ISSN: 0067-2904

Molecular investigation of SARS-CoV-2 among participants in the 2021 Arbaeen March

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Received: 30/4/2024 Accepted: 11/8/2024 Published: 30/7/2025

Abstract

Molecular epidemiology techniques played a crucial role during the COVID-19 pandemic, as the genotyping of emerging variants was essential for effective surveillance, predicting transmission rates, and understanding the evolutionary dynamics of the virus. In Iraq, the epidemiological status before and after the 4th wave of Omicron emerging variant is unknown as it was preceded by a mass gathering event of Arbaeen pilgrimage. In this study, TaqPath RT-PCR assay was exploited along with epidemiological approaches to monitor and track dominant mutation and to identify hot spot region in Iraq through analyzing 3,748 SARS-CoV-2 positive specimens collected from different Iraqi governorates from September 5th, 2021, to March 28th, 2022. Current analysis showed that Karbala province represents a hot spot region of infection rather than the capital, Baghdad. Furthermore, the results showed high prevalence of histidine/valine deletion at codon 69/70 (H69/70V) of SARS-CoV-2 spike gene. This genetic signature is a basic characteristic of Alpha and Omicron variants. In silico analysis of genomic data retrieved from Global Initiative on Sharing All Influenza Data (GISAID) repository showed high prevalence of Delta variants and descended sublineages and confirmed the existence of Alpha variants during the studied period. The current study demonstrated that mass gathering events contribute to an increased transmission rate, elevating the prevalence of the H69/70 deletion found in local viral isolates, and offering a thorough epidemiological perspective on the factors that led to the rapid surge of the Omicron fourth wave in Iraq.

Keywords: SARS-CoV-2, Mass gatherings events, TaqPath assay, Epidemiological Curve. H69/70V deletion

التحقق الجزيئي لـ SARS-CoV-2 بين المشاركين في مسيرة الأربعين 2021

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الخلاصة

لعبت تقنيات علم الأوبئة الجزيئية دورا حاسما خلال جائحة COVID-19 ، حيث كان التنميط الجيني للمتغيرات الناشئة ضروريا للترصد الفعال والتنبؤ بمعدلات الانتقال وفهم الديناميات التطورية للفيروس. في العراق، الوضع الوبائي قبل وبعد الموجة 4 من متغير أوميكرون الناشئ غير معروف حيث سبقه حدث تجمع جماهيري للحج الأربعيني. في هذه الدراسة ، تم استغلال اختبار TaqPath RT-PCR جنبا إلى جنب مع الأساليب الوبائية لرصد وتتبع الطفرة السائدة وتحديد منطقة النقاط الساخنة في العراق من خلال تحليل 3748 عينة إيجابية من SARS-CoV-2 تم جمعها من مختلف المحافظات العراقية من 5 سبتمبر 2021 إلى 28 مارس 2022. وأظهر التحليل الحالي أن محافظة كربلاء تمثل منطقة ساخنة للعدوى بدلا من العاصمة بغداد. علاوة على ذلك ، أظهرت النتائج انتشارا مرتفعا لحذف الهستيدين / فالين في الكودون 70/69 (H69 / 70V) لجين ارتفاع SARS-CoV-2. هذا التوقيع الجيني هو سمة أساسية لمتغيرات ألفا وأوميكرون. في تحليل السيليكو للبيانات الجينومية المسترجعة من مستودع المبادرة العالمية لتبادل جميع بيانات الأنفلونزا (GISAID) أظهر انتشارا مرتفعا لمتغيرات دلتا والسلالات الفرعية المنحدرة وأكد وجود متغيرات ألفا خلال الفترة المدروسة. أظهرت الدراسة الحالية أن أحداث التجمعات الجماهيرية تساهم في زيادة معدل انتقال العدوى ، مما يزيد من انتشار حذف 70 / H69 الموجود في العزلات الفيروسية المحلية ، ويقدم منظورا وبائيا شاملا للعوامل التي أدت إلى الانتشار السريع في موجة Omicron الرابعة في العراق.

1. Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome (SARS-CoV-2) virus, poses a global threat due to its high transmission rate and large number of acquired mutations [1, 2]. Since the first diagnosed case in Al-Najaf City in February 2020, Iraq has reported over 2.3 million confirmed cases and 25,198 deaths [3]. The pandemic course witnessed different lineages that were designated by the World Health Organization as Variants of Concerns (VOCs). The first reported VoC was Alpha B.1.1.7, followed by Beta B.1.351, Delta B.1.617.2, and the last reported Omicron variant, which poses an increased risk to public health due to its disease severity, enhanced immune evasion, and increased viral transmissibility [4-9]. Sequencing technology has enabled the development of rapid PCR tracking protocols that target genetic signatures such as Spike 69/70 deletion of histidine/valine amino acids [6-10]. PCR-based detection techniques are currently considered the gold standard for viral detection, owing to their simplicity, high specificity, and sensitivity. As a result, real-time RT-PCR assays were implemented for rapid diagnosis and monitoring of SARS-CoV-2 variants [11, 12]. The TaqPath combo kit, manufactured by Thermo Fisher is a multiplex PCR assay that allows a simultaneous detection of three targets which includes the ORF1ab, nucleocapsid, and spike genes of the SARS-CoV-2 [13]. TaqPath spike gene primers target the 69/70 deletion in the N-terminal domain of the spike gene. The absence of this unique deletion leads to the successful amplification of the targeted area (S+). Otherwise, spike gene target failure (SGTF) represented by signal dropdown indicates the presence of the 69/70 deletion. Exploitation of SGTF/S+ results of PCR assays for variants genotyping and have been exploited in early detection of Omicron variant [14-16]. In this study, TaqPath SGTF detection pattern was used to investigate the abundance of 69/70 deletion in SARS-CoV-2 positive samples collected between September 5th, 2021, and March 28th, 2022. The study period encompassed the Arbaeen pilgrimage, a major mass gathering event held annually in Karbala (approximately 100 km south of Baghdad). The pilgrimage is one of the largest religious ceremonies that occurred each year with the participation of 20 million people and the number continues to grow [17]. Extensive analysis of the SGTF detection pattern combined with epidemiological approaches was conducted using 3,748 positive samples collected from various regions in Iraq to evaluate the prevalence of H69/70V deletion in local SARS-CoV-2 isolates. Current study covers all 18 Iraqi governorates and provides a comprehensive summary of the SARS-CoV-2 epidemiological curve, infection occurrences rates of SARS-CoV-2 and hot spot regions of Iraqi provinces.

2. Methodology

2.1 *Sampling Methodology*

Nasopharyngeal specimens were collected randomly from routine clinical diagnostic PCR SARS-CoV-2 tests performed by various Iraqi governmental laboratories. These specimens originally referred to the Iraqi National Influenza centre in the Central Public Health Laboratory for confirmation procedures. To ensure geographical diversity (representation from various Iraqi governorates), demographics (various ages), and varied clinical outcomes of COVID-19 disease [18].

2.2 *TaqPath Assay*

All positive specimens were randomly selected and underwent viral RNA extraction using the ExiPrep 96 Lite (A-5250, BIONEER) and the ExiPrep Viral DNA/RNA extraction kit (K-4614, BIONEER). The amplification and detection were carried out using the TaqPath assay following Thermo Fisher's recommended procedure on the 7500 Fast Applied Biosystem RT-PCR platform.

2.3 *Statistical Analysis*

The Epi-Info7 statistical dashboard was used to analyze gender frequency, cumulative frequency, and Wilson 95% upper and lower confidence limits (UCL and LCL, respectively) for all 3,748 positive specimens. Epi-Info 7 software is powered by the Center of Disease Control and Prevention (CDC) that widely used in public health data analysis such as to Zika and Ebola viruses related outbreaks [19]. RT-PCR results used as raw data for building the epidemiological curve (Epi Curve). The epi curve is frequently used in the epidemiological studies to investigate the incubation periods, transmissibility, and mode of transmission of different infectious diseases [20, 21].

2.4 *Occurrences Rate Calculation*

Microsoft Excel 365 was used to calculate the occurrence rate and reveal the trend of SARS-CoV-2 infection in Iraq over the study period [22]. The population count of Iraq in 2021 was considered using the official data reported by the Central Statistical Organization/Iraq Ministry of Planning [23]. Epi-Info7 was used to demonstrate the occurrence rates of SARS-CoV-2 in the studied population.

2.5 *Shape Files and Country Map*

The shape files for the administrative boundary level of Iraq and its governorates downloaded from Humanitarian Data Exchange enabled by Information Technology outreach Services [24].

2.6 *Genomic Data Mining*

SARS-CoV-2 genomic data was downloaded from GISAID repository which included a total of 478 whole-genome sequences, representing the entire entries of Iraq during the targeted period, retrieved genomic data analysed using Audacity Instant tool powered by GISAID. The tracking of 69/70 deletion within global frequency considered using EpiCoV^{TM}/GISAID.

3. Results

A total of 3,748 positive cases were enrolled randomly including 1,654 female and 2,094 male patients across all 18 governorates of Iraq. Using the Epi-Info7 statistical dashboard, sample frequency, percentage, cumulative percentage, and 95% upper and lower control limits (Wilson method) were calculated, the highest ratio in both genders were in Baghdad, Karbala, followed by Diyala and Al-Qadissiya, comprehensive representation of analyzed data for both genders represented in supplementary file 1. RT-PCR TaqPath assay revealed a total number of 2,983 cases with the SGTF amplification pattern, which possess the H69/70V deletion. On other hand, a total number of 555 cases were positive for all 3 targets (S+). Figure 1 illustrates the distribution of S+ TaqPath results across Iraqi governorates, while Figure 2 presents the SGTF pattern across Iraqi provinces. Supplementary file 2 provides detailed percentages and frequencies of TaqPath results for each governorate.

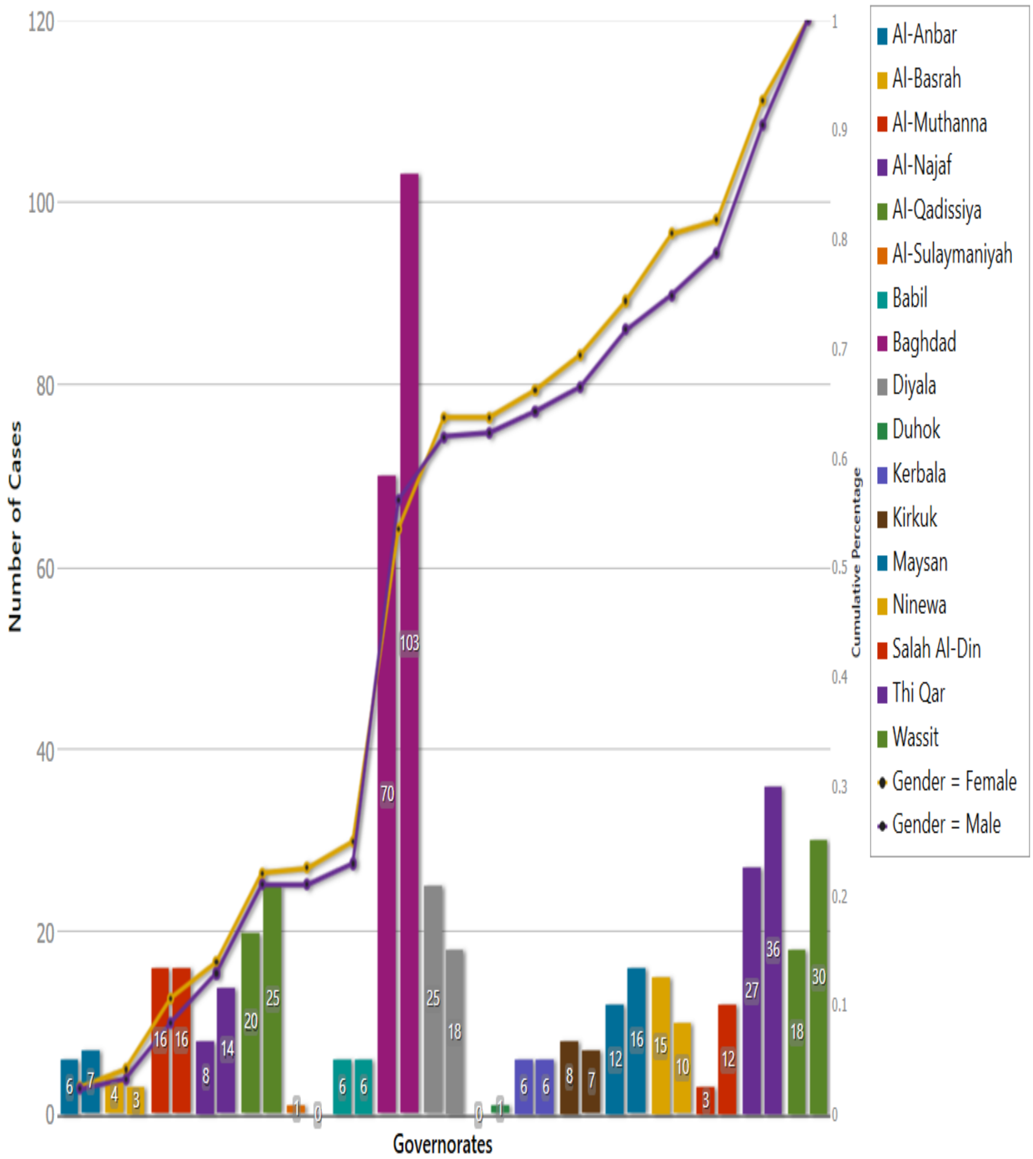


Figure 1: Distribution of S+ TaqPath results sorted by governorates and gender, each governorate indicated in different colour, male and female percentage indicated by the cumulative percentage of purple and yellow colours, respectively.

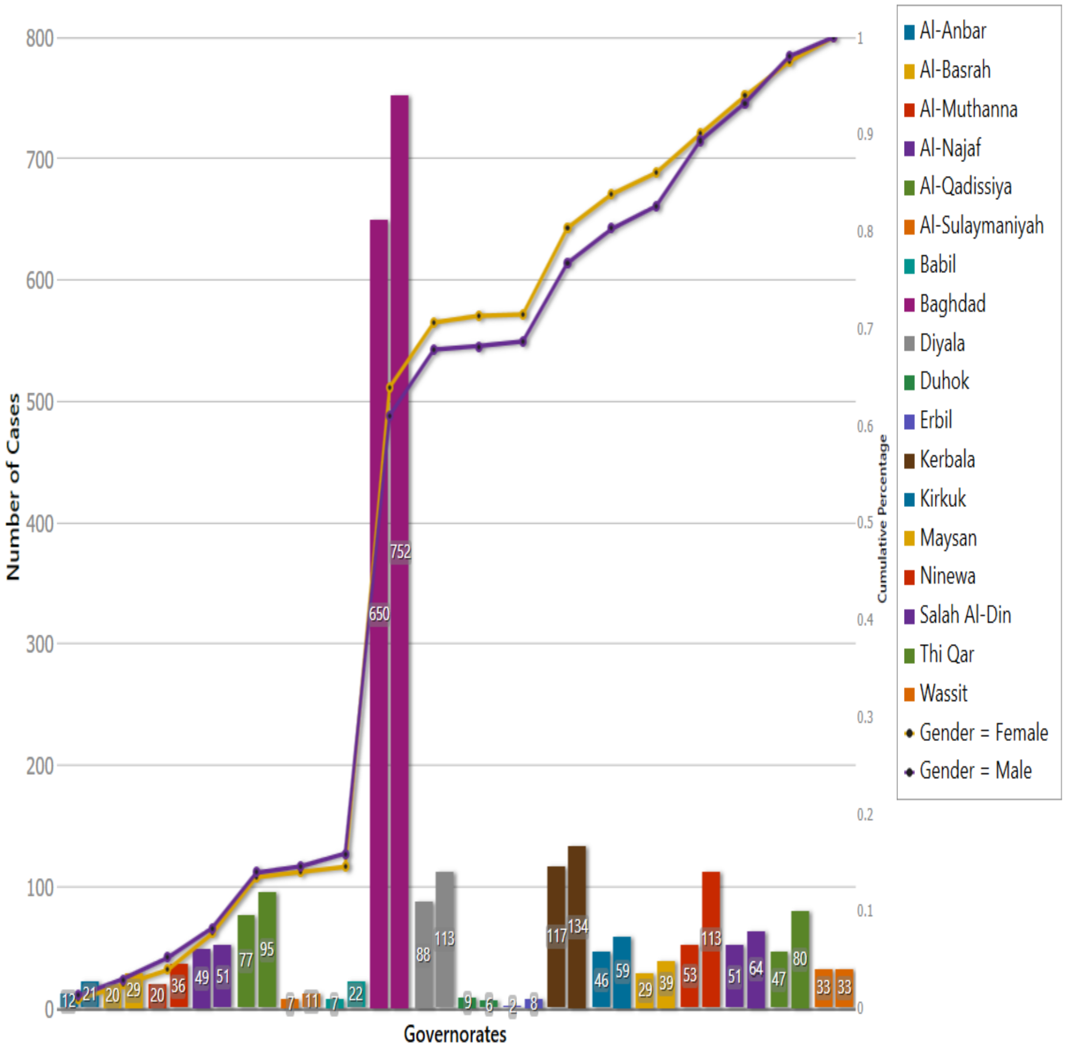


Figure 2: Distribution of SGTF RT-TaqPath results by governorates and gender, each governorate indicated in different colour, male and female percentage indicated by the cumulative percentage of purple and yellow colours. The results indicate high prevalence of SARS-CoV-2 with H69/70 deletion in Baghdad in both genders.

To assess and estimate the occurrence of SARS-CoV-2 within the studied period, Epi curve was used to summarize positive RT-PCR outcomes, the results were analyzed and displayed per specimen collection date using the Epi Info 7 statistical dashboard, as shown in Figure 3.

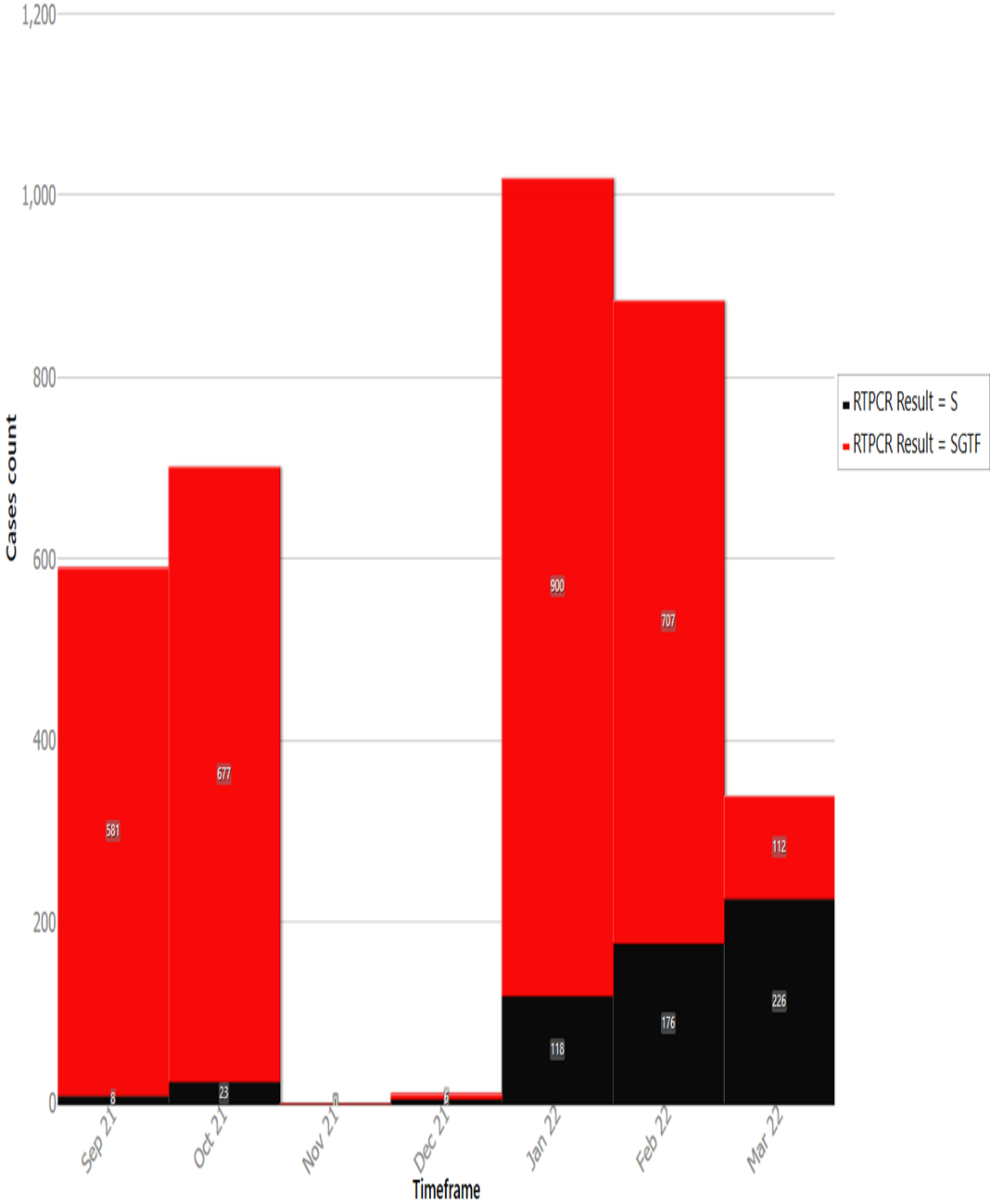


Figure 3: An epidemiological curve illustrating the distribution of SARS-CoV-2 positive TaqPath assay results. Black columns indicate S+ results, red columns represent SGTF results distributed by samples collection date. Gradual decrease in the prevalence of local strains that harbor the H69/70V deletions.

To estimate the population infection rate and hot spot regions, occurrence rate used instead of incidence rate, as the current study did not include all positive cases reported from Iraq within the studied period. Karbala Governorate shows the highest occurrence rates of SGTF and S+ SARS-CoV-2 isolates followed by Baghdad. Supplementary file 3 list all occurrence ration through all 18 Iraqi governates and list the population numbers per each province.

Epi info 7 used to create a map representing the results of both patterns as illustrated in Figures 4 and 5.

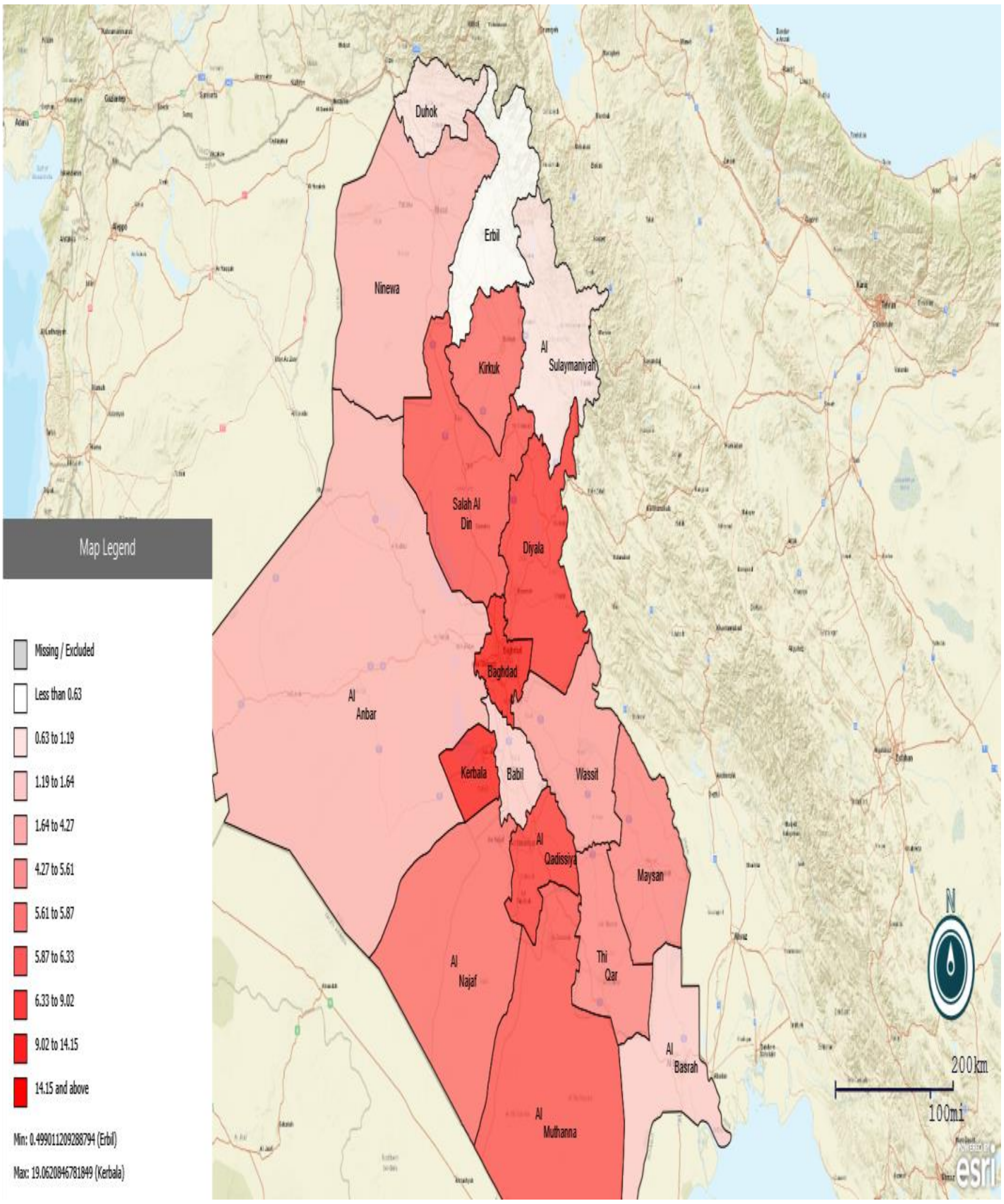


Figure 4: Geographical distribution of predicted occurrence rate of SARS-COV-2 cases that show SGTF detection pattern per 100000 population. The highest occurrences rate was in Karbala governate followed by Baghdad and Al- Al-Qadisiya governorates.

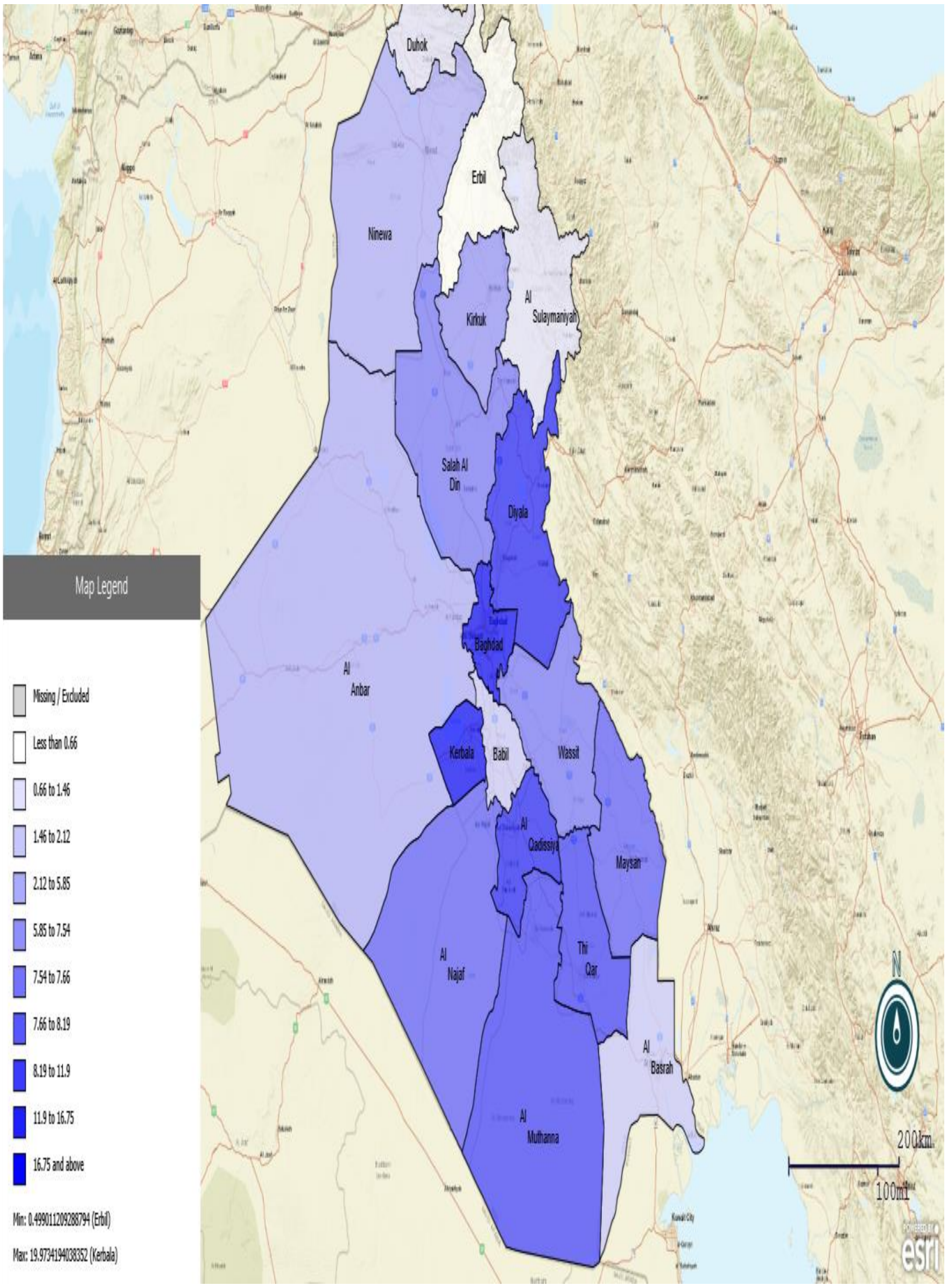
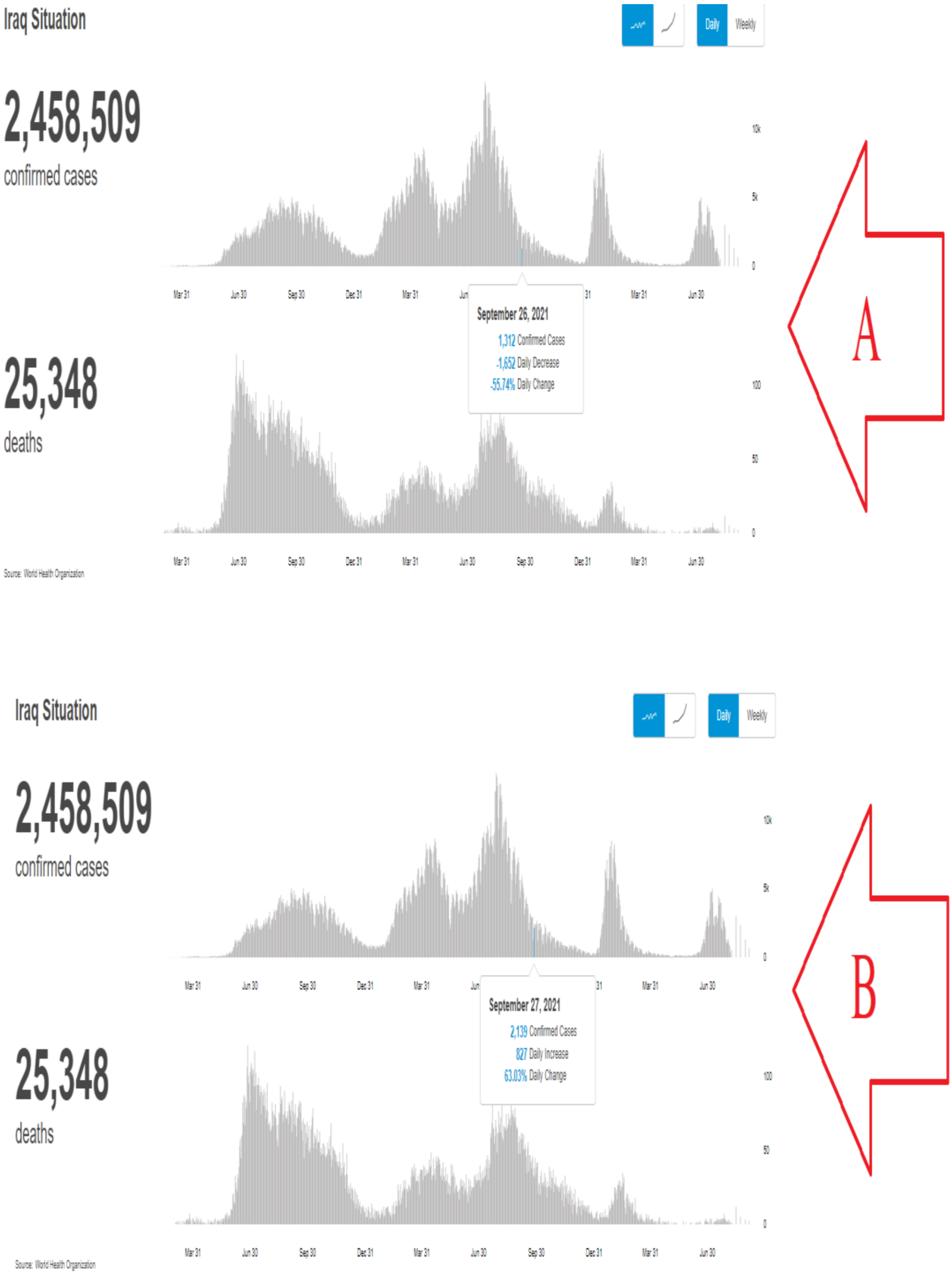


Figure 5: Geographical distribution of predicted occurrence rate of SARS-COV-2 TaqPath positive cases pattern per 100,000 population. The highest occurrences rate was in Karbala governate followed by Baghdad and Al- Al-Qadisiya governorates.

To assess the national epidemiological status of COVID-19 infections during Arbaeen pilgrimage that occurred in the end of September 2021, focusing on number of reported cases from Iraq to the WHO. On day 26, the number of daily cases was 1312, but a sudden and dramatic surge was reported the next day, with 2139 positive cases, representing a staggering daily change of -55.74% to 63.03% in just one day, coinciding with the peak day of the pilgrimage, as illustrated in figure 6.


Iraq Situation

2,458,509
confirmed cases

25,348
deaths

September 26, 2021
1,312 Confirmed Cases
-1,652 Daily Decrease
-55.74% Daily Change

Source: World Health Organization

Iraq Situation

2,458,509
confirmed cases

25,348
deaths

September 27, 2021
2,139 Confirmed Cases
827 Daily Increase
63.03% Daily Change

Source: World Health Organization

Figure 6: Iraq epidemiological status during p retrieved from WHO GLOBAL SARS-COV-2 status Dashboard. Graph A resembles the status on 26th of September 2021 while graph B resembles the status on 27th of September 2021. Each single column within the graph's peaks is proportional to the total Iraqi case counts per day. The data was inspected on 19-09-2022. The results highlight the increased transmission rate during the mass gathering event.

To investigate the circulating variants during the studied period, a total number of 478 FASTA files were retrieved from GISAID repository, spanning the period from July 1 to December 31, 2021. The dominant circulating variants were Delta B.1.617.2, other variants include AY.106, AY.112, AY.121, AY.122, AY.98, AY.65, AY.5, AY.126, AY.20, AY.29, AY.29.1, AY.33, AY.38, AY.39, AY.4 and AY.43 Delta sublineages. However, eleven Alpha B.1.1.7 samples have been reported to GISAID, their collection date was 06-07-2021, 01-10-2021 and 01-12-2021 reported from Baghdad, Duhok and Sulaymaniyah indicating low circulating frequency. These files filtered to 406 high quality genomes which were analyzed using Audacity Instant tool, alignment performed against the entire genomic entries in GISAID. Iraqi genomic data matched a total number of 33,071 global sequences. Genomic distances and frequency of main circulating variants are indicated in Figure 7.

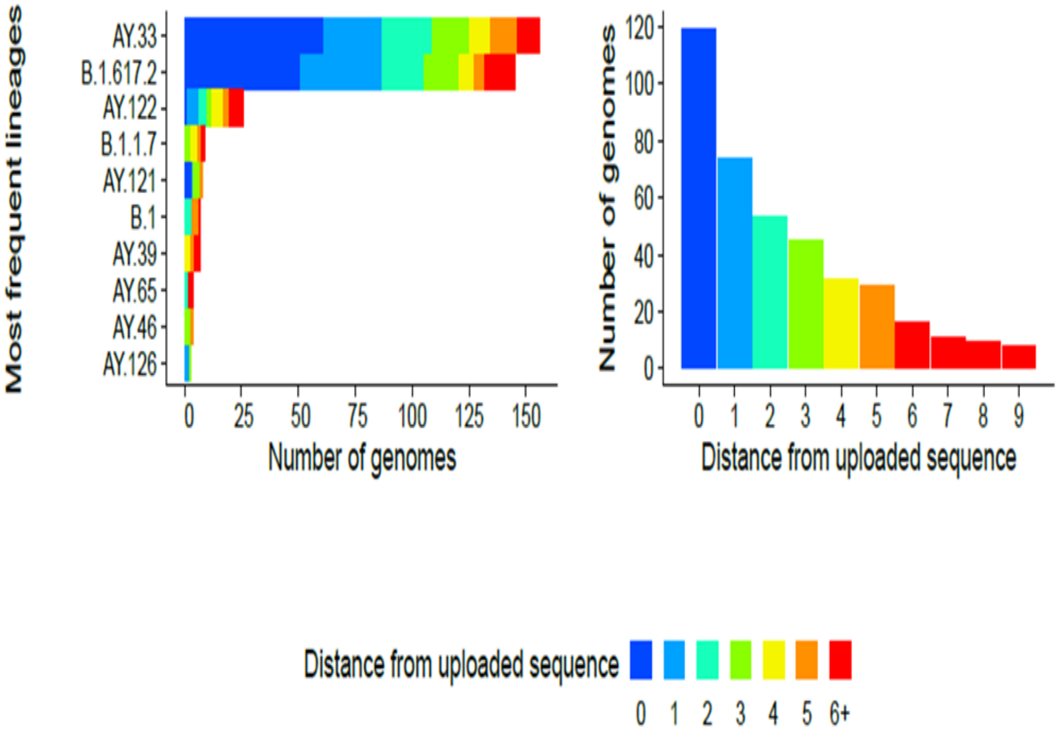


Figure 7: SARS-CoV2 linages frequency and their distances from uploaded sequences, blue color indicate high relevancy to global isolates. The great majority of reported sequences from Iraq during the targeted timeframe belong to Delta lineage or relates sublineages.

The global frequency of H69/70 deletion within reported H60/70V deletions assessed using the EpiCoV^{TM}/GISAID, among a total number of 16,682,646 reported SARS-CoV-2 isolates, the histidine deletion on codon 69 reported within 6,489,726 viral genomes, while the valine deletion at codon 70 reported within 6,494,970 viral genomes indicating that both deletions are presented as one set. Furthermore, the H69/70V deletion were harbored by current dominant circulating JN.1 lineage.

4. Discussion

One of the distinctive genetic signatures of SARS-CoV-2 that used widely for rapid for epidemiological discriminative purposes using PCR approach is the H69/70V deletion which appeared for the first time in Alpha B1.1.7 and harbored later by Omicron variants [25,26]. Studies have shown that the H69/70V deletion enhances the infectivity of the spike protein by increasing its cleavage efficiency of the S2 polypeptide subunit and aiding in syncytial formation [27]. In general, the clinical manifestations documented in patients consent forms that enrolled in this study indicate a wide range of symptoms including coughing, dyspnea, headache, nausea, fatigue, diarrhea, fever above 38°C, agitation, wheezing, runny nose, and cyanosis. These symptoms varied among patients, with no specific pattern that can be distinguished [28, 29]. However, reliable determination of viral transmissibility based on presented symptoms is challenging due to noisy surveillance data which greatly affected by completeness of enrolled patients consent forms [30]. Infection rate comparison between genders showed higher number of infected males than females, which indicate that males are at higher risk of being infected with COVID-19 than females because of daily lifestyle and working environments [31]. TaqPath assay results showed dominancy of SARS-CoV-2 isolates that harbor 69/70 deletion during September and October 2021. The absence of the 69/70 deletion among 555 samples was noticed as a gradual increasing trend in the epi curve. The results of epidemiological curve along with of large increasement of daily reported cases during the pilgrimage give a clear indication of high transmissibility rate within the studied period. Additionally, it caused a gradual spreading of viral isolates with the S+ pattern and boosted the prevalence ratio of circulating variants harboring the H69/70V deletion which constitute a large part of the studied isolates, such phenomenon is noticed with other reported mass gathering events like Hajj in Saudi Arabia, Carnival in Brazil, and Namugongo Martyr's Day in Uganda [32-34]. The high frequency of circulating SARS-CoV-2 isolates that harbor the 60/70 deletion is not consistent with the frequency of the identified viral lineages identified using deposited Iraqi data in GISAID repository, this discrepancy may be explained by the low number of reported sequences available for analysis and limited resources like Next generation sequencing machines. These results highlight concerns about that Omicron variants could be present locally before the official declaration in November 2021. This hypothesis is supported by the observation that Delta and its subsequent sub lineages do not possess the 69/70 deletion, which were prevalent during the studied period as pointed out by analyzed FASTA files, contrarily, the TaqPath assay results indicate different outcome, as it showed high prevalence of isolates possess the 69/70 deletion which is a characteristic of Alpha and Omicron variants not Delta or its sub lineages. Such results support the argument that Omicron was circulating worldwide without detection [35-37]. High spreading ratio that noticed during the studied timeframe, focusing on the mass gathering event justified by the fact that implementation of mitigation controls during such events possess a challenge due high population density, inadequate health infrastructure, low awareness among participants regarding health risks, and ineffective health education [38-40]. Despite a comprehensive investigation and a large enrolled dataset, this study is limited by a one-month gap in data from November to the end of December 2021, the number of SARS-CoV-2 positive cases greatly decreased during this period, as declared by Iraq's public health authorities, such decreasing trend in total cases can limit the available resources for further epidemiological investigation [41, 42]. The assessed frequency of 69/70 deletions of histidine and valine amino acids within global sequences were relatively high, this indicates recurrent emergence and persistence of this deletions across different lineages including the current viral lineage, the JN.1. as it considered as evolutionary advantage the enhance immune escaping increase viral transmissibility [43-45].

Conclusion

This study faced the challenge of a one-month gap in the epidemiological curve. Despite this limitation, our findings reveal a high prevalence of the 69/70 deletion in locally circulating isolates during the world's largest mass gathering event. Furthermore, these results suggest that this event was a significant contributor that increased infection rate. Additionally, current findings provide evidence that the 69/70 deletion mutation was present in various SARS-CoV-2 variants during the period when the Delta variant was dominant, which does not typically harbor this deletion. Additionally, the results indicate recurrent incidence of H69/70V deletion within circulating variants. These findings underscore the need for continued tracking and monitoring of the evolution and transmission of SARS-CoV2.

Acknowledgment

The authors would like to acknowledge all researchers and contributors that submit their data to GISIAD repository, furthermore, the authors thank National Influenza Center, Central Public Health Laboratory for their support.

Ethical Approval

The research topic approved by the ethics committee at College of Science, University of Baghdad (CSEC/0222/0155) on January 28th, 2022.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

The current work didn't receive any funds.

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