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Original Article

Molecular Epidemiology of SARS-CoV-2 Variants in Vaccinated and Non-Vaccinated Patients of Erbil Province, Kurdistan Region-Iraq

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Abstract

Multiple new SARS-CoV-2 variants of concern (VOC) have emerged globally since the onset of the COVID-19 pandemic. With the virus continuing to evolve, more are expected. This emphasizes the need for rapid diagnostic methods for the detection of circulating lineages. Variants-specific real-time reverse transcription (rRT)-PCR method can be used as an alternative to genome sequencing, which is expensive and labored for identifying these variants, especially in settings with limited resources. We assessed the prevalence of various SARS-CoV-2 variants spreading in the Erbil province using a diagnostic screening RT-PCR-based method. A total of 144 SARS-CoV-2 positive samples were prospectively tested for known SARS-CoV2 variants using ViroQ® SC2 Variant rRT-PCR. Furthermore, the technique was validated using 25 SARS-CoV-2 negative nasal samples. Out of 144 SARS-CoV-2 positive samples, 118 (81.9%) were B.1.617.2 (Delta), 5 (3.5%) were Epsilon B.1.427/B.1.429, 1(0.7%) was Eta B.1.525, 2(1.4%) were SARS-CoV-2 Wild type, while 18 (12.5%) were undefined variant, and the delta strain was the most prevalent SARS-CoV-2 strain. Our study showed that variant-specific RT-PCR could be a useful tool for the rapid screening of SARS-CoV-2 variants.

Keywords: COVID-19, SARS-CoV-2, Variants, RT-PCR, Erbil, Iraq.

INTRODUCTION

Sever acute respiratory syndrome 2 (SARS-CoV2) is the cause of the respiratory disease known as coronavirus disease 2019 (COVID-19), which is a highly infectious disease [1]. The infection was first discovered in the Wuhan city. The World Health Organization (WHO) has declared a pandemic as a result of the disease's fast worldwide spread [2]. Fever, cough, shortness of breath, and tiredness are all common signs of a viral infection [3]. According to preliminary data, nearly 20% of patients have severe COVID-19 and require hospitalization. The main route of disease transmission is direct contact with contaminated droplets. The spread of COVID-19 and the fatality rate caused by SARS-CoV-2 remain noteworthy and historical concerns [4,5]. Iran was one of the first countries to have a significant epidemic. On February 19, 2020, The country's first confirmed case was from a traveler from China who was a merchant in Qom [6]. Several of the earliest Middle Eastern nations with infections were related to Iranian travelers, such as Lebanon, Kuwait, Bahrain, Iraq, Oman, and the UAE [7]. The first verified incidence of COVID-19 in Iraq was reported on February 24, 2020, in Al-Najaf, a city south of Baghdad [8]. As of January 22, 2022, more than two million people in Iraq had the infection, with over 24,000 fatalities [9]. On March 1, 2020, the first case of COVID-19 was detected in the Kurdistan region of Iraq. COVID-19 hit the Kurdistan region of Iraq in four consecutive waves, as it did in the rest of the globe [10].

The newly discovered SARS-CoV-2 virus belongs to the *Coronaviridae* family, which includes at least six current types of human pathogenic Coronavirus, such as SARS-CoV and MERS-CoV [11]. Compared to SARS-CoV from 2002 and MERS-CoV from 2013, SARS-CoV-2 is a more severe virus [12]. There is an urgent need for

more detailed research on the virus's pathogenesis, infectivity, and virulence, as well as the development of effective therapy techniques [13,14]. There was instant interest in researching the zoonotic source, transmissibility, mutation, and variants of SARS-COV-2 [15].

Whole-genome sequencing indicated that the SARS-CoV-2 species is the Betacoronavirus genus and are enveloped, positive-sense single-stranded RNA viruses and the genome is more than 29 thousand nucleotides in length [16]. The genomic organization of SARS-CoV-2 is similar to other coronaviruses consisting of 5′ end of the genome encoding over two-thirds of the ORF1ab polyproteins. Simultaneously, the 3′ end contributes to one-third of the whole genome and contains genes encoding structural proteins such as spike (S), envelope (E), membrane (M), and nucleocapsid (N). SARS-CoV-2 also comprises 6 accessory proteins that encode by the ORF3a, ORF6, ORF7a, ORF7b, and ORF8 genes[17].

Through a combination of missense, deletion, insertion, and other mutations, SARS-CoV-2 has developed into many variants of concern and variants of interest. The SARS-CoV-2 virus is rapidly transmissible because of changes in the strain's receptor-binding (S1) and fusing (S2) regions, which are attached to the human angiotensin-converting enzyme 2 (ACE2) receptor by the spike protein. Transmission may become easier if more mutations accumulate [18].

Several SARS-CoV-2 strains discovered across the world since the pandemic began. Each has one or more changes in the viral genome, especially in the gene that codes for spike proteins [19]. As a result, viral transmission and the host immune response may be affected. At the beginning of the pandemic, a viral variant with D614G, a spike protein alteration was found in northern America and afterwards reported in several European countries [20]. To date, the SARS-CoV-2 strains that have been detected are: Alpha B.1.1.7, B.1.1.7 (484K), B.1.1.1/C.36/B.1.1.7, Eta B.1.525, B.1.526, Beta B.1.351, Gamma P.1, Zeta P.2, B.1.1.28, Kappa B.1.617.1/B.1.617.3, Delta B.1.617.2, Epsilon B.1.427/B.1.429, and Omicron B.1.1.529. These variants have the potential to increase transmissibility or significantly alter SARS-CoV-2 epidemiology; increase pathogenicity or significantly alter clinical illness appearance; or reduce the effectiveness of public health responses and existing diagnostic methodologies, vaccinations, or treatments [21].

Alpha variation is a SARS-CoV-2 variant discovered in January 2020 and characterized by the COVID-19 Genomic UK Collaboration in the UK (COG-UK) [19]. It was identified by 17 changes in the whole viral genomes, mainly in the spike proteins, such as N501Y in the receptor-binding domain (RBD), P681H at the Furin c terminus, and 69-70del in the immune response evasion domains. In October 2020, the B.1.351 lineage, also identified as Beta, initially was reported in South Africa. It was identified by 8 mutations in the spike protein area, such as K417N, E484K, and N501Y [22]. In December 2020, the Delta variant (B.1.617.2 lineages), which is now a prevalent variety of the virus in numerous places across the world, was discovered for the first time in India. The presence of various changes in the spike protein, such as L452R, T478K, and P681R, distinguish it. According to studies, this type of SARS-CoV-2 is sixty per cent more pathogenic than the Alpha variant [23]. Additional variants exist, including the Gamma (P.1 lineage), which is most prevalent in Brazil and contains Seventeen alterations, three of which are found in the spike protein parts, such as K417T, E484K, and N501Y [24]. The Omicron variant (B.1.1.529 lineage), which is presently circulating in numerous regions across the globe, is a severely mutated virus that was originally discovered on November 24, 2021, in South Africa. The objective of this study was to conduct an identification of circulating a variant among the tested samples for SARS-CoV-2 in Erbil province and determination of the dominant variant.

MATERIALS and METHODS

Sample Collection

In this study, we screened prospectively 144 laboratory-diagnosed SARS-CoV-2 cases using the RT-PCR method during September 2021. 69 samples were females and 75 were males aged from 2-85 years. Samples were collected and tested at the

Central Public Health Laboratory (CPHL) SARS-CoV-2 test center in Erbil city, Kurdistan Region-Iraq. Clinical samples (Nasopharyngeal and throat swabs in viral transport media) and relevant data (age, gender, vaccination status) were collected by trained laboratory technicians. This study was conducted by the molecular diagnostic department at the Central Public Health Laboratory - Erbil city in collaboration with the department of Biology, College of Education, Salahaddin University-Erbil and the research center of Salahaddin University-Erbil, Iraq.

SARS-CoV-2 RT-PCR Screening Test

According to World Health Organization guidelines, Real-time RT-PCR was used to detect SARS-CoV-2 in all samples (nasopharyngeal and throat swabs). Briefly, viral RNA was extracted by an automated method using the SphaeraMag DNA/RNA isolation kit using an automated Phoenix-Pure96 system (Procomcure Biotech, Germany) following the manufacturer's recommendations. The commercial kit DIAGNOVITAL®SARS-CoV-2 Real-Time PCR Kit (RTA Laboratories Biological Products Pharmaceutical and Machinery Industry) was used on the Rotor-Gene Q (QIAGEN) Real-Time PCR Detection System to perform RT-PCR. The DIAGNOVITAL®SARS-CoV-2 PCR kit detects two target genes of the SARS-CoV-2 genome: the E and RdRp genes.

RT-PCR SARS-CoV-2 Variant Analysis

Positive samples were randomly selected for RT-PCR variant screening assay using RT-PCR the ViroQ® SC2 Variant kit (BAG Diagnostic Gmbh, Germany) following the manufacturer's instructions on Rotor-Gene Q (QIAGEN) Real-Time PCR Detection System. The RT-PCR variant typing was set up in two reactions (Kit 1 & Kit 2) that detected eight different mutations (484K, 1176F, 501Y, 484E, 452R, Del 69-70, 681R, 484Q) in the SARS-CoV-2 spike protein that is associated with the respective 12 variants (Alpha B.1.1.7, B.1.1.7 (484K), B.1.1.1/C.36/B.1.1.7, Eta B.1.525, B.1.526 , Beta B.1.351, Gamma P.1, Zeta P.2, B.1.1.28, Kappa B.1.617.1/B.1.617.3, Delta B.1.617.2, Epsilon B.1.427/B.1.429), and the identification of SARS-CoV-2 wild type (Wuhan).

RESULTS

This study randomly selected 144 samples from SARS-CoV-2 diagnostic positives between September 1st and 30th, 2021. The samples were 69 (49.9%) females and 75(52.1%) males; the age range was between 2 and 85 years old (Table 1). There were no substantial differences in the overall number of men and females.

Table 1. Demography of samples included in this study

Sample	Gender	No.
Gender	Female	69
	Male	75
	Total	**144**
Age ranges	Range	Year
	<10	4
	10 – 17	10
	18 – 29	32
	30 – 39	45
	40 – 49	25
	50 – 59	14
	60 – 69	9
	70 – 79	2
	>80	3
	Total	**144**

SARS-CoV-2 variant incidences were considerably lower in younger ages compared to the other age groups ($p<0.05$). Compared to other age groups, the 30 to 39 age group had a significantly higher percentage of SARS-CoV-2 variants ($p<0.05$), but no significant difference was observed in the age group 18 - 29 years. SARS-CoV-2 variant infections were substantially higher in unvaccinated patients (115, or 79.9%) than in fully 19 (13.2), or partially 10 (6.9%) vaccinated patients (Figure 1).

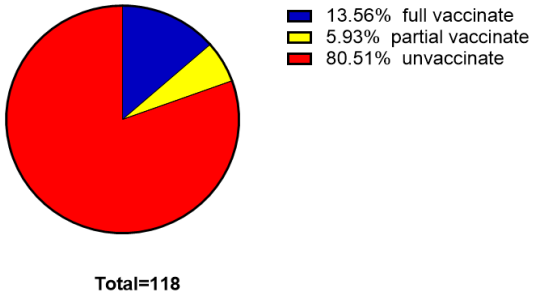

Figure 1. Vaccination status among 144 SARS-CoV-2 positive cases

In the delta variant unvaccinated patients, 95 were significantly increased compared to fully 16 or partially 7 vaccinated patients. While in Epsilon variant, 3 unvaccinated patients and 2 partially vaccinated patients were detected. On the other hand, there was only one unvaccinated case detected in the Eta variant and two unvaccinated cases detected in the SARS-CoV-2 Wild type. Moreover, in undefined variant unvaccinated patients, 14 were significantly increased compared to fully 3 or partially 1 vaccinated patients (Figure 2).

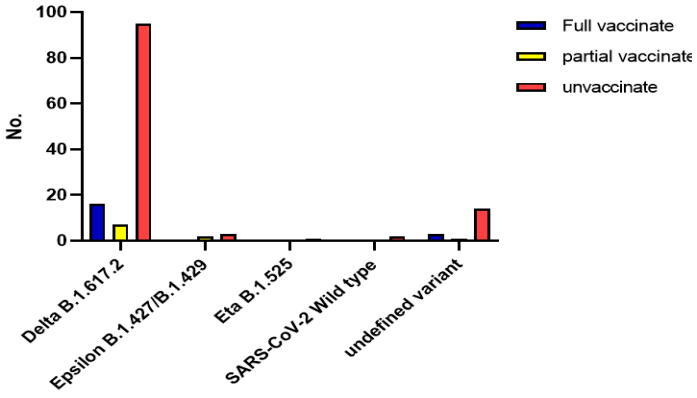

Figure 2. Circulation of SARS-CoV-2 variant within Vaccinated and Non-Vaccinated case

The ViroQ® SC2 Variant RT-PCR results showed that 118 (81.9%) were Delta (B.1.617.2), 5 (3.5%) were Epsilon (B.1.427/B.1.429), 1 (0.7%) was Eta (B.1.525), 2 (1.4%) were SARS-CoV-2 Wild type. In comparison, 18 (12.5%) were undefined variants (Table 2), revealing that these variations were prevalent in the region's population throughout September, with the Delta variant dominant one. Among the 18 samples of undefined variants, the mutations (484E, 681R) and (681R, 452R) were detected in nine and seven samples, respectively. At the same time, two samples were negative for all mutations covered by the assay used. The assay's specificity was verified by using 25 SARS-CoV-2 negative nasopharyngeal specimens. All SARS-CoV-2 negative samples showed no amplification.

DISCUSSION

In situations when genome sequencing capacity is restricted, an alternate method such as using a variant-specific RT-PCR-based strategy for SARS-CoV-2 variants screening can be a beneficial option. Furthermore, sequencing the whole viral genome is both costly and time-consuming. To overcome the limitation in our instance, we used the multiplex RT-PCR approach to assess the prevalence of SARS-CoV-2 strains in the Erbil province of Kurdistan region/Iraq. The prevalence of SARS-CoV-2 variations in a particular region of Iraq was being assessed for the first time in the present study.

Table 2. Summary of the variants detected from 144 positive cases of SARS-CoV-2

Lineage	Number (%)
SARS-CoV-2 Wild type	2 (1.4)
Alpha B.1.1.7	0
B.1.1.7 (484K)	0
B.1.1.1/C.36/B.1.1.7	0
Eta B.1.525	1 (0.7)
B.1.526	0
Beta B.1.351	0
Gamma P.1	0
Zeta P.2	0
B.1.1.28	0
Kappa B.1.617.1/B.1.617.3	0
Delta B.1.617.2	118 (81.9)
Epsilon B.1.427/B.1.429	5 (3.5)
Undefined variant	18 (12.5)

There have been several commercially available multiplex RT-PCR techniques for quick detection of the different mutations in SARS-CoV-2 variants of concern and variants of interest [25]. Our study's findings revealed the usefulness of SARS-CoV-2 variant-specific RT-PCR techniques for identifying key variant-identifying alterations that may be useful in tracking and controlling COVID-19 infections. Among a total of 144 non-replicated, nasopharyngeal swabs RT-PCR positive SARS-CoV-2 specimens from the diagnostic routine, three different SARS-CoV-2 variants were detected.

The findings of this study were consistent with prior research that indicated using a SARS-CoV-2 variant-specific RT-PCR as an effective approach for detecting SARS-CoV-2 variants in positive PCR samples was successful [26]. Our findings demonstrated that there was no substantial difference among males and females in terms of infection with SARS-CoV-2, which was consistent with previous epidemiological data [27]. According to this study, SARS-CoV-2 infection rates were substantially higher in patients between 18 - 49

years than in the younger age group (<17) and older (>60). This can be related to this age group's social activity and social interactions. The most of SARS-CoV- 2 disease were in unvaccinated individuals.

The study findings revealed one major VOC, Delta, and two VOI, including Epsilon and Eta, were circulating in Kurdistan in September 2021. Delta strain was determined to be dominant in our local population during that period. Likely, Delta was introduced into Kurdistan. The lineage first emerged in late 2020 in India, where it was responsible for a large number of infections and deaths in the second wave of COVID-19 cases in April 2021 [28,29]. Following that, this variety quickly expanded over the world, outcompeting other VOCs to become the dominant strain in many regions, it was recognized as a VOC by the World Health Organization (WHO) in May 2021.

Delta is a dangerous variation that has more than a dozen substitution alterations (T19R, V70F, T95I, G142D, E156-, F157-, R158G, A222V, W258L, K417N, L452R, T478K, D614G, P681R, D950N) in the spike protein. Delta has the L452R mutation in the receptor-binding domain (RBD), as well as other variants of concern. The K417N enhances antibody neutralization and evasion. The Delta virus's ability to replicate is enhanced by the P681R mutation at the proximal furin cleave region [27].

According to confirmed data from the Kurdistan Ministry of Health, the Alpha strain first appeared there in early March 2020, and was dominantly circulating variants of SARS-CoV-2 infections until reporting the Delta strain in July 2021. Subsequently, the daily cases have increased dramatically following the appearance of the Delta strain in the country [30]. Our study presented the Delta strain (81.9%) as the highest variant in the local community in September and showed the replacement of the Alpha with Delta. The Delta strain was estimated to spread more readily (40–60%) than Alpha and twice more than the initial SARS-CoV-2 strain from Wuhan [31].

Epsilon variants, first discovered in the USA, comprise two closely related lineages. The Eta was first detected in the United Kingdom/Nigeria – in December 2020. The Epsilon variant contains several spike mutations of interest (L452R, D614G; S13I) and exhibits a 20% increase in transmissibility to co-circulating lineages [32]. The Eta harbor key spike mutations (D614G, delH69V70, E484K, F888L, P681H). It is circulating at low levels in several countries, with Nigeria having the greatest proportion of infections. Our data showed a low prevalence of Epsilon (3.5%) and Eta (0.7%). The Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) are supporting this by de-escalating these two variations due to extremely low levels of detection and the effectiveness of vaccines and treatments against it [33,34].

The limitations of this study were that this study only covered one province of Iraq rather than the whole country and in a limited time period. Small sample size and use of RT-PCR technique rather than genome sequencing are the main limitations of our study, RT-PCR approach detects only the predefined set of mutations and fails to identify any new potential mutations in the genome of the virus. However, this approach may be used to select specimens for sequencing to confirm any novel variant because this technique is less costly, less labour intensive and can screen larger sample sizes.

CONCLUSION

Study findings revealed that the variant-specific RT-PCR approach seems useful to detect known SARS-CoV-2 variations quickly. It can be used as a surveillance tool to monitor suspected variants' distribution and population frequency and to priorities samples for sequencing. Results also demonstrate that Delta was a dominant variant in our local population in September 2021. More research is required to confirm the prevalence of variants and their detection.

Ethical Consideration

The Erbil directorate of health confirmed and approved this study for collecting samples and data from referred patients at CPHL-Erbil as part of the national COVID-19 pandemic screening program.

Conflict of Interest

The authors declare they have no conflicting interests.

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