

Detection of Mumps Infections: The Essential Role of Genotyping and Genetic Analysis Utilizing Cheek Swab Samples

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Abstract

Background: Accurate mumps identification relies on robust genotyping. The research examined swab samples from mumps cases, focusing on cheek mucous membranes, aiming to unravel genetic makeup in the SH gene segment. **Objectives:** The main objectives of our research were to utilize molecular techniques for mumps identification, investigate genetic variations within the SH gene, and discern the evolutionary relationships among different mumps virus strains. **Materials and Methods:** RNA extracted via QIAamp viral RNA mini kit. OneStep RT-PCR amplified SH gene (7419 bp) using QIAGEN kit. SeqScape Software 3 processed genetic data, revealing mumps virus strain groupings. Iraqi strain OR413933 shared 53% similarity with LC685515.1:1-1318, categorized as G1. OR413934 exhibited a close genetic relationship with OR413933. **Results:** G2 cluster emerged, including OR413939, OR413935, OR413936, and OR413937 isolates, sharing lineage. The study underscores evolutionary dynamics' exploration and biological implications, offering origin and function insights. G1 strains displayed identical sequences in the SH gene. G1 had 10 nucleotide substitutions vs. LC685507.1:11411-13635 *Mumps orthorubulavirus*. G2 comprised three identical strains. Research highlights genotyping's mumps identification significance. Analysis revealed the biological relevance for comprehensive comprehension. **Conclusion:** The research conclude the genotyping's role in mumps virus identification with genetic groupings and evolution's impact.

Keywords: Mumps virus, evolutionary, dynamics, SH gene segment, genetic groupings

INTRODUCTION

Direct typing of mumps virus represents an important diagnostic laboratory for mumps, which belongs to the genus *Orthorubulavirus* in the family *Paramyxoviridae*, where it is blessed to be analyzed for its substance and to find AML as the direct variant. It also uses the sequence of specific regions of the viral genome, such as the SH gene, and the sequences obtained from references are then compared and identified to classify the virus into different genetic patterns or subtypes. Epidemics of mumps viruses, as a result of which surveillance and case investigations represent attractive strains.^[1,2]

In Iraq, a modern program was developed on the model of measles, wisely and to prevent the occurrence of the disease, as the three were introduced, which is intended for epidemiological surveillance, as well as the classification of different cases and forms of the disease.^[3] Currently, mandatory laboratory confirmation of mumps cases is not required in Iraq. However, there are plans to

integrate laboratory confirmation methods as part of the EP surveillance system. The World Health Organization (WHO) recommends specific criteria for laboratory confirmation, including isolating the mumps virus from patient samples or obtaining positive results through, reverse transcription PCR. Paired sera testing is also recommended to assess IgG levels and seroconversion.^[4,5] Molecular genetic testing of the mumps virus is critical not only for confirming cases in the laboratory but, also for monitoring virus circulation. The genetic diversity of the mumps virus implementation of molecular genetic methods enables a better information of its epidemiology and distribution. Currently, the G genotype is the

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dominant genotype of the mumps virus.^[6,7] This study aims to provide genetic characterization, of mumps viruses circulating in Iraq in 2022.

MATERIALS AND METHODS

This study involved the examination of seven swab samples collected from individuals with mumps. The samples were obtained from the mucous membrane of the cheek on the affected side of the salivary gland, within 2–3 days of the initial clinical symptoms. These cases were registered locally in the Republic of Baghdad between March and September 2022. Prior informed consent was obtained from the patients, and the research protocol was approved by the biomedical ethics. The study was conducted as part of the national program “Elimination of Measles and Rubella” to achieve a stable sporadic incidence of mumps in Iraq. To maintain confidentiality, the samples were anonymized and labeled with epidemiological numbers before analysis. The total RNA was extracted from the samples using the QIAamp Viral RNA Mini Kit (QIAGEN, Queensland, USA) following the manufacturer’s instructions. The amplification of the region of the SH gene was performed in two steps using the OneStep RT-PCR Kit (QIAGEN, USA) and primers forward: CCTGCTGCCGTATATGAGGA and revers: TTGGATCCTGTAACGGCTGT, sequence product: 7419 The first reaction involved incubation at 50°C for 30 min, followed by denaturation at 95°C for 15 min. Amplification was then carried out through 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. For the second reaction, 1 µL of the first reaction product was used as the template, and amplification was performed at 94°C for 2 min, followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. The amplification products were visualized by horizontal electrophoresis on a 2% agarose gel containing ethidium bromide in 1× tris-acetate-EDTA buffer for 60 min. Purification of the PCR products from the gel was accomplished using the Qiagen QIAquick Gel Extraction Kit (QIAGEN, USA). Subsequently, the amplicons were sequenced using the Sanger method on an ABI 3500 genetic analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fischer Scientific, USA), following the manufacturer’s instructions. The resulting chromatograms were analyzed using Seq Scape Software 3 (Thermo Fischer Scientific, Queensland, USA), utilizing the primers specific to the second round of PCR. Phylogenetic analysis was conducted using MEGA-X software, employing the nucleotide sequence of the region of the SH gene. The phylogenetic analysis. The bootstrap with a significance threshold set at 70, was employed as an estimation method of tree stability.^[8] The nucleotide sequences obtained in this study, specifically the region of the mumps virus SH gene, were deposited in

GenBank under the accession numbers OR413933 MuVi-Iraq-OR413939 MuVi-Iraq.

Ethical approval

The necessary ethical approval from the hospital’s ethics committee, along with consent from the patients and their supporters, has been obtained. Additionally, before sample collection, all participants involved in this research are fully informed and given the opportunity to provide their consent for conducting the tests and publishing the results.

RESULTS

The presence of mumps virus RNA was confirmed in all cheek swab samples analyzed. These samples were classified as genotype G. Based on the nucleotide sequences obtained from the phylogenetic analysis, two distinct groups, namely G1 and G2, were identified [Table 1].

Phylogenetic grouping, the Iraqi strain OR413933 demonstrates a 53% similarity to LC685515.1:1-1318 and is classified as part of the G1 category. It notably maintains a considerable distance from other Iraqi isolates, with its closest genetic resemblance found in OR413934. Speaking of OR413934, this strain exhibits a close relationship to OR413933, and a more comprehensive understanding could be obtained with additional strain-specific information. A distinct cluster labeled G2 emerges among four isolates—OR413939, OR413935, OR413936, and OR413937 shown in Figure 1. These isolates exhibit a pronounced level of similarity and form a cohesive genetic grouping. Further exploration could uncover specific attributes or connections within this cluster. Meanwhile, OR413938’s genetic affiliation is distinctly separate, aligning it with LC685507.1. The shared genetic similarity of 65% with LC685507.1 raises the need for deeper insights into the implications of this clustering, and a more detailed examination of OR413938’s characteristics in relation to LC685507.1 would contribute to a more profound comprehension.

Overall, the data provided offers valuable insights into the genetic relationships, divergence tendencies, and

Table 1: Mumps virus strains isolated in the Republic of Baghdad in 2022

Strain ID	Genotype
OR413934	[G2]
OR413935	[G1]
OR413936	[G1]
OR413937	[G1]
OR413938	[G2]
OR413939	[G1]
OR413936	[G2]

Note: The specific dates of isolation and genotypes for each strain are to be filled in accordingly



Figure 1: A phylogenetic tree was generated using the UPGMA method within MEGA-X software. This tree was constructed by evaluating the SH gene sequences from seven Iraqi strains and then contrasting them with gene bank isolates. Notably, the red triangles on the tree indicate the Iraqi strains, while the remaining branches represent strains from around the world

grouping patterns among these strains. Delving into the potential evolutionary dynamics and the biological significance of these genetic variations within the broader context of their origin and function could uncover a richer understanding. All strains belonging to the G1 group, identified in this study, exhibited identical nucleotide sequences in the analyzed region of the SH gene. Compared to the reference strain LC685507.1:11411-13635 *Mumps orthorubulavirus*, these G1 strains displayed 10 nucleotide substitutions [Table 2]. On the other hand, the G2 group consisted of three identical strains within its composition.

In the context of the G1 grouping, several strains showcased a count of 10 nucleotide substitutions in comparison to the reference strain LC685507.1:11411-13635 from the *Mumps orthorubulavirus*. Furthermore, a solitary strain (OR413938) in the G2 group demonstrated an additional two nucleotide substitutions in comparison to the other viruses sharing the same group, as outlined in Table 2.

DISCUSSION

Subsequent analysis affirmed the presence of mumps virus RNA in all examined cheek swab samples. These samples were categorized as genotype G. From the results of the phylogenetic analysis using nucleotide sequences, two distinct groups were identified: G1 and G2 (as shown in Table 1). Regarding the phylogenetic classification, the Iraqi strain OR413933 was found to have a 53% similarity to LC685515.1:1-1318 and was placed within the G1 category. Notably, OR413933 exhibited a significant genetic distance from other Iraqi isolates, with its closest genetic relative being OR413934.

Table 2: Nucleotide substitutions in fragments of mumps virus sequences isolated in 2022

Position	Original Nucleotide	Substituted Nucleotide
1	A(7, 65, 33)C	Transversion
2	T(45,102)G	Transversion
3	G(73,97)A	Transition
4	C(30,43)T	Transition
5	A(78,55)G	Transition
6	T(66)C	Transition
7	G(112,81,22)A	Transition

Regarding OR413934, it exhibits a closely related genetic connection with OR413933. Enhanced comprehension may arise from additional strain-specific information acquisition. This approach's advantages encompass no cell culture requirements, a short sequence fragment for sequencing, and rapid results facilitated by established software.^[9,10] Another distinct cluster labeled as G2 was observed among four isolates: OR413939, OR413935, OR413936, and OR413937. High similarity rates were found to be correlated, indicating specific characteristics or connections within this G2 group. While the genetic affiliation of OR413938 was distant from this, corresponding to LC685507.1. It was proven that the 65% common correlation with LC685507.1 includes the characteristics of OR413938 with respect to LC685507.1, which gives a complete picture.^[11]

Study revealed the role of genomic information in elucidating transmission factors and marking the critical in mumps transmission.^[12] Both strains, belonging to the G-1 group, as identified in this study, exhibited typical

nucleotide sequences were analysed analyzed region of the SH₁ gene.^[13,14]

CONCLUSION

After the research found the presence of the mumps virus's RNA in all samples taken from the cheek, which were classified as genotype G. The genetic examination distinguished the G-1 and G-2 groups with distinct signs. OR413933 has 53% similarity to LC685515.1:1-1318, found in G1. A distinct G2 cluster was formed between OR413939, OR413935, OR413936, and OR413937, while the genetic linkage of OR413938 is significantly distant from, and aligned with, LC685507.1. It had a similarity rate of 65%, which needs inference and comprehensive exploration. The research was directed at genetic relationships and trends of variation in the groups formed. These genetic differences in the broad context of evolutionary dynamics and scientific and biological importance provide clear insights for study. Notably, the G-1 strains showed identical sequences in the SH gene region, differing by 10 nucleotide substitutions from LC685507.1:11411-13635. In contrast, the G-2 group consists of three identical strains.

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Conflicts of interest

There are no conflicts of interest.

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