Surveillance for SARS-CoV-2 Variants of Concern and Initial Detection of Omicron using RT-PCR in the Kurdistan region of Iraq

Dlshad Abdullah Hasan[a], Sazan Qadir Maulud[b], Sharmeen Qadr Faqi[a], Rzgar Farooq Rashid[c], Jivan Qasim Ahmed[d*], Karzan Abdulmuhsin Mohammad[e]

[a]Molecular Diagnostic Department, Central Public Health Laboratory-Erbil, Kurdistan Region, Iraq.

[b]Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq.

[c]Department of Medical Laboratory Science, College of Science, Knowledge University, Erbil 44001, Iraq.

[d]Department of Pathology and Microbiology, University of Duhok, Kurdistan Region, Iraq.

[e]General Directorate of Scientific Research Center, Salahaddin University-Erbil, Erbil, Iraq.

Abstract

Background: Omicron (B.1.1.529), a novel SARS-CoV-2 variant (VOC), is a highly diverse variant with many mutations. Immune evasion is possible, as is increased transmissibility within the populations. The RT-qPCR method may be effectively utilised for variant surveillance. This is to rule in or rule out significant variants quickly.

Objectives: As a result, the goal of this study was to track the prevalence of the SARS-CoV-2 Omicron variant in the local community using the SGTF test in conjunction with the SARS-CoV-2 S-gene mutations RT-PCR assays.

Patients and methods: The study included 255 SARS-CoV-2 positive specimens collected in Erbil central public health laboratory between January 1 to February 6, 2022, for routine testing purposes. The SARS-CoV-2 variant profiling was performed on extracted RNA using PowerChek SARS-CoV-2 S-gene Mutation Detection Kit Ver.3.0 plus S-gene Target Failure (SGTF) of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit.

Results: The samples were surveyed, resulting in a positivity rate of (86.6%) for Omicron BA.1, (3.1%) Omicron BA.2, (1.7%) Delta variant (B.1.617.2), and (8.6%) were inconclusive variants. Among Omicron COVID-19 cases, 89 (38.5%) were fully vaccinated, and 4 (1.7%) received full vaccination plus a booster dose. Nevertheless, 16 (7%) of the confirmed Omicron COVID-19 cases had a documented previous SARS-CoV-2 infection.

Conclusion: The SARS-CoV-2 S-gene mutations RT-PCR assay is a cost-effective and fast method for the surveillance of SARS-CoV-2 variants of concern. Currently, Omicron BA1 is the predominant SARS-CoV-2 variant in the Kurdistan region/Iraq, and the emergence of the Omicron BA2 variant is of high concern.

Keywords: SARS-CoV-2; Omicron;Variants of concern.

Introduction

On November 26, 2021, the World Health Organization (WHO) recognised Omicron as a variation of concern (VOC) for a new emergent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant (Lineage B.1.1.529) (*Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern* no date). Compared to alpha, beta, gamma, and Delta variants, the Omicron variant is considered the most mutated one that has emerged so far with a huge number of mutations (including 30 mutations in the S gene). Of these mutations, some are unique to the Omicron variant (eg, E484A, T547K, N764K, D796Y, N856K, Q954H, N969K, L981F, L212I), while other mutations (e.g. 69–70del, T95I, G142D/143–145del, K417N, T478K, N501Y, N655Y, N679K, and P681H) have been conserved from the earlier variants (**Kannan et al.**, **2021**; **Kannan et al.**, **2022**; **Karim and Karim**, **2021**). Several of these mutations could affect viral binding affinity, immune escape, and transmissibility (**Karim et al.**, **2021**; **Ahmed et al.**, **2022**; **Ahmed and Maulud**, **2023**).

A deletion (△69-70) inside the S gene, which is also observed in the Alpha variant, causes "S gene target failure (SGTF)" (or "S gene dropout") in several SARS-CoV-2 PCR assays. This can be used as a marker for screening for this variation, pending confirmation by genome sequencing (**Metzger et al.**, **2021**).

Furthermore, the Omicron variant was split into four sub-lineages: B.1.1.529, BA.1, BA.2, and BA.3. The BA.1 lineage is now the most prevalent worldwide and there has been a recent trend in several regions (e.g., India, South Africa, the United Kingdom, and Denmark) indicates an increase in the amount of BA1 lineage. The spike: 69/70 deletion is not found in BA.2 as for the BA1; therefore, it will not be detected by S-gene target failure PCR like BA.1 (**Saxena et al., 2022**).

According to the data released by WHO, the Omicron variation had been found in 171 countries as of January 20, 2022. In most countries, the Omicron variant quickly displaced Delta and became the dominant variant. The present study aims to detect COVID-19 cases attributed to the Omicron variants and enhance surveillance of current circulating strains, using S-Gene Target Failure (SGTF) PCR along with SARS-CoV-2 S-gene mutations panel RT-PCR.

Patients and Methods

Study design

To track the circulation of the SARS-CoV-2 omicron variant and other variants of concern (VOC) in the city of Erbil, we implemented a system based on screening an initial diagnostic SARS-CoV-2 PCR samples for S-gene target failures (SGTFs) by polymerase chain reaction (PCR) followed by a multiplex RT-PCR SARS-CoV-2 mutations panel. The present study was conducted in the Department of molecular diagnostics at Central Public Health Laboratory (CPHL), where routine detection of SARS-CoV-2 using reverse transcription PCR (RT-PCR) is performed for confirmation. From January 1 to February 6, 2022, a total of 255 SARS-CoV-2 positive specimens were included prospectively for VOC surveillance by both S-Gene Target Failure (SGTF) and variant mutation RT-PCR panel. Relevant data on demographic characteristics, vaccination status, and previous SARS-CoV-2 infection cases were extracted from the routine register-based surveillance of COVID-19 at CPHL-Erbil.

SARS-CoV-2 RT-PCT detection test

Viral RNA was extracted from respiratory specimens (nasopharyngeal and/or throat swabs) using a viral RNA extraction kit, by automated nucleic acid magnetic particle extraction method. Utilising SphaeraMag DNA/RNA Isolation Kit on automating Phoenix-Pure96 system (Procomcure Biotech GmbH com) as per manufacturer's instructions. The SARS-CoV-2 RT-PCR test was conducted using LightMix®. SarbecoV E-gene plus EAV control and LightMix Modular SARS-CoV-2 (COVID-19) RdRP-gene (TIB Molbiol/Roche Diagnostics, Germany, 5 μL aliquots) following producer instructions on Rotor-Gene Q (QIAGEN) Real-Time PCR Detection System.

S-Gene Target Failure (SGTF) RT-PCR

255 SARS-CoV-2 positive samples were collected from January 1 to February 6, 2021. The samples were subjected to SGTF RT-PCR using a commercial assay approved by WHO (Thermo Fisher, TaqPath COVID-19 CE-IVD RT-PCR Kit). Multiple real-time RT-PCR targeting three SARS CoV-2 genes (N protein, S protein, and ORF1ab) were performed on Applied Biosystems™ 7500 Real‑Time PCR Instrument as per manufacturer's instructions.

RT-PCR SARS-CoV-2 Variant Assay

All randomly selected SARS-CoV-2 positive samples were subjected to RT-PCR variant screening assay using PowerChek SARS-CoV-2 S-gene Mutation Detection Kit Ver.3.0 (Kogen Biotech, Seoul, Korea) following the manufacturer's instructions. This assay is a multiplex real-time reverse transcription (rRT)-PCR developed for detecting key mutations (K417N, L452R, E484A / K, N501Y, T547K, P681R) in SARS-CoV-2 S-gene of all variant of concern (VOCs) classified by WHO including Omicron (BA.1, BA.2), Delta, Alpha, Beta, and Gamma.

Statistacal analysis

Software such as SPSS version 20 and Microsoft Excel 20087 were used to analyse the data. Tables were created using cross tabulation and frequency distribution. Numbers and percentages were used to express all of the qualitative data. Statistical significance was determined to have a p value less than 0.05 .

Results

During the study period, from January 1 to February 6, 2022, a total of 6390 persons attended the CPHL-Erbil for SARS-CoV-2 testing. 699 (10.9%) of the tested individuals tested positive for SARS-CoV-2 by RT-PCR assay (table 1). Weekly case counts rose considerably (**Fig.1**), with about 10% weekly increases. In total, the variant screening study randomly included 255 out of 699 positive samples. The age ranged from 8 – 79 years old, with 132 (51.8%) cases being females. 133 (52.2%) were fully vaccinated, and 9 (3.5%) had received a booster dose (**Table.1**).

Table 1. Characteristics of Positive SARS-CoV-2 Total and Omicron variant cases

| Variables	Total Positive Samples		Omicron Positive Samples	
Characteristics				
	Number	*%*	Number	*%*
Gender				
• **Female**	132	51.8	124	54.1
• **Male**	123	48.2	105	45.9
Age, years				
• **10 – 17**	18	7.1	13	5.7
• **18 – 29**	31	12.2	30	13.1
• **30 – 39**	79	30.9	75	32.3
• **40 – 49**	72	28.2	66	28.8
• **50 – 59**	33	12.9	29	12.7
• **60 – 69**	12	4.7	9	3.9
• **≥ 70**	4	1.6	4	1.7
• **Unknown**	6	2.4	3	1.3
Vaccine status				
• **Unvaccinated**	91	35.7	80	35
• **Partial vaccinate**	11	4.3	9	3.9
• **Full vaccinate**	133	52.2	127	55.5
• **Booster vaccination**	9	3.5	4	1.7
• **Unknown**	11	4.3	9	3.9
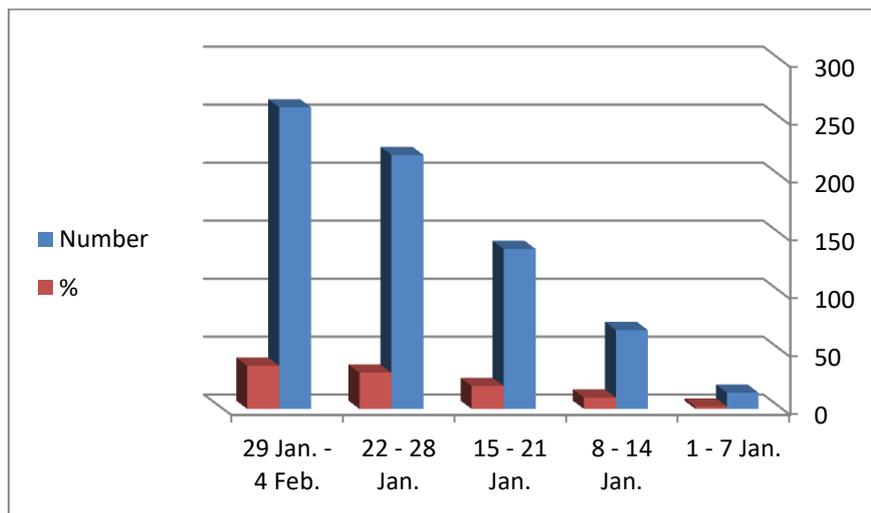
Fig.1.Weekly SARS-CoV-2 positive rate

Among 153 cases of known vaccine types, 115 (75.2%) received NT162b2 mRNA (BioNTech-Pfizer), 29 (18.9%) were vaccinated with the ChAdOx1 nCoV-19 vaccine (Oxford–AstraZeneca), and 9 (5.9%) received Sinopharm inactivated virus COVID-19 (BBIBP-CorV) (**Table.2**). The most common symptoms were fatigue, scratchy throat, cough, mild fever, abdominal pain, and runny nose. Exposures associated with social events, household transmission, and international and domestic travel were recorded.

Table 2. Vaccination status by vaccine type among 153 SARS-CoV-2 positive cases

Vaccine type	Partially Vaccinated	Fully Vaccinated	Booster vaccination	Total No.	%
Pfizer	9	97	9	115	75.2
AstraZeneca	2	27	0	29	18.9
Sinopharm	0	9	0	9	5.9

The S-gene target failure (SGTF) analysis of the samples was performed. The samples were classified as SGTF when only N and ORF1ab were successfully amplified or non-SGTF if all three targets N, ORF1ab, and S were amplified. The SGTF assay result revealed that 232 (91%) had SGTF while 23 (9%) did not have SGTF. Later, by using SGTF RT-PCR and SARS-CoV-2 RT-PCR variant screening assay, 221 (86.6%) were confirmed to be Omicron BA1 COVID-19 cases, 8 (3.1%) Omicron BA.2. In comparison, 4 (1.7%) were infected with SARS-CoV-2 Delta variant (Pango lineage B.1.617.2), and 22 (8.6%) were inconclusive variants (**Table.3**). Seven percent of the Omicron COVID-19 cases (n = 16) had a documented previous SARS-CoV-2 infection. Among Omicron COVID-19 cases 89 (38.5%) were fully vaccinated more than 14 days before the onset of symptoms or diagnosed with SARS-CoV-2 positive, and 4 (1.7%) were fully vaccinated plus a booster dose.

Table 3. SARS-CoV-2 lineages identified

Lineage	Count (%)
Omicron BA.1	221 (86.6%)
Omicron BA.2	8 (3.1)
Delta B.1.617.2	4 (1.7)
Inconclusive	22 (8.6)

Validation of the SARS-CoV-2 S-gene mutations panel RT-PCR assay specificity was done with SARS-CoV-2 negative nasopharyngeal samples (30 samples). No amplification was detected in all samples.

Discussion

According to the findings of this study, the Omicron variant has dramatically spread the local inhabitants, replacing other variants and becoming the dominant one. On January 6, 2022, the first attributed cases of COVID-19 to the Omicron variant were detected in the Kurdistan region/Iraq,

a substantial increase in the number of cases has been observed following that date released by the ministry of health in the Kurdistan regional government (*COVID-19: Dashboard - GOV.KRD* no date). The loose restrictions on masks and public meetings might be the primary causes of the dramatic increase in case records. Furthermore, the capacity of the Omicron variant to evade the immune system of infected persons and its faster transmission rate among the community might have resulted in an increased number of COVID-19 cases. Study results demonstrate the emergence and subsequent dominance of the Omicron BA1 lineage and report the first cases of Omicron BA2 in the Kurdistan region/Iraq. A substantial weekly increase in the incidence of cases was also reported. As of February 6, 2022, the Eastern Mediterranean Region reported a weekly increase of 36% in the number of new cases (**Del Rio et al.**, **2022**; **Saïah et al.**, **2022**). This is consistent with the rapid global expansion of the Omicron variant.

The BA.1 and BA.2 variants share several alterations; however, BA.2 has many differences. A recent relative increase in the amount of BA.2 lineage has been found in different countries (e.g., Denmark, India, and the United Kingdom). Because the spike 69/70 deletion is related to S-gene target failure (SGTF) in some PCR assays, BA2 will not be detected by S-gene target failure used as a proxy for BA1 detection (**Saïah et al.**, **2022**).

According to a WHO technical brief released on December 23, 2021, the rapid increase in Omicron cases is due to the substantial role of immune evasion when previously infected or vaccinated persons ('Enhancing readiness for Omicron (B.1.1.529): Technical brief and priority actions for Member States View

most current version', **2021**). Immune escape is another concern, given that (38.5%) of cases were fully vaccinated or others who have received at least one dose of the COVID-19 vaccine, and few have even received booster doses (**Hasan et al.**, **2022**). Structural analysis of Omicron variant mutations indicated a reduction rate of antibody binding generated by vaccination or previous infection with the SARS-CoV-2 virus as a result of uniquely positioned mutations (**Kannan et al.**, **2021**).

In agreement with previous reports from other countries, our outcomes support that vaccination may not provide complete protection from infection. By December 9, 2021, Denmark identified a total of 785 SARS-CoV-2 Omicron variant cases. Among these, (76%) of the cases were fully vaccinated, and (7.1%) have received a booster vaccination. Also, 34 (4.3%) have had a previous SARS-CoV-2 infection. Similarly, a study of the first outbreak in Norway found that most of the cases (n = 79/81; 98%) were fully vaccinated (**Brandal et al.**, **2021**).

Due to resource constraints, genome sequencing to discover variants of concern cannot be performed. The use of real-time multiplex PCR to find various SARS-CoV-2 variants targeting critical mutations in the S gene can be an essential alternative technique. This may be such a strategy is a rapid, less expensive, and less technically demanding method of generating data on the spread of various SARS-CoV-2 variants during the pandemic and identifying variants as soon as possible .

Several studies have been conducted to explore the use of RT-qPCR to detect SARS-CoV-2 variants of concern rapidly. **Nörz et al. (2021)** demonstrated

the viability of multiplexed RT-qPCR Spike-gene SNP assay for the detection of B.1.617 lineage variants (delta/kappa) . Another study by **Zhao et al. (2021)** constructed a method for the detection of multiple mutations sites of SARS-CoV-2 variants based on a multiplex PCR-mass spectrometry mini-sequencing system (mPCR-MS mini-sequencing) with a high rate of specificity and sensitivity .Similarly, **Banada et al. (2021)** described a reverse transcriptase PCR (RT-PCR) melting-temperature (Tm) screening assay that identifies the first three major SARS-CoV-2 VOCs.

Conclusion

The results of this research show a rapid increase in records and steeply spread of the SARS-CoV-2 Omicron variant lineage BA1 in the Kurdistan region/Iraq. In addition, the emergence of the BA2 lineage is considered to be a concern. More studies and surveillance are required to better understand the epidemiological and clinical features of the Omicron variant and other SARS-CoV-2 variants in circulation. The large number of infections occurring in fully vaccinated patients is noteworthy. It is critical to track the COVID-19 pandemic, which requires accurate identification of possible variants is very important. Multiplex RT-qPCR variant detection assay can achieve the identification of SARS-CoV-2 mutations in a short time and at a low cost.

Ethical statement

This study was conducted under the approval of the Erbil Health Directorate/Kurdistan region Ministry of Health as a part of routine national surveillance for COVID-19 cases; therefore, ethical approval was not required.

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