Original Article

Detection of SARS-CoV-2 variants among travelers crossing the northern international border checkpoint in Duhok province, Iraq

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Abstract

Background: Variants of SARS-CoV-2 have been detected along with the worldwide COVID-19 transmission. Insufficient data exists regarding SARS-CoV-2 variants in Iraq, particularly concerning travelers at Iraqi border crossings. This study aimed to screen SARS-CoV-2 variants among individuals entering Iraq from Turkey through the Ibrahim Khalil international border checkpoint.

Methods: A total of 116 (52 females, 64 males) COVID-19-positive cases from June 6, 2021, to July 30, 2021, were randomly selected from the Ibrahim Al-Khalil border crossing point between Turkey and Iraq. The patients were Iraqis tested positive for COVID-19 through nasopharyngeal and oropharyngeal swab collections. The confirmation of SARS-CoV-2 cases involved RNA extraction and real-time reverse transcription polymerase chain reaction (RT-PCR), following the World Health Organization guidelines. All samples were subjected to variant detection using the reverse transcription RT-PCR method. The RNA was extracted using a Zybio nucleic acid extraction kit and the RT-PCR was conducted using SARS-CoV-2 S-gene mutation detection RT-PCR kit.

Results: In the 116 patients, we detected variants B.1.1.7, B.1.617.1.2, B.1.617.1.3, and Omicron sub-lineages (B.1.1.529, BA.1) at frequency rates of 13, 16, 45, and 36 cases, respectively. The highest prevalence rate was the delta variant among the tested subjects (38.8%) followed by the Omicron sub-lineages (B.1.1.529, BA.1) at 31%.

Conclusion: This finding, which is the first of its kind at the northern Iraqi border checkpoint, suggests that these two variants could be a source of COVID-19 circulating in the Duhok province in Iraq. Additional studies are warranted across the country's border entry points.

Keywords: Alpha Variant, COVID-19, Delta Variant, Sars-CoV-2, Omicron Variant, Iraq

Background

Different variants of SARS-CoV-2 have appeared as COVID-19 spreads around the world [1,2]. The disease is characterized by respiratory symptoms with high morbidity and mortality rates attributed to the variants [3]. Several SARS-CoV-2 variants have been reported in different countries, including B.1.1.7, B.1.351, Delta (B.1.617.2 lineage), and the Omicron variant (B.1.1.529 lineage) with varying rates of infectivity and spreading abilities [1-3]. Variants occur as a result of differential viral mutations, especially within the S protein that affects viral cellular entry and the efficacy of antibody manufacturing [4,5]. As the SARS-Co V- 2 genome is susceptible to antigenic drift type of mutations that keep the virus from immunological recognition, concerns are emerging on variants that eventually become of high virulence [4,5]. This situation calls for continuous monitoring of the existing variants and the search for newly developed variants that could be the basis of disease prevention and vaccination [1,5]. Limited information is available on the SARS-CoV-2 variants in Iraq, a condition that is mostly uncharacterized [6-9]. One report indicates the existence of the Omicron variant in the Kurdistan region of Iraq [6]. Another study reported 22 distinct mutations of SARS-CoV-2 spike protein regions between 2020 to 2021 in Iraq isolates, with the majority of the variants belonging to B.1.1.7 lineage [9]. Border gates are focal points of disease entry among neighboring states [10]. A recent study from the Kurdistan Region, Iraq reported the occurrence of COVID-19 cases among travelers crossing the Iraqi border from Turkey (August 21, 2020, to August 21, 2021), without any attempt to detect the SARS-CoV-2 variants [11]. Taking into account the possibility of the emergence of COVID-19 reinfection in this

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region that could be related to new variants [12], the purpose of the present study was to screen/distinguish SARS-CoV-2 variants among Iraqi travelers entering the northern international border checkpoint (Ibrahim Khalil) in Duhok province, Iraq.

Methods

Study design and participants

This is a cross-sectional study to detect SARS-CoV-2 variants among randomly selected Iraqi travelers entering the northern international border checkpoint (Ibrahim Khalil) in Duhok province, Iraq. This study took place during the third wave of COVID-19 in the Kurdistan Region of Iraq with almost 2000 positive cases each day during the summer of 2021. A total of 116 COVID-19-positive cases from June 6, 2021, to July 30, 2021, were randomly obtained from the international Ibrahim Al-Khalil border crossing point between Turkey and Iraq. The patients were Iraqis, 52 females (age 19-61 years) and 64 males (age 20-60 years).

Samples Size

The sample size of the study was within the acceptable range for clinical studies as obtained by the online tool (https://www.benchmarksixsigma.com/calculators/sample-size-estimationproportion-data/), taking into consideration the 95% confidence level, 80% power of the test, with 10% acceptable difference.

Inclusion and exclusion criteria

The study included Iraqi citizens with COVID-19 positive tests crossing the international Ibrahim Al-Khalil border checkpoint to enter Iraq from Turkey during the period from June 6, 2021, to July 30, 2021. Non-Iraqi citizens and COVID-19 negative cases were excluded from the study.

Ethics and approvals

This study was conducted according to the guidelines of the Helsinki Declaration. Ethical approval of the present study was obtained from the Directorate of Health, Duhok, Kurdistan Region, Iraq (No. 24102021-10-14, October 24, 2021). Consent of the patients was obtained as a control measure of border entry. For the sake of confidentiality, all information including the identity, age, and gender of the patients were kept unspecified from the research group.

Sampling

Nasopharyngeal and oropharyngeal swabs were collected from the travelers to initiate the process of diagnosing COVID-19, and SARS-CoV-2 cases were confirmed by RNA extraction and real-time switch translation (RT)-PCR according to the guidelines of the World Health Organization [13]. The lung involvement of the COVID-19 patients was confirmed upon clinical examination. Proper health care and protective measures were administered in dealing with the patients and their samples by the health authorities of Duhok governorate at the lab of Ibrahim Al-Khalil border Crossing Coronavirus Testing Center and Lalav Infectious Diseases Hospitals (Duhok).

Extraction of RNA and real-time polymerase chain reaction

All samples were subjected to variant detection using reverse transcription RT-PCR method as per standard guidelines [13]. The RNA was extracted using a Zybio nucleic acid extraction kit (Magnetic bead method), and the RT-PCR was conducted using a SARS-CoV-2 S-gene mutation detection RT-PCR kit (Kogene Biotech, Seoul, South Korea). We also estimated the RT-PCR cycle threshold (≤ 34= positive) for each variant that measures SARS-CoV-2 viral load in a semi-quantitative manner [14].

Statistics analysis

The statistical software PAST4.13 (https://www.nhm.uio.no/english/research/resources/past/) was used to statistically analyze data presented as mean $\pm$ SD by the one-way analysis of variance followed by Tukey's multiple comparison test. Descriptive statistics were also used to characterize the data. The level of statistical significance was at $p < 0.05$.

Results

From the samples of the 116 patients (52 females and 64 males; mean age $\pm$ SD= 36 ± 11 years), we detected variants B.1.1.7 UK (Alpha), B.1.617.1.2 India (Delta), B.1.617.1.3 India (Delta), and Omicron sub-lineages (B.1.1.529, BA.1) at frequency rates of 13, 16, 45, and 36 cases, respectively (Table 1). No gender-specific difference was found across the variants detected. The highest prevalence rate was the delta variant among tested subjects (38.8%) followed by the Omicron sub-lineages (B.1.1.529, BA.1) at 31%. However, unidentified variants comprised only 5.2% despite the existence of the disease in the patients (Table 1). The RT-PCR cycle threshold values of the detected variants in the present study ranged from 23.88 ± 7.39 to 28.25 ± 2.4 (mean $\pm$ SD), which were < 34, the cut-off point for positivity (Table 1). Furthermore, the viral load of the India delta (B.1.617.1.3) variant appeared to be more than the Omicron sub-lineages (B.1.617.1.3) with prevalence rates of 38.8%, and 31%, respectively (Table 1). These two variants were normally distributed when the normal plot of the residuals was examined, and the data were without outliers (Figure 1).

Table 1: COVID-19 variants detected in patients entering the northern international border checkpoint in Duhok province, Iraq

Variants detected	Mean No. of variants/isolate $\pm$ SD	Detection frequency	%	Cycle threshold (≤ 34= positive), Mean $\pm$ SD
B.1.1.7 UK (Alpha)	7.0 ± 3.89	13	11.2	24.30 ± 8.37
B.1.617.1.2 India (Delta)	21.5 ± 4.76	16	13.8	24.18 ± 8.36
B.1.617.1.3 India (Delta)	52.0 ± 13.13	45	38.8	23.88 ± 7.39
B.1.1.529, BA.1 Omicron sub-lineages	92.5 ± 10.53	36	31.0	$28.25 \pm 2.4^{*}$
No variant	$113.5 \pm 1.87^{\dagger}$	6	5.2	20.16 ± 8.18
Total		116	100	25.13 ± 6.86

*Significantly different from the B.1.617.1.3 variant, $p < 0.05$. †Significantly different from the other variants, p < 0.
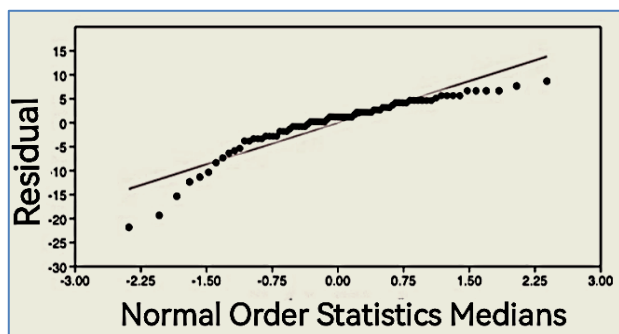
Figure 1: Normal distribution of Omicron sub-lineages and B.1.617.1.3 India (Delta) variants showing statistically significant correlations (p value= 0.001) using ANOVA residual-several sample test; p-value < 0.05 is considered significant

Discussion

In the present study, we detected in COVID-19-positive patients variants B.1.1.7 UK (Alpha), B.1.617.1.2 India (Delta), B.1.617.1.3 India (Delta), and Omicron sub-lineages (B.1.1.529, BA.1) at varying frequencies. This finding, which is the first of its kind at an Iraqi international border checkpoint, suggests that these variants could be a source of COVID-19 circulating in Duhok province in Iraq. The highest frequency rate (38.8%) was that of the variant B.1.617.1.3 India (Delta), and the lowest (11.2%) was that of B.1.1.7 UK (Alpha). Variants that could not be identified comprised only 5.2% despite the existence of the disease in the patients. Further follow-up, which was beyond the scope of the present study, could have delineated these cases.

Previous reports have revealed the existence of several SARS-CoV-2 variants, mainly Omicron (6), B.1.1.7 lineage (8,9), and B.1.428.1 lineage [9]. Having increased affinity for the ACE2 receptors, these variants are also characterized by a high transmission rate avoiding the neutralizing antibodies [1,4,5]. The variants could be the basis of local COVID-19 transmission, and they might also have resulted in a surge in daily cases during 2020-2022 in Iraq [6-9]. However, it is not clear from the present findings the source (s) of the infection, whether it was from Turkey or the patients themselves originally carried the infection with them when traveling to Turkey. Nevertheless, similar variants have been detected in Turkey [15]. Further, tight border COVID-19 tests on both sides (Iraq and Turkey) should clarify this point and possibly detect the transmission route of the disease. Within this context, individuals traveling play a central part in spreading recent variants of SARS-CoV-2 that could have devastating health hazards [10,16]. Stressing this point, it has been reported that COVID-19 cases were found among travelers crossing Turkey to Iraq [11], without attempting to delineate the SARS-CoV-2 variants infecting them. Hence, our results further add to and ascertain the previous study [12] concerning the existence of COVID-19 in travelers crossing Turkey to Iraq from the same border gateway. However, within the unpredictable course of COVID-19 events, identifying regional and local SARS-CoV-2 variants would be an asset in the recommendations of vaccination type(s) decided by local health authorities [5,17]. The RT-PCR cycle threshold values of the detected variants in the present study were below the cutoff point 34, indicating a relatively high viral load (Table 1). This cycle threshold measures SARS-CoV-2 viral load in a semi-quantitative manner [14]. Viral recovery in the samples decreases as the cycle

threshold readouts exceed 35 [14]. Depending on the cycle threshold, the viral loads among the variants except the Omicron sub-lineages (B.1.617.1.3) were close to each other. However, the prevalence rate of the India delta (B.1.617.1.3) variant (38.8%) was also close to that of the Omicron sub-lineages (B.1.617.1.3) (31%) as both variants were normally distributed with no outliers (Figure 1).

Conclusion

The finding of the present study suggests that the detected variants could be a source of COVID-19 circulating in the Duhok province in Iraq. Detection of SARS-CoV-2 variants at the northern Iraqi international border checkpoint signifies the importance of the spread of COVID-19 into the country, and hence further similar studies are warranted across the country's border entry points. Providing multiple variants detection kits for SARS-CoV-2 is a crucial matter in identifying the most strengthening strains and their prevalence rates in the region, which is crucial in an efficient crisis management approach in case of a pandemic.

Abbreviation

COVID-19: Coronavirus disease; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; (RT)-PCR: Reverse Transcription Polymerase Chain Reaction

Authors' contributions
Ghazwan Ahmed Mohammed Raouf (GAMR): Concept, data acquisition, statistics, writing; Marwan Khalil Qader (MKQ): Concept, statistics, writing; Ibrahim Muhammed Abdullah (IMA): Concept, data acquisition, statistics; Omar Mohammed Younis (OMY): Design, sampling and data acquisition; Fouad Kasim Mohammad (FKM): Concept, design, statistics, drafting the manuscript; Muayed Aghali Merza (MAM): Concept, design, statistics, writing. All authors have read, reviewed, and approved the final manuscript.

Ethics approval and consent to participate
We conducted the research following the declaration of Helsinki. Ethical approval of the present study was obtained from the Directorate of Health, Duhok, Iraq (No. 24102021-10-14, October 24, 2021). Written consents of the patients were obtained as a control measure of border entry.

Consent for publication
Not applicable

Competing interest
The authors declare that they have no competing interests.

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