact of herpes zoster and post-herpetic neuralgia on quality-of-life. *BMC Med* 2010; 8: 37.
4. Lukas K, Edte A, Bertrand I. The impact of herpes zoster and post-herpetic neuralgia on quality of life: patient-reported outcomes in six European countries. *Z Gesundh Wiss* 2012; 20: 441-451.
5. Nagel MA, Gilden D. Update on varicella zoster virus vasculopathy. *Curr Infect Dis Rep* 2014; 16: 407.
6. Koshy E, Mengting L, Kumar H, Jianbo W. Epidemiology, treatment and prevention of herpes zoster: A comprehensive review. *Indian J Dermatol Venereol Leprol* 2018; 84: 251-262.
7. Patil A, Goldust M, Wollina U. Herpes zoster: A review of clinical manifestations and management. *Viruses* 2022; 14: 192.
8. Gubareva LV, Mishin VP, Patel MC, Chesnokov A, Nguyen HT, De La Cruz J, et al. Assessing baloxavir susceptibility of influenza viruses circulating in the United States during the 2016/17 and 2017/18 seasons. *Euro Surveill* 2019; 24: 1800666.
9. Arvin A, Campadelli-Fiume G, Mocarski E, Moore PS, Roizman B, Whitley R, et al. Human herpesviruses: Biology, therapy, and immunoprophylaxis. cambridge: cambridge university press; 2007.
10. Carmichael JC, Yokota H, Craven RC, Schmitt A, Wills JW. The HSV-1 mechanisms of cell-to-cell spread and fusion are critically dependent on host PTP1B. *PLoS Pathog* 2018; 14(5): e1007054.
11. Nikolic D, Kohn D, Yen-Lieberman B, Procop GW. Detection of herpes simplex virus and Varicella-Zoster virus by traditional and multiplex molecular methods. *Am J Clin Pathol* 2019; 151: 122-126.
12. Kennedy PGE, Gershon AA. Clinical features of Varicella-Zoster virus infection. *Viruses* 2018; 10: 609.
13. Gershon AA, Gershon MD. Pathogenesis and current approaches to control of varicella-zoster virus infections. *Clin Microbiol Rev* 2013; 26: 728-743.
14. Campsall PA, Au NH, Prendiville JS, Speert DP, Tan R, Thomas EE. Detection and genotyping of varicella-zoster virus by TaqMan allelic discrimination real-time PCR. *J Clin Microbiol* 2004; 42: 1409-1413.
15. Loparev VN, Rubtcova EN, Bostik V, Tzaneva V, Sauerbrei A, Robo A, et al. Distribution of varicella-zoster virus (VZV) wild-type genotypes in northern and southern Europe: evidence for high conservation of circulating genotypes. *Virology* 2009; 383: 216-225.

16. Aubaid AH, Abed A, Alrodhan MA. A surveillance of varicella zoster virus among school age children in Al-Diwaniyah governorate. *Indian J Public Health Res Dev* 2020; 11: 1662.
17. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018; 35: 1547-1549.
18. Bryant D, Moulton V. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. *Mol Biol Evol* 2004; 21: 255-265.
19. Birlea M, Cohrs RJ, Bos N, Mehta SK, Pierson DL, Gilden D. Search for varicella zoster virus DNA in saliva of healthy individuals aged 20-59 years. *J Med Virol* 2014; 86: 360-362.
20. Nei M, Kumar S (2000). Molecular evolution and phylogenetics. New York, NY: Oxford University Press.
21. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016; 33: 1870-1874.
22. Yawn BP, Itzler RI, Wollan PC, Pellissier JM, Sy LS, Saddier P. Health care utilization and cost burden of herpes zoster in a community population. *Mayo Clin Proc* 2009; 84: 787-794.
23. Gnann JW, Whitley RJ. Clinical practice. *Herpes zoster. N Engl J Med* 2002; 347: 340-346.
24. Patil A, Goldust M, Wollina U. Herpes zoster: A Review of clinical manifestations and management. *Viruses* 2022; 14: 192.
25. Nikolic D, Kohn D, Yen-Lieberman B, Procop GW. Detection of herpes simplex virus and Varicella-Zoster virus by traditional and multiplex molecular methods. *Am J Clin Pathol* 2019; 151: 122-126.
26. Jevšnik M, Lusa L, Uršič T, Glinšek Biškup U, Petrovec M. Detection of herpes simplex and varicella-zoster virus from skin lesions: comparison of RT-PCR and isothermal amplification for rapid identification. *Diagn Microbiol Infect Dis* 2020; 97: 115015.
27. Pan D, Wang W, Cheng T. Current methods for the detection of antibodies of Varicella-Zoster virus: A review. *Microorganisms* 2023; 11: 519.
28. Harpaz R, Leung JW. The epidemiology of herpes zoster in the United States during the era of varicella and herpes zoster vaccines: changing patterns among older adults. *Clin Infect Dis* 2019; 69: 341-344.
29. Cohen JI. clinical practice: herpes zoster. *N Engl J Med* 2013; 369: 255-263.
30. Gershon AA, Breuer J, Cohen JI, Cohrs RJ, Gershon MD, Gilden D, et al. Varicella zoster virus infection. *Nat Rev Dis Primers* 2015; 1: 15016.
31. Eisenberg RJ, Atanasiu D, Cairns TM, Gallagher JR, Krummenacher C, Cohen GH. Herpes virus fusion and entry: a story with many characters. *Viruses* 2012; 4: 800-832.
32. Connolly SA, Jardetzky TS, Longnecker R. The structural basis of herpesvirus entry. *Nat Rev Microbiol* 2021; 19: 110-121.
33. Aberle SW, Aberle JH, Steininger C, Puchhammer-Stöckl E. Quantitative real time PCR detection of Varicella-zoster virus DNA in cerebrospinal fluid in patients with neurological disease. *Med Microbiol Immunol* 2005; 194: 7-12.
34. Bai X, Rogers BB, Harkins PC, Sommerauer J, Squires R, Rotondo K, et al. Predictive value of quantitative PCR-Based viral burden analysis for eight human herpesviruses in pediatric solid organ transplant patients. *J Mol Diagn* 2000; 2: 191-201.
35. Loparev VN, Rubtcova EN, Bostik V, Govil D, Birch CJ, Druce JD, et al. Identification of five major and two minor genotypes of varicella-zoster virus strains: a practical two-amplicon approach used to genotype clinical isolates in Australia and New Zealand. *J Virol* 2007; 81: 12758-12765.
36. Kennedy PG. Varicella-zoster virus latency in human ganglia. *Rev Med Viro* 2002; 12: 327-334.
37. Norberg P, Depledge DP, Kundu S, Atkinson C, Brown J, Haque T, et al. Recombination of globally circulating varicella-zoster virus. *J Virol* 2015; 89: 7133-7146.
38. Sadaoka T, Depledge DP, Rajbhandari L, Breuer J, Venkatesan A, Cohen JI. A variant allele in varicella-zoster virus glycoprotein B selected during production of the varicella vaccine contributes to its attenuation. *mBio* 2022; 13(4): e0186422.
39. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. *Annu Rev Genomics Hum Genet* 2006; 7: 61-80.
40. Kim JI, Jung GS, Kim YY, Ji GY, Kim HS, Wang WD, et al. Sequencing and characterization of Varicella-zoster virus vaccine strain SuduVax. *Virol J* 2011; 8: 547.
41. Lezhnyova V, Davidyuk Y, Mullakhmetova A, Markelova M, Zakharov A, Khaiboullina S, et al. Analysis of herpesvirus infection and genome single nucleotide polymorphism risk factors in multiple sclerosis, Volga federal district, Russia. *Front Immunol* 2022; 13: 1010605.
42. López-Muñoz AD, Rastrojo A, Martín R, Alcamí A. Herpes simplex virus 2 (HSV-2) evolves faster in cell culture than HSV-1 by generating greater genetic diversity. *PLoS Pathog* 2021; 17(8): e1009541.
43. Domingo E, Sheldon J, Perales C. Viral quasispecies evolution. *Microbiol Mol Biol Rev* 2012; 76: 159-216.
44. Nicola AV, Straus SE. Cellular and viral requirements for rapid endocytic entry of herpes simplex virus. *J Virol* 2004; 78: 7508-7517.
45. Isaacson MK, Ploegh HL. Ubiquitination, ubiquitin-like modifiers, and deubiquitination in viral infection. *Cell Host Microbe* 2009; 5: 559-570.

46. Wang Z, Moult J. SNPs, protein structure, and disease. *Hum Mutat* 2001; 17: 263-270.
47. Sievers F, Higgins DG. Clustal Omega, accurate alignment of very large numbers of sequences. *Methods Mol Biol* 2014; 1079: 105-116.
48. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. *Annu Rev Genomics Hum Genet* 2006; 17: 61-80.
49. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, et al. GenBank. *Nucleic Acids Res* 2013; 41: D36-D42.
50. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, et al. GenBank. *Nucleic Acids Res* 2017; 45: D37-D42.