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RESEARCH PAPER

Tracking SARS-CoV-2 variants in Kurdistan Region of Iraq

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A B S T R A C T:

SARS COV-2, the etiology of the ongoing COVID-19 pandemic, is a positive single-stranded RNA virus which was first identified in the city of Wuhan in 2019. Since its first discovery, several variants of the virus have been reported around the world. Identifying the emerged SARS-CoV-2 variants overtime is important since this could alter the pathogenicity and transmissibility of the virus as well as the efficacy of the vaccine. Here, 34 genome sequence of SARS COV-2 samples, which were collected between Feb. 2021 to Dec. 2021 in Kurdistan Region of Iraq (KRG), were compared and analyzed in the spike (S) protein region. The nucleotide sequences were collected from the GISAID database (https://gisaid.org/), and the SARS-COV-2 variants were determined based on the mutations that they carry. Additionally, the frequency of each mutation was determined in the S protein region of the isolates. Overall, 29 mutations were detected in the sequence samples; the majority (44.8%) were located in the N-terminal domain (NTD) of the protein, whereas only 10.3% located in the ribosomal binding domain (RBD). D614G was found in all sequenced strains, followed by 67.6% mutation of P681H. Moreover, seven deletions were found in the NTD of the S protein, with 61.8% of the deletions was occurred at the position Y144. Alpha variants (B.1.1.7) were found to be dominant lineage during the period of this study, accounted for 65% of the sequence samples. Overall, our data support the speedy increase of the SARS-COV-2 genome diversity. Based on our knowledge, this is the first mega sequence analyses of different SARS-COV-2 strains from KRG of Iraq.

KEY WORDS: SARS-COV2, COVID-19, Spike protein, RBS, Viral infection
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1.INTRODUCTION:

The COVID-19 which is caused by acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide since its emergence in 2019 in Wuhan city, China (Wu et al., 2020). The disease has infected more than 300 million people around the worldwide with approximately 5.5 million deaths (Suphanchaimat et al., 2022). In Kurdistan Regional Government (KRG), the first case of COVID-19 was reported on March 2020 in the city of Sulaymaniyah (Abdulah et al., 2021, Mohammad et al., 2021, Abdullah et al., 2020).

Since then, more than 430,000 cases were confirmed, and 7,450 deaths have been reported as of 15th May 2022 (http://gov.krd/coronavirus-en/dashboard/).

SARS CoV-2 is a positive single-stranded RNA virus, which has nearly a 30 kbp genome size, belonging to the *Coronaviridae* family (Mohammad et al., 2021, Sabir et al., 2020). Its genome is encoded for structural, nonstructural, and accessory proteins (Ciotti et al., 2019). The structural proteins include envelope (E), membrane (M), spike (S), and nucleocapsid (N) protein (Wang et al., 2020). The gene that is

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encoded for the proteins surface plays an important role in the virus binding and entering into the host cells by recognizing the receptor angiotensin-converting enzyme 2 (ACE-2) (Zhou et al., 2020). Therefore, the spike protein is being targeted for vaccine development by researchers worldwide (Duan et al., 2020, Zhou et al., 2020). Structurally, the spike protein is made up of 1276 amino acids and composed of two sub-domains, S1 and S2 subunits (Tegally et al., 2021). The virus interacts with the human ACE-2 receptor via a region is called receptor-binding domain (RBD) (residue 319 to 541) (Thomson et al., 2021).

Since the first report of SARS-COV-2 was in the city of Wuhan/China, the numbers of variants of the virus has been reported worldwide carrying significant genetic changes comparing to the first reported strain (Choi and Smith, 2021, Suphanchaimat et al., 2022). These mutations in the virus can affect the pathogenicity of the strain as well as the severity of the disease (Lauring and Hodcroft, 2021). Additionally, it can also affect the spreading of the virus, and importantly it can affect the efficacy of the – vaccines that are currently available (Lauring and Hodcroft, 2021). SARS-CoV-2 carrying D614G mutation in the spike protein region has been shown to be dominant mutation around the globe, which increases the efficiency of the virus to cause disease (Mansbach et al., 2021). Many other variants were identified including, N439K mutation in the RBD of spike protein in Europe and the USA (Thomson et al., 2021). Alpha variant, is classified as a virus of concern (B.1.1.7 lineage) was reported in the UK in early 2020 (Wise, 2020). In December 2020 Delta variant was first reported in India and characterized by carrying several mutations in the spike proteins including L452R, T478K and P681R (Plante et al., 2021a, Brief, 2021). It is variant is more concerning since it is 60% more transmissible than the Alpha variant (Plante et al., 2021a, Brief, 2021). P.1 lineag (,20J/501Y.V3) was first identified in Brazil and characterized by having 17 mutations in which K417T, E484K, and N501Y mutations are located in the RBD domain (Faria et al., 2021).

A new Variant of concern (VOC) of SARS-CoV-2 called *Omicron* (B.1.1.529) was reported from South Africa in November 24, 2021 (Gowrisankar et al., 2022). The omicron variant has posed a serious global health problem due to its high transmissibility capacity. It has also possessed more than 50 mutations in which 26-32 of them are located in the S protein region(Gowrisankar et al., 2022, Poudel et al., 2022, Ahmed et al., 2022). Although it was observed that the Omicron variant multiplies nearly seventy times faster than the Delta variant, the severity of COVID-19 by Omicron variant is less when compared to the previous strains, especially compared to the Delta variant (Rao et al.).

Here, we analyzed the sequence of the SARS-CoV-2 S-proteins from KRG collected between February 27, 2021, to the end of December 2021. The aim of the research is to report the variants that circulate in the region during that period of time, and the results of this study could help the more efficient vaccination program control the disease.

2. MATERIALS AND METHODS

The S protein sequence of the SARS-COV-2 strains that were used here obtained from the public database of Global Initiative on Sharing All Influenza Data (GISAID, https://gisaid.org/) database (Shu and McCauley, 2017). Samples used in this study were collected from 27 February 2021 to the end of 2021. The accession numbers and data of sample collections were recorded from the database. If the gender and age of the patients were provided, they would have also been recorded. Crystalized SARS-COV2 spike protein (PDB: 6VXX) was used to visualize the location of the mutations using PyMOL,

3. RESULTS AND DISCUSSION

3.1. Mutation in the spike protein

The mutation carrying by SARS-CoV-2 genome is a matter of concern if it occurs in the spike region of the virus because this could modify the pathophysiology, transmissibility and the virulence characteristics of the virus, and also the effect of the vaccine and therapeutic antibodies against the virus (Sabir, 2022). Nearly one-third of the spike protein sequence is associated with mutations. Variations of the spike protein SARS-COV2 is an RNA virus and mutations can naturally occur during viral replications, which can lead to variation (Wise, 2020). In this study, we have found 29 mutations among the genome sequences of KRG SARS-COV-2, the mutation sites are shown in Figure 1.

In this study, 34 locally sequenced SARS-COV-2 strains were obtained and analyzed in the S protein region. The protein sequences were

compared to the first sequenced SARS-COV-2 from Wuhan/China (Accession number: NC_045512.2) (Wu et al., 2020). The sequence accession numbers, the date of sample collection, the age and gender of the patients are shown in Table 1.

Table 1: The accession numbers of the viral genome, gender, and age of the patients who samples were taken in six difference months in 2021.

No.	Accession numbers	Collection date	Gender	Age	City
1	EPI_ISL_4574528	3/4/2021	Female	64	Sulaimani
2	EPI_ISL_4574537	3/4/2021	Male	37	Sulaimani
3	EPI_ISL_2153106	3/9/2021	unknown	unknown	Erbil
4	EPI_ISL_2629240	3/9/2021	unknown	unknown	Erbil
5	EPI_ISL_4574538	3/16/2021	Female	20	Sulaimani
6	EPI_ISL_4574544	3/27/2021	Male	41	Sulaimani
7	EPI_ISL_4574545	3/27/2021	Male	42	Sulaimani
8	EPI_ISL_4574546	3/27/2021	Female	41	Sulaimani
9	EPI_ISL_4574533	4/3/2021	Male	38	Sulaimani
10	EPI_ISL_4574532	4/4/2021	Male	65	Sulaimani
11	EPI_ISL_4574534	4/13/2021	Male	31	Sulaimani
12	EPI_ISL_4574536	4/14/2021	Male	31	Sulaimani
13	EPI_ISL_4574551	4/23/2021	Female	49	Sulaimani
14	EPI_ISL_4574535	4/25/2021	Female	45	Sulaimani
15	EPI_ISL_4574547	4/28/2021	Female	36	Sulaimani
16	EPI_ISL_4574548	4/28/2021	Male	57	Sulaimani
17	EPI_ISL_4574540	5/14/2021	Female	54	Sulaimani
18	EPI_ISL_4574529	5/16/2021	Female	38	Sulaimani
19	EPI_ISL_4574541	5/17/2021	Male	59	Sulaimani
20	EPI_ISL_4574542	5/17/2021	Male	59	Sulaimani
21	EPI_ISL_4574552	6/13/2021	Male	65	Sulaimani
22	EPI_ISL_4574553	7/6/2021	Female	58	Sulaimani
23	EPI_ISL_5804796	7/20/2021	Male	42	Duhok
24	EPI_ISL_5804797	7/20/2021	Male	40	Duhok
25	EPI_ISL_5804791	7/22/2021	Female	60	Duhok
26	EPI_ISL_5804792	7/22/2021	Male	60	Duhok
27	EPI_ISL_5804793	7/22/2021	Female	69	Duhok
28	EPI_ISL_5804794	7/22/2021	Female	15	Duhok
29	EPI_ISL_5804795	7/22/2021	Male	25	Duhok
30	EPI_ISL_5804798	7/25/2021	Female	51	Duhok
31	EPI_ISL_5804799	7/28/2021	Female	30	Duhok
32	EPI_ISL_5804800	7/28/2021	Female	39	Duhok
33	EPI_ISL_6583105	11/1/2021	Female	45	Sulaimani
34	EPI_ISL_7405941	11/11/2021	Male	45	Erbil

Mutations that are carried by SARS-CoV-2 genome, particularly in the S protein region, are a matter of concern since this could alter the virus' pathophysiology, transmissibility, as well as the virulence characteristics of the virus. It can also affect the efficacy of the vaccines and therapeutic antibodies against the virus (Sabir, 2022). Since SARS-COV-2 is a RNA virus, mutation can spontaneously occur during viral genome replication which can lead to variation (Wise, 2020).

3.2. Frequency of Mutation

Previously, we have analyzed 91 spike protein sequences of SARS-COV-2 from Iraq (Sabir, 2022), and uploaded into the GISAID database. In this study, 34 locally sequenced SARS-COV-2 strains collected from Kurdistan region of Iraq were analyzed and compared to the first sequenced SARS-COV-2 from Wuhan China (Accession number: NC_045512.2) (Wu et al., 2020). Among the studied strains, 29 mutations were found in the S protein region of SARS-CoV-2 strains. The majority (13 mutations, 44.8%) of the mutations were located in the NTD of the protein, 3 mutations (10.3%) were located in RBD, and the remaining 2 mutations (6.8%) were located at each of S1/S2 and HR1 domains (Figure 1C). Among all of them the most predominant mutation that was found in all the sequenced samples was D614G (100%), followed by P681H mutation which was found in 23 (64.7%) samples. N501Y, A570D, T716I, S982A, and D1118H were all found in 64.7% of the sequenced samples. The list of all mutations with their percentages are shown in Figure 1C.

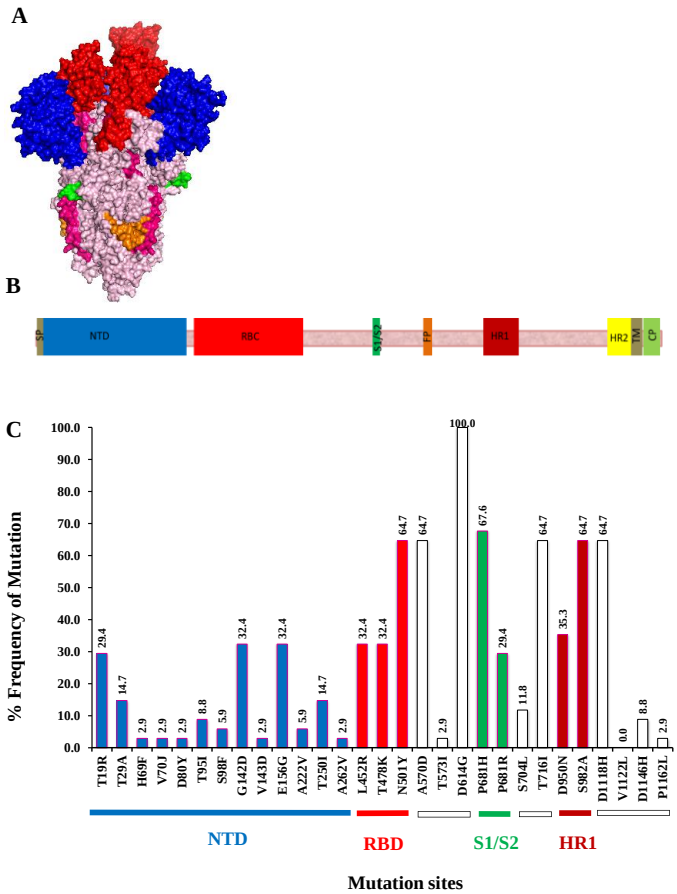

Figure 1: **Spike protein structure and %frequency of mutation.** **(A)** Trimer structure of SARS-COV2 protein (PDB: 6VXX). **(B)** Schematic representation of Spike domains. **(C)** Percentage of frequency of mutations and approximate locations of the mutations in the different S proteins domains. NTD: N-terminal domain; RBD: Ribosomal binding domain; S1/ S2 protease cleavage site; HR1: heptad repeat 1.

A variant that carries D614G mutation in the S protein region of the virus was first recorded in the early stage of the pandemic in the north of America, and then reported in many European countries (Shen et al., 2021). D614G has been previously reported in the sequence samples of KRG (Sidiq, 2021), and it is the only mutation that has been recorded in the S-proteins of all the variants (Guruprasad, 2020). This mutation increases the transmissibility of the virus in compared to the wild type (MN908947.1) (Shen et al., 2021, Plante et al., 2021b, Yurkovetskiy et al., 2020, Volz et al., 2021, Ugurel et al., 2020). In addition, D614G mutation can significantly increase the affinity of the spike protein to its receptor (ACE2) as well as increase immune escape and transmissibility (Singh et al., 2022b, Hu et al., 2022b, Arora et al., 2021, Li et al., 2020a).

The other abundant mutations which were found in the S protein was P681H mutation, which is located at S1/S2 domain (Figure 1A). This mutation, with D1118H, are found among 64.7% of the sequenced samples, and can increase the virus (Khateeb et al., 2021).

Among the other mutations, three of them were located in the ribosomal binding domain (RBD; 319–541) which were: L452R, T478K, and N501Y (Figure 1C). Mutations in the RBD region can affect the sensitivity of the neutralization action of humoral monoclonal or polyclonal antibodies (Wise, 2020). Variants that carry L452R mutation have shown to resist the monoclonal antibodies (mAbs) X593 and P2B-2F6 (Khateeb et al., 2021, Li et al., 2020b). T478K mutation can increase the virus's transmissibility and affinity for ACE2, as well as its ability to evade the immunological response of the host (Khateeb et al., 2021). N501Y mutation has been recorded among Alpha, Beta, or Gamma, but not in Delta and Kappa variants, this mutation, similar to T478K, increases the transmissibility of the virus (Khateeb et al., 2021), whereas N501T decreases the binding affinity of the S protein to the ACE2 receptors in human (Shang et al., 2020).

Thirteen mutations were found in the N-terminal domain (NTD) of the S protein which were: T19R, T29A, H69F, V70J, D80Y, T95I, S98F, G142D, V143D, E156G, A222V, T250I, A262V (Figure 1C). The domain of the protein has shown to play an important role in the virus entry into the cell through binding to the host cell receptors (Xia, 2021). Among the NTD mutations, 32.4% of the mutations were found at both G142D and E156 positions; followed by 32% in E156G; and 14.7% in T29A and T250I (Figure 1C). The other mutations were 8.8% carrying T95I, 5.9% carrying both S98F and A222V; and 2.9% mutation in H69F, A70J, D80Y, V143D, and A256V (Figure 1C).

Moreover, T19R, G142D, and E156G helps the virus escaping antibody response (Singh et al., 2022a, Hu et al., 2022a, Arora et al., 2021, Capozzi et al., 2021).The presence of E156G mutation along with L452K, D614G and P681R of the S protein of Delta was linked with the increased transmission and elevation in host cell attachment (Johnson et al., 2021).

In addition to those mutations, we also found seven deletions in the S protein of KRG strains. Deletion, different from substitutions, are particularly concerns since it could generate viruses with the whole different S protein amino acid sequence and structure resulting from different nucleotide reading frame (McCarthy et al., 2021). Among the studied isolates, all the

deletions were found at the N-terminal of the protein. The locations of deletions were: H69, V70, S71, Y144, Y145, F157 and R158. Previously, it has been reported that 90% of 1108 SARS-CoV-2 sequences have deletion(s) in the NTD of the S protein, and 97% of the deletion maintain its open reading frame (McCarthy et al., 2021). The most frequent deletion among the studied isolates was found at that location of ΔY144 with 61.8%, following by V70 and H69 with 58.8% and 55.9%, respectively (Figure 2). Deletion at the position of both ΔF157 and ΔR158 were accounted for 32.4% in the whole sequence samples, and 2.9% deletions were occurred at the positions of both S71 and Y145 (Figure 2).

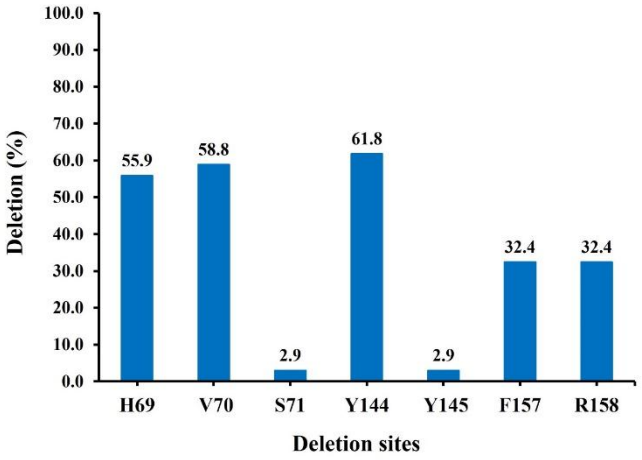

Figure 2: Percentage of deletions in the Spike proteins of the studied isolates from Kurdistan region of Iraq. Letters H, V, S, Y, F, and R represent the single letter amino acids of histidine, valine, serine, tyrosine, phenylalanine, and arginine, respectively. The numbers next to the letters, represent the position of the amino acid residue in the amino acid sequence of the S protein. The numbers on the Y axis show the percentage of the deletions.

Deletions in the position (69-70) was first reported in the mink-related strains from Denmark, known as cluster 5 variant (Assessment and Control, 2020). The strain is shown to be rapidly circulating among the mink farms, however, the transmissibility of the virus or the severity of the COVID-19 was not significantly different compared to the previously recorded variants (Assessment and Control, 2020).

It also been reported that ΔH69/V70 usually occurs with substitution of N501Y, N439K and Y453F in RBD (Kemp et al., 2021).
Additionally, the B.1.1.7 lineage requires ΔH69/V70 deletion for the effective cell entry, cell-cell fusion activity, as well as rapid syncytium formation (Meng et al., 2021). Moreover, Wang *et al* (2021) found that ΔY144 plays vital role in resistance to N-terminal domain directed monoclonal antibodies (Wang et al., 2021). Y145 residue in accordance with H146 was found to switch positions to accommodate the loop-bound state better, consequently salt-bridge interaction between residue H146 and D627 (Bangaru et al., 2020).

3.3. SARS-COV2 variants in Kurdistan

Among the studied sequences, alpha variants (VOC/B.1.1.7) were the dominant lineage during February to December of 2021, that was firstly detected in Mexico during December 2020 (Zárate et al., 2022) , and it was accounted for 65% of the studied SARS-COV2 strains (Figure 3). This variant was characterized by 43 - 90% which increased rate of transmission compared to the previously known variants (Davies et al., 2021). Additionally, the data analysis of 2341 patients in the United Kingdom affected with alpha variants of SARS-COV-2 between November 2020 to January 2021 revealed that this variant is not causing a more sever COVID-19 among men. However, women infected with alpha variants have a greater risk to be admitted to ICU or increase the mortality rate (Stirrup et al., 2021). During early 2021, a study conducted among 58 total genome sequence samples of SARS-COV-2 strains from Iraq, and it was shown that only 6% of the samples were belonging to alpha variant (Sabir, 2022). This increase in the number of B.1.1.7 variant could perhaps support the higher transmissibility rate of this variant (Frampton et al., 2021). The second most dominant lineage among SARS-COV-2 sequences was Delta variant (B.1.617.2 and AY.33) were compromised 22% of the sequenced strains (AY.33 is a sublineage of the Delta variants B.1.617.2 (Doerksen et al., 2021)). Previously, the delta variant was recorded among SARS-COV-2 sequence samples of KRG (Rashid and Salih, 2022), Morocco, England, Brazil (Hemlali et al., 2022, Smallman-Raynor and Cliff, 2022, Lima et al., 2021), and other countries (Planas et al., 2021b). The major concern about the delta variants is the ability to escape from neutralizing monoclonal and polyclonal antibodies that were produced from previous infection of the virus or by vaccination (Planas et al., 2021a). The other variant (2%) was not classified, but it appears to be closely related to alpha variant based on the mutations it carried. In conclusion, our data emphasizes the increase of genome diversities of the SARS-COV2 in

Kurdistan. Twenty-nine substitutions were found in the S protein of the virus, with seven deletions in the NTD. Alpha variant-appeared as a dominant strain during the period of this study, followed by delta variant. The result of this study can be used to understand the diversity of the virus, as well as to design stronger therapeutic agents to control the spread of the virus.

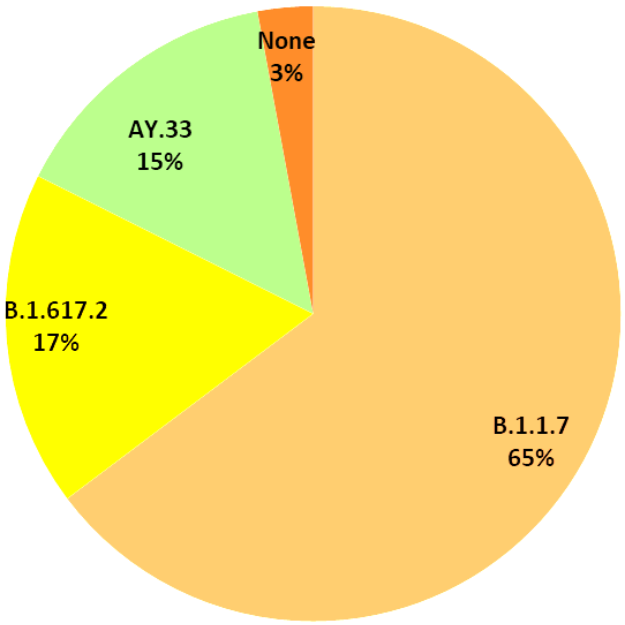

Figure 3. Pie chart of Percentage of the different variants of the SARS-COV2. Alpha variant B.1.1.7 were the dominant lineage (65%) appears in Kurdistan between February 2021 and December 2021.

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