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Coronavirus Pandemic

Genome Analysis of SARS-CoV-2 Delta variant in the Kurdistan region of Iraq

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Abstract

Introduction: Since its isolation in the UK, the SARS-CoV-2 Delta variant has become an epidemic. This study aimed to decipher the viral appearance and genomic characterization of the Delta variant isolated from patients in the Kurdistan region of Iraq.

Methodology: Samples were collected from the West Erbil Emergency Hospital, and infection by SARS-CoV2 was confirmed using Real-Time PCR. The sequenced samples were analyzed and compared to the previously published data on Delta variants.

Results: The analysis showed that the variant belongs to SARS-CoV2 Delta variant B.1.617.2, including most of the previously detected mutations in India. The variant includes 13 mutations (11 substitutions and 2 deletions) on the spike region. Some substitutions are the same as the previous Delta isolate (T19R, G142D, T478K, D614G, L452R, P681R, and D950N). However, other substitutions (E156G, T250I, T19A, and L861W) were unique in the spike protein of the Delta variant (EPI_ISL_7405941) found in the Iraq variants.

Conclusions: The impact of the novel mutations needs more study, but the common ones are shown to enhance transmission and escape from immunity. Future studies need to focus on the impact of the different vaccines in the Kurdish population on the Delta variant and the effect of the novel mutations on transmissibility and escape from immunity.

Key words: SARS-CoV-2; delta variant; B.1.617.2; illumina covidseq method; mutation.

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Introduction

The city of Wuhan in China is widely recognized as the epicenter of the SARS-CoV-2 virus, which, after emerging in late 2019, rapidly spread across the globe. This led the World Health Organization (WHO) to classify it as a global pandemic [1]. Since its inception, the SARS-CoV-2 genome has been subject to mutations [2,3], resulting in the emergence of several Variants of Concern (VOC) in different countries. Notable among these are the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) variants [4,5]. The spike (S) protein of SARS-CoV-2 has undergone numerous mutations and is a pivotal component in these variants [6]. Frequent mutations within the spike gene can influence the virus's infectivity, its ability to evade the immune response, and its interaction with host cells [7-9]. The structure of the SARS-CoV-2 virus, an enveloped beta-coronavirus, comprises a single positive-stranded RNA genome of approximately 29 kilobase pairs (kbp) [10]. The spike (S) protein, crucial for pathogenicity through its role in receptor recognition and cell membrane fusion, consists of two subunits: S1 and S2 [11,12]. The receptor-binding domain (RBD) within the S1 subunit (residues 319 to 541) plays a pivotal role in binding to the human ACE2 receptor [11]. The initial identification of the protein's structure was achieved using cryo-electron microscopy (PDB: 6VSB) [10]. The Delta variant (B.1.617.2) of SARS-CoV-2 rapidly became the dominant strain in Iran and spread to over 142 countries

by mid-2021 [12]. This variant was classified as a Variant of Concern (VOC) by the WHO due to mutations in the RBD that affect its affinity for the ACE2 receptor [13]. The Delta VOC is considerably more transmissible than the Alpha (B.1.1.7) VOC, with a 40–60% increase in transmissibility, and it is associated with a heightened risk of hospitalization [14]. Individuals who are unvaccinated or only partially vaccinated are particularly vulnerable to the Delta variant [15]. The Delta variant exhibits several mutations in the S protein, including substitutions in the RBD (L452R and T478K), the D614G substitution, mutations in the N-terminal domain (NTD), and additional substitutions near the furin cleavage site and the heptad repeat 1 (HR1). The Delta variant has shown the ability to evade neutralization by certain monoclonal antibodies [16]. A subvariant of Delta, known as Delta Plus, with a K417N mutation in the RBD, demonstrates even greater immune evasion capabilities [17]. Studies have indicated a decrease in serum neutralizing antibody titers following vaccination against Delta, with a more pronounced decline compared to vaccination against Alpha and Beta variants [15,18]. In the United States, the Delta strain was responsible for over 98% of newly identified COVID-19 cases [5], and it was first identified in Iraqi Kurdistan on July 20, 2021 [19]. The primary objective of this study is to investigate the development and characteristics of the SARS-CoV-2 B.1.620 lineage within the Kurdistan Region of Iraq. This includes an examination of its transmission patterns and potential implications for virulence. We will outline the specific mutations and deletions present in the B.1.620 lineage, many of which have been observed in distinct VOCs. Additionally, we will report on the local transmission of the Delta variant originating from Erbil city in the Kurdistan region of Iraq, comparing it to other published strains using phylogenetic analyses and data from our dataset.

Methodology

Sample collection and ethical approval

The study on SARS-CoV-2 strains isolated from nasal and oropharyngeal (N/OP) swabs received ethical approval from the Salahaddin University-Erbil ethical committee (Reference No. 7d/21301, dated 3/11/2021). Nasopharyngeal/oropharyngeal swab samples were collected from 150 cases at the West Erbil Emergency Hospital. All patients provided written informed consent for their participation in the study, including permission for the publication of the study's findings. The confirmed agreement to publish the research results was obtained from all participating patients between May and July 2021. The presence of the Delta variant in the participants was confirmed through RT-PCR analysis. Patients included in the study were diagnosed with COVID-19 following a standardized procedure, excluded were pediatric patients, individuals on immunosuppressive medications for 3 months or longer, and those with malignant tumors.

RNA extraction

To extract viral RNA from the 150 samples, 200 µL of viral samples was used, as mentioned in the kit of MagPurix viral RNA extraction (Reference No. ZP02013, Zinexts Life Science, China). The viral RNA was isolated and purified applying a Zybio EXM3000 Nucleic Acid Isolation System (Reference No.: ZBI-EXM3000, Thailand).

The isolated RNA from the samples was then reverse-transcribed into cDNA, according to the instructions included with the miRCURY LNA RT Kit (Cat. No. / ID: 339340, QIAGEN, Hilden, Germany). Sequence specific probe was used for qPCR, as mentioned in QuantiTect Probe PCR Kit (200) (Cat. No. / ID: 204343, QIAGEN, Hilden, Germany). This kit includes an appropriate ready-to-use master mix for quantification cDNA targets. The expression level of the cDNA was then determined using a Real-Time (RT)-PCR machine (QIAGEN, Hilden, Germany). The machine was programmed to synthesis cDNA at 50°C for 15 minutes at one cycle, and then the denaturation step was directly started at 95°C for 5 minutes at one process. The thermocyclers were set up for forty cycles of denaturation at 95°C for 10s, followed by annealing at 60°C for 30s and elongation at 72°C for 30 seconds.

Data analysis

Fluorophore curves for ROX, FAM, cy5, JOE, and Quasar were studied. The instrument determines the threshold lines based on the Ct value specified on the CoA for each Kit.

Whole Genomic Sequencing and Library Preparation

In the present study, sample processing and genomic sequencing were carried out for the samples with Ct < 30 [20]. Regarding whole-genomic sequencing, these samples were subjected to the Illumina Covidseq technique to generate RNA libraries (Cat. No.: KP201-11, Illumina Inc, USA), and 300 ng of the isolated RNA was received by applying Ribo-Zero Gold rRNA depletion to remove human ribosomal RNA.

In this study, the TruSeq Stranded Total RNA kit (Illumina, Cat no. 20020599) and the IDT for Illumina TruSeq RNA UD Indexes (96 indexes, 96 wells) (Illumina, Cat no. 20022371) were used to create the RNA libraries from reduced RNA. First- and second-strand cDNA molecules were generated using RT-qPCR-detected variant strains, as described in the TruSeq Standard Total RNA Kit. Then Poly (A) tail was added by adenylation, and followed by adapter ligation was performed [17]. Following amplification performance, the libraries were measured, pooled, and sequenced using the Illumina NextSeq 500 technique. They were thereafter accepted as normalization. To obtain 75-bp paired-end reads, the sample sequencings were carried out using an Illumina NextSeq 500 system with a 150-cycle high-output kit (v2.5). The sequencing findings were retained for future investigation (Nextera). After converting the Bcl files to fastq, the CLC GenomicsWorkbench version 11.0 (CLC, QIAGEN, Germany) was used to evaluate them. Using Geneious software, genetic alterations were confirmed and displayed with the BAM files

Bioinformatic analysis

Using the default parameters, the FastQC tool, v3, was utilized to check read quality control [21]. The fast tool, v0.19, was utilised to trim adapter sequences and low-quality bases [22]. Using the CD-HIT-DUP tool, v4.6.8, duplicates and low-complex sequences, those with a length of fewer than 40 bases were filtered out [23]. Off-target sequences were screened using Bowtie2 v2.3.4.3 and then were compared to human genome version GRCh38.p13 [24].

To assemble the viral genome, the sequencing reads received are utilized as the input to make viral genomes by applying the Wuhan-Hu-1 reference genome sequence (MN908947). The sequencing reads gained for each sample were then aligned by applying Bowtie2 v2.3.4.3. Then, the mapped sequences were utilised de novo assembly in the tool with the SPAdes,v3.14.0. The majority threshold criterion was used to generate consensus genome sequences. Only line reads with a coverage level greater than eighty percent and a mean depth of ≥ 8x were measured for the analyses.

To obtain an accession number, the whole-genomic sequence was decided to submit to GISAID (Global Initiative for Sharing All Influenza Data) on December 9, 2021. The GISAID database staff assigned the accession number EPI_ISL_7405941 after it was confirmed. All GISAID researchers can now access the entire genome sequence (https://www.epicov.org/epi3/frontend#4042bc).

Genomic Data Analysis

To determine the SARS-CoV strain in the Iraqi Kurdistan region, our sequence (Accession ID: EPI_ISL_7405941) was run as a BLAST query to find out the closely related sequences in GISAID EpiCov database. Then, the whole genomic sequence of SARS-CoV-2 variants was collected and arranged. After that, the MUSCLE tool in Molecular Evolutionary Genetics Analysis (MEGA) software, v11, was used to align the sequences and the Maximum Likelihood tool was used to then construct a phylogenetic tree.

Table 1. Clinical characteristics of participants.

Clinical features	No. of cases (%)	OR (95% C1)	Significance
Gender			
Male	87 (58)	7.41 (5.44-9.22)	*
Female	63 (42)	6.16 (4.11-9.67)	
Age			
< 50	110 (73.3)	7.44 (4.11-10.50)	**
≥ 50	40 (26.7)	5.32 (4.43-7.06)	
BMI Categories			
Underweight ≤ 18.5	10 (6.7)	4.31 (3.22-5.98)	
Normal weight = 18.5–24.9	95 (63.3)	6.68 (4.31-10)	*
Overweight = 25–29.9	40 (26.7)	7.15 (5.05-10.22)	
Obesity = BMI of 30 or greater	5 (3.3)	5.01 (4.67-5.67)	
Common Symptoms			
Fever	138 (92)	6.46 (5.22-10.15)	***
Chills	112 (74.7)	6.51 (5.18-9.76)	ns
Cough	118 (78.7)	6.81 (5.76-10.98)	ns
Sore throat	85 (55.7)	5.22 (5.11-9.76)	ns
Runny nose	105 (79)	7.53 (6.53-9.48)	*
Vaccination			
Vaccinated	33(22)	6.22 (4.33-8.13)	*
Non-vaccinated	117(78)	6.62 (5.11-10.27)	
Hospitalized state			
Non-hospitalized	120 (80)	8.93 (5.38-11.47)	***
Hospitalized	30 (20)	4.65 (4.03-7.12)	

Results

Clinical Characteristics of the Patients

In this study, 150 patients were included, 58% were male, and 42% were female. Most participants (73%) were over 50 years old. The patients had different extents of the severity of the common symptoms (Table 1). The most common symptom was fever in 92% of participants, followed by a runny nose, cough and chills observed in 79%, 78.7%, and 74.7% of the patients, respectively. The sore throat was the lesser and found among 55.7% of the patients. Remarkably, 78% of the participants were not vaccinated; out of the 150 patients, only 30 were hospitalized.

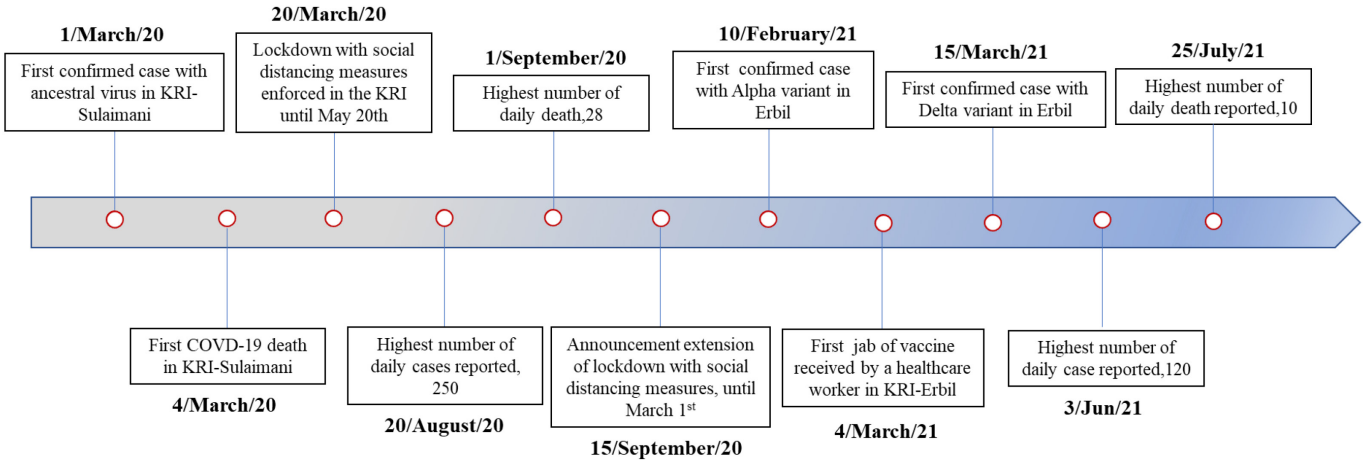
Timeline of the SARS-CoV-2 variant occurrence in KRI

At the beginning of COVID-19 emergence, the first case was recorded in the Kurdistan region of Iraq (KRI)-in the Sulaimani governate on March 01, 2020. KRI reported that symptoms were similar to the coronavirus disease. The case, identified based on Real-Time PCR results, was a resident of Sulaimani who traveled to Iran. Then, the rate of COVID-19 infection with this virus has risen dangerously in other KRI governates, such as Erbil, Duhok, and Halabja. The KRI Health Minister reported on social media that a dozen cases had been infected from the first case. On March 04, the first case was dead in Sulaimani. Then, the KRI announced the COVID-19 lockdown in the governate of Sulaimani, Erbil, Duhok, and Halabja from March 20 to May 20.

On August 20, 2020, the case rate of COVID-19 began to multiply around the KRI governates. COVID-19 has infected more than 200 people, and over 25 have died. After months of lockdown, the number of COVID-19 patients and deaths started to see a flattened line and a reduction in cases. On February 10, 2021, The KRI Health Minister authorities declared a new strain of SARS-COV-2, called Alpha (B.1.1.7) variant, in Erbil. On March 04, 2021, a frontline health worker took the vaccine's first jab. The Health Minister of Iraq contracted with Sinopharm, Pfizer, and AstraZeneca companies to supply a sufficient vaccine. The KRI took 17% of the vaccine dose from the health minister of Iraq. On March 15, 2021, The KRI health minister declared the first case of delta variant. This concerned variant was known to be more contagious, easily transmissible, and highly mutated to evade the immune system. On June 03, 2021, the health minister authorities declared the highest number of infected cases (120) with the Delta variant and the highest death rate on July 25 (Figure 1).

The Kurdish Regional Government (KRG) in northern Iraq has confirmed the presence of the highly infectious Delta variant of the coronavirus. During a press conference in the provincial capital of Erbil, KRG Health Minister Saman Barzinji announced that they had been investigating the variant and suspicions were now confirmed. However, Barzinji did not disclose the number of infections or where the cases were detected. The region has seen a significant increase in coronavirus infections, with daily cases rising from less than 500 to over 1,000 in recent months. The KRG health authorities have also noted that the epidemiological indicator has turned dark orange and is trending towards red. The Kurdistan region has reported 198,741 virus cases, including 4,553 fatalities, while across Iraq, the number of cases has reached 1,457,192, including 17,677 deaths and 1,326,073 recoveries. The World Health Organization has reported that the Delta variant has been detected in approximately 100 countries and regions.

Figure 1. This timeline details specific events during the COVID-19 pandemic in Iraqi Kurdistan Region.



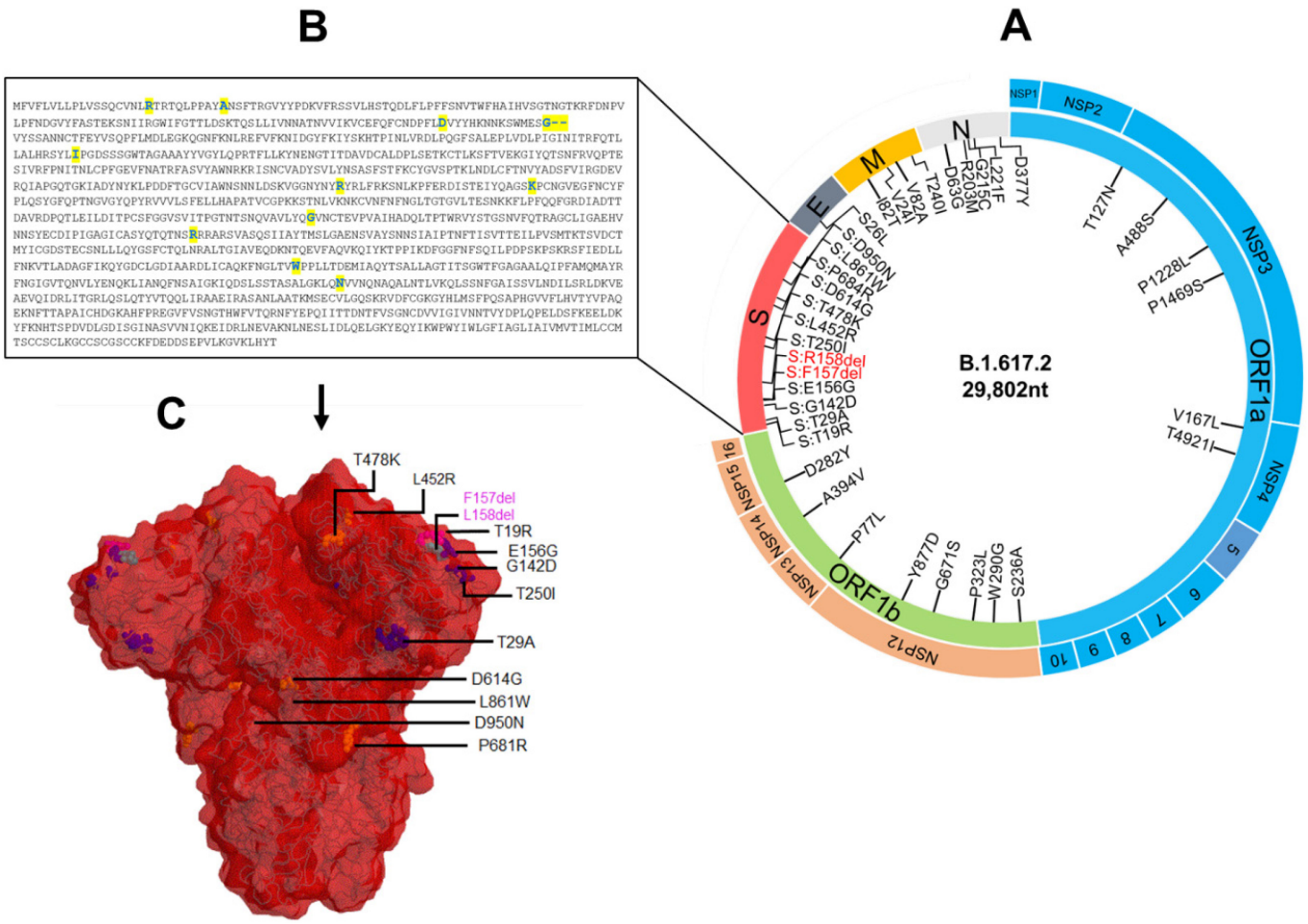
Genomic analysis of the Delta variant of Iraq

Compared to the viral reference genome of hCoV-19/Wuhan/WIV04/2019, EPI_ISL_7405941 was highly identical (98.979%) to a variant of Delta GK/478K.V1 (B.1.617.2+AY.x), first detected in India. The genome was composed of 29,802 nucleotides and encoded for four proteins, including S, E, M, and N. The Delta variant (B.1.1.7.2) found in Iraq carries 37 mutations (Figure 2). Some are known from the Alpha strain and have accumulated several additional changes in the spike gene. The variations found in the spike protein were 13 amino acids (T19R, T29A, G142D, E156G, F157del, R158del, T250I, L452R, T478K, D614G, P684R, L861W, D950N). As a result, it may be bearing epitopes different from those against which the current vaccines are produced. A vast number of variations are found in the entire genome of the SARS-CoV2 Delta variants, including mutation in the ORF1a (T127N, A488S, P1228L, P1469S, V167L, T4921I), ORF1b (S236A, W290G, P323L, G671S, Y877D, P77L, A394V, D282Y), E (S26L), M (I82T, V24I, V82A, T240I) and N (D63G, R203M, G215C, L221F, D377Y).

Mutations of the Delta spike protein in Iraq

Genetic variations can occur naturally in the RNA genome of SARS-CoV-2 during viral replication, resulting in the formation of variants. These nucleotide changes can alter the virus's transmissibility, virulence, and effectiveness of vaccines and therapeutic antibodies, particularly if they affect the spike gene. The amino acid sequence of the Delta variant's spike protein (EPI_ISL_7405941) differs from the reference genome of hCoV-19/Wuhan/WIV04/2019 in thirteen ways, including eleven substitutions and two deletions (Figure 2). Some of these substitutions, such as T19R,

Figure 2. Delta strain genome organization and its spike structure.



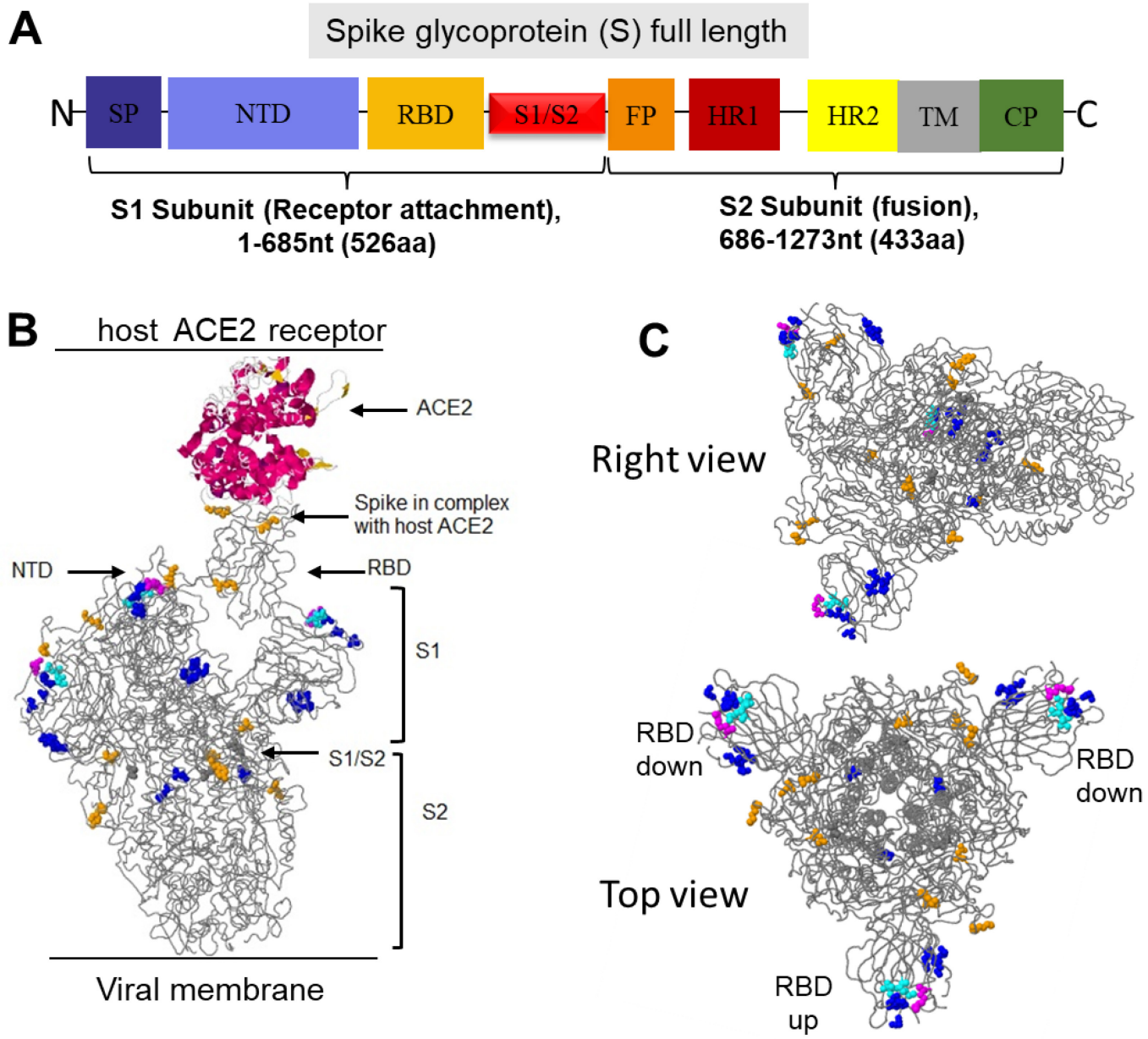
A. The Delta genomic RNA structure includes two 5′-proximal ORFs (ORF1a and 1b) that encode several non-structure proteins (NSPs), S –spike protein; E – envelope protein; M – membrane protein, and N – Nucleocapsid protein. **B.** Spike glycoprotein sequence along with the amino acid alterations. **C.** Structural representation of spike glycoprotein. Locations of 11 substitutions (black colour) and two deletions (pink colour) are shown.

G142D, T478K, D614G, L452R, P681R, and D950N, are also present in Delta variants found in other countries. However, other substitutions, such as E156G, T250I, T19A, and L861W, were first detected in the spike protein of the Delta variant (EPI_ISL_7405941) found in Iraq. Additionally, two amino acids, F157 and L158, were found to be deleted (Figure 3).

Phylogenetic analysis of SARS-CoV2 Delta variants from the Kurdistan region of Iraq

The phylogenetic analysis of the entire length of the SARS-CoV2 Delta variant from Erbil city/Kurdistan region of Iraq showed a close relation and high similarity with delta variants from Turkey, Tehran, United Arab Emirate, Thi-Qar/ Iraq, Duhok/ Iraq and interestingly with Delta variant from Sweden (EPI_ISL_3158110) (Figure 4). The mentioned variants formed a separate clade compared with other Delta

Figure 3. Spike protein genome map.



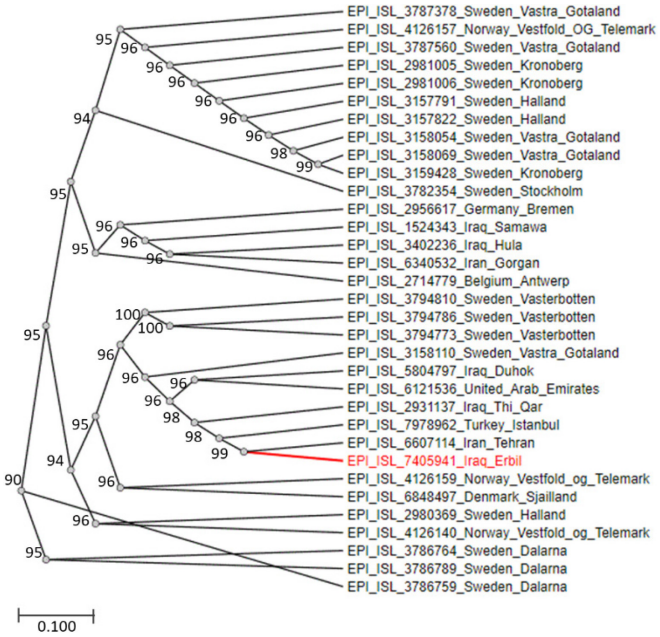
A. The genomic parts of the spike protein. **B.** The spike protein–ACE2 receptor complex. The primary structure displays the receptor-binding domain (RBD) and site (S1/S2), where the subunits of S1 and S2 will be detached (postfusion). **C.** Top view of the prefusion state.

variants from other countries. The similarity in this clade ranges between 96-98%.

Discussion

The emergence and global spread of the SARS-CoV-2 Delta variant (B.1.617.2) as a Variant of Concern has raised significant public health concerns due to its increased transmissibility and potential impact on vaccine effectiveness [15,25]. The Delta variant carries a constellation of mutations, many of which are located in the spike protein of the virus. This genetic diversity within the Delta variant lineage has contributed to its ability to evade the immune system and has implications for its transmissibility and disease severity [15,25,26]. One of the key findings is the genetic diversity within the Delta variant lineage. This lineage comprises three primary subtypes, including B1.617.1, B.1.617.2, and B.1.617.3. Each of these subtypes harbors alterations in the receptor-binding domain (RBD) and N-terminal domain (NTD) of the spike protein [16]. These mutations within the spike protein are of particular concern as they may improve the virus's ability to evade immune responses. Studies have shown that some of these mutations may enhance viral transmissibility [27]. The variants found in Kurdistan have been classified as B.1.617.2 subtype. It is important to recognize that specific variants of SARS-CoV-2 may predominate in certain regions, leading to localized outbreaks and unique epidemiological patterns. The prevalence of the B.1.617.2 subtype in Kurdistan highlights the global nature of the pandemic and the need for coordinated international efforts in monitoring and addressing emerging variants. The genetic makeup of the Delta variant from Kurdistan is of particular interest. Compared to the original Alpha variant, the Delta variant carries a higher number of mutations, with 23 additional mutations [28], including 12 mutations in the spike protein [29]. Some of these mutations are shared with previously published Delta sequences, such as T19R, G142D, T478K, D614G, L452R, P681R, and D950N [30]. These mutations are associated with increased viral transmission, potentially contributing to the variant's higher infectivity. Furthermore, some substitutions unique to the Delta variant from Kurdistan, such as E156G, T250I, T19A, and L861W, may have implications for treatment responses and the severity of cases. These findings underscore the dynamic nature of SARS-CoV-2 and the importance of genomic surveillance in tracking and responding to emerging variants. The impact of specific spike protein mutations on viral infectivity and immune evasion is

Figure 4. Phylogenetic analysis traces the evolutionary tree of the COVID-19 coronavirus. The genomic research suggests the delta variant spreads in the Iraqi Kurdistan region are derived from those delta variants spread around Iran and Turkey.



also a crucial point of discussion. Mutations such as L452R and P681R have been found to increase attachment to the ACE2 receptor [27]. The interaction between the spike protein and the ACE2 receptor is a critical step in viral entry into host cells, and alterations in this interaction can affect viral infectivity and disease severity [31,32]. The T478K substitution further enhances the interaction with ACE2 [33]. These mutations play a significant role in the Delta variant's ability to infect host cells and evade the immune system, highlighting the need for ongoing research on viral mutations and their functional consequences. The Delta variant carries two mutations, L452R and T478K, in the receptor-binding domain (RBD) that reduce its responsiveness to the host ACE2 receptor. However, it is important to note that the Delta variant's reduced responsiveness should be contrasted with the Omicron variant, which carries fifteen mutations in this region, making it even more challenging to neutralize [14]. These comparisons emphasize the ever-evolving nature of the virus and the importance of understanding the significance of individual mutations in the context of overall viral fitness. The absence of the E484K mutation in the Delta variant from Kurdistan is another key observation. The E484K mutation has been associated with reduced antibody efficacy and immune evasion [16]. Its absence in the Delta variant from Kurdistan suggests differences in the antigenic

properties of this variant compared to others. This observation may have implications for the effectiveness of vaccines and monoclonal antibody therapies against the Delta variant in the Kurdistan region. The Delta variant of SARS-CoV-2 presents heightened challenges due to its increased transmissibility and the potential for immune evasion. While vaccines like Pfizer and AstraZeneca still offer a level of protection against the Delta variant, their efficacy is somewhat reduced compared to their performance against earlier variants, such as Alpha. Two doses of the Pfizer vaccine, for example, provide 79-87% protection against Delta, whereas AstraZeneca offers 60% effectiveness [34]. Notably, a single dose of these vaccines has only a minimal inhibitory effect on the Delta variant [16]. Real-world data from various countries have demonstrated that while current vaccination practices may not completely prevent Delta variant infections, they do provide significant protection against severe illness and death. Nearly 95% of individuals develop a neutralizing response after completing a two-dose vaccine regimen, even though the antibody response to Delta is somewhat lower than that against Alpha. These findings underscore the importance of widespread vaccination to mitigate the impact of the Delta variant and the need for ongoing adjustments to vaccination strategies in response to evolving variants. In conclusion, our discussion highlights the dynamic and complex nature of the SARS-CoV-2 Delta variant, with its genetic diversity, regional prevalence, unique mutations, and implications for transmissibility and immune evasion. The ongoing monitoring of variants, genomic surveillance, and research into the functional consequences of specific mutations are essential to inform public health measures, vaccine development, and treatment strategies. The importance of global vaccination efforts is underscored, as vaccines remain a critical tool in reducing the impact of SARS-CoV-2 variants, including Delta, on public health [15].

Conclusions

The SARS-CoV-2 strain isolated from the Kurdistan region of Iraq is genetically related to published and original sequences from India, categorizing it as a Delta variant with certain distinct mutations. This study underscores the significance of vigilant patient management when dealing with the Delta variant, given its unique genetic profile. The development and equitable distribution of vaccines continue to be pivotal in curbing the prevalence of the Delta variant and other emerging variants. Ongoing research is essential to explore the potential impacts of new variants on vaccine efficacy, evaluate the long-term effectiveness of current vaccines against Delta, and assess the effectiveness of public health interventions in containing the spread of the Delta variant.

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Ethics approval

Ethical approval for the present study of the SARS-CoV-2 variants for the nasal and oropharyngeal (N/OP) swabs were received from Salahaddin University-Erbil ethical committee (21301).

Availability of Data and materials

The whole-Genomic sequence is publicly available to all researchers in GISAID (https://www.epicov.org/epi3/frontend#4042bc) under the accession number EPI_ISL_7405941.

Authors' contributions

Sevan Omer Majed contributed to the data acquisition and analysis, Suhad Asad Mustafa reviewed, edited, and re-wrote the manuscript. Paywast Jamal Jalal wrote the main manuscript, while Mohammed Hassan Fatah, Monika Henryka Misako, and Sahar Hassannejad assisted with manuscript drafting and methodology. Abdulkarim Yasin Karim participated in data acquisition and analysis and manuscript drafting. All authors have read and approved the final manuscript.

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