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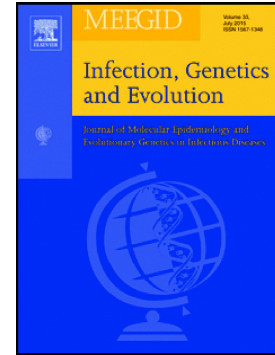
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GENOME SEQUENCING OF SARS-CoV-2 REVEALS THE PREVALENCE OF VARIANT B 1.1.7 IN EGYPT

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Abstract

Recently, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) resulted in Coronavirus Disease 2019 (COVID-19) outbreak. A new SARS-CoV-2 strain is expected to emerge in late 2020, including B.1.1.7. The high transmission rate of SARS-CoV-2 B.1.1.7 has raised public health concerns in several countries. Hence, in this study, we assessed the sequencing of SARS-COV2 to reveals the prevalence of the SARS-CoV-2 Alpha variant (B 1.1.7) in Egypt. We found that the viral transmission of the alpha variant is expanding. Moreover, based on hospitalizations and case fatality rates, there is a potential for increasing severity. There was no effect on susceptibility to Emergency Use Authorization monoclonal antibody treatments. However, there was minimal impact on neutralization by convalescent and post-vaccination sera. Samples have been clustered into the 20D sub clade for the majority of them. The eight samples shown in our study are considered the first recorded samples with the Alpha variant in Egypt. Therefore, The Egyptian government, represented by the Ministry of Health, must take all measures to examine the compatibility of the currently used vaccines with this new

strain and the feasibility of the treatment protocol presently used with such strains developed in the Arab Republic of Egypt.

Keywords: SARS-CoV-2; COVID-19; Alpha variant; B 1.1.7; Egypt.

Abbreviations

BLAST	Basic Local Alignment Search Tool.
BWA	Burrows-Wheeler Aligner software.
COVID-19	Coronavirus Disease 2019.
Ct	Cycle thresholds.
EUS	Emergency Use Authorization.
NSP1-16	Non-structural proteins 1 1-6
NTD	N-terminal domain.
ORF	Open reading frame.
RBM	Receptor binding motif
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
VOC	Variant of Concern.
VOI	Variant of interest
WHO	World health organization

1. Introduction

Recently, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) 2 resulted in Coronavirus Disease 2019 (COVID-19) outbreak. There have been more than 260 million cases of COVID-19 pandemic since the outbreak, with the mortality impact at roughly 3.9 million (Jamwal et al., 2021). Controlling COVID-19 can be enhanced by vaccination, separating from others socially, general cleanliness, and a large number of diagnostic tests (Voysey et al., 2021; Ferretti et al., 2020).

The genome of SARS-CoV-2 has been estimated to be 29.9kb (Lu et al., 2020). It is composed of a nucleocapsid protein (N) and an envelope. Envelopes are related with three types of structural proteins: spike protein (S), membrane protein (M),

and envelope protein (E). N, S, M, and E are four structural proteins in the SARS-CoV-2 genome, although there are total of 16 non-structural proteins (NSP1-16). Also known as NSP 12, RdRp which plays a key part in the proliferation of (Naqvi et al., 2020).

SARS-CoV-2 was found to be transmissible in roughly 55% of cases, with approximately 30% of people remain asymptomatic (Li et al., 2020). Spike protein receptor binding domain alterations appear to be responsible for the increased transmissibility of novel variations (Cherian et al., 2021; Funk et al., 2021). A significant number of people must be tested in order to detect SARS-CoV-2 infections and, more critically, to identify asymptomatic carriers who may unknowingly infect a huge number of people (Ju et al., 2020). A new SARS-CoV-2 strain is expected to emerge in late 2020, and world health organization (WHO) has classified it as a Variant of Concern or VOI (VOCs). As of now, VOCs include Alpha, Beta, Gamma, Delta, and Epsilon, while VOIs include Epsilon, Zeta, Eta, Theta and Kappa and B.1.427/B.1.429 (Epsilon) (WHO, 2021). There has been viral evolution since the discovery of SARS-CoV-2, however these VOCs have increased transmissibility and severity as well as altered antigenicity, which could have consequences for acquired immunity or efficiency of current vaccinations (Collier et al., 2021; Challen etal., 2021; Madhi et al., 2021; Wang et al., 2021).

The high transmission rate of SARS-CoV-2 B.1.1.7 in the United Kingdom (UK) has raised public health concerns. In addition to the D614G mutation, this variation features eight additional non-synonymous variants in spike: H69-V70, Y144, N501Y, A570D, P681H, T716I, and D1118H. At least three B.1.1.7 spike mutations should be of concern, including the two amino acid deletion in the N-terminal domain (NTD), as well as the N501Y mutation in the receptor binding motif (RBM) and P681H mutation at the Furin cleavage site (P681H) (England et

al., 2020; Rambut et al., 2020; Funk et al., 2021; Voloch et al., 2021). Interestingly, each of these three mutations can be found in other variants of interest, as well. Mathematical modeling data and epidemiological evidence suggest that this variant is more transmissible than the SARS-CoV-2 variants that were in circulation prior to its introduction; evidence of an increased mortality rate has also been reported, despite initial reports that it was not more pathogenic (England , 2021; Volz et al., 2020).

The first case detected in Egypt with the Alpha variant was recorded in March 2021 (GISAID, 2021). Hence, in this study, we assessed the sequencing of SARS-CoV-2 to reveal the prevalence of SARS-CoV-2 variant B 1.1.7 in Egypt.

2. Material and Methods

2.1. Sampling

Oropharyngeal swabs (n=1235) were taken from patients with suspected COVID-19 in fever hospital of army forces in Egypt. The samples were transported under biosafety requirements (transport bag 95Kpa) in viral transport medium Kit (Cat. no. A48498) at (2-4) C° to the biological prevention department at central chemical laboratories of Almaza in Cairo, Egypt.

2.2. Extraction of RNA and RT-PCR detection

Viral RNA was extracted using QIAamp Viral RNA Mini Kit (Cat. no. 52906). Then, samples were tested for three SARS-CoV-2 genes, i.e., ORF1ab and N protein, using the VIASURE kit (Cat. no.VS-NCO213H) according to the manufacturers' instructions. For each sample, we added 5 µL of extracted RNA to 15 µL of dehydrated master mix. Another tube for positive control was prepared by adding 5 µL of Positive Control to 15 µL of dehydrated master mix. The last tube was prepared for negative control by adding 5µL of Negative Control to 15µL of

dehydrated master mix. Then, qPCR was performed on an Ariamx thermal cycler (Agilent, Germany), using the following conditions: 45 ◦C for 15 minutes, 95 ◦C for 2 minutes, 40 cycles of 95 ◦C for 10 seconds, and 60 ◦C for 50 seconds. Cycle thresholds (Ct) were analyzed using auto-analysis settings, with the threshold lines falling within the exponential phase of the fluorescence curves and above any background signal. We used HEX dye as a fluorescent label for each reaction to determine the Ct value for the internal positive control.

2.3. Library Preparation and Next-Generation Sequencing

According to manufacturers' instructions, library preparation was done using the AviSeq COV19 NGS Library Prep kit (Ref. AVG202096). Next-generation sequencing was done by using the Illumina machine (Iseq-100 instrument). RNA was measured on Qubit 2.0 Fluorometer by using Qubit™ RNA HS 100 Assay Kit (Cat.No.Q32852). Then, cDNA was prepared by adding RT Primer Mix DP (BATCH No.020402C). In addition, a multiplex PCR reaction was done to amplify the entire SARS-CoV-2 genome. Digestion was done to remove any nonspecific PCR products. After library preparation, the quality of the library and the amplification of viral RNA were visualized via gel electrophoresis and measured using DNA HS 100 Assay Kit (Cat.No.Q32852).

2.4. Data Analysis

FASTA files were generated using Abiomix software. Then Nextclade Tool (version 1.5.2) was used for viral genome alignment, mutation calling, quality checks, phylogenetic placement, and clade assignment. Genome sequencing alignment was done by using Burrows-Wheeler Aligner (BWA) software. Basic Local Alignment Search Tool (BLAST) was used to find similarities between nucleotide and amino acid sequences.

3. Results

3.1. Molecular identification

We used RT-PCR for molecular identification. From April 2020 to July 2021, we collected 1235 COVID-19 positive samples, but only a few hundred models were chosen for sequencing (Cq from 14 to 20).

3.1.1. Sequence Data Analysis and Phylogenetic Analysis

Whole Genome sequencing alignment using BWA software resulted in 99 successful high-quality genomes. These sequences were covered the entire genome of reference SARS-CoV-2 (RefSeq: NC_045512.2), Basic Local Alignment Search Tool (BLAST) was used to find similarities between nucleotide and amino acid sequences. Hence, nucleotide identity ranged from 99.8% to 100%, and amino acid identity ranged from 99.7% to 100%

The genetic mutation using Nextclade Tool showed the viral genome, including ORF1a, ORF1b, ORF3a, ORF6, ORF7a, ORF7b, ORF8, S, E, M, and N. [Supplementary file; Figure A. 1-4]. Phylogenetic tree analysis showed six clades in Egypt, as shown in Figure 1. These clades were shown in Rectangular and Radial Phylogeny as shown in Fig.1A and Fig .1B respectively. The most common clade was 20D with 61.6%, and the Alpha variant was 8%.

There were about seven sequences clustered as 19A with no mutation in S-gene. However, a single mutation with high quality in ten sequences was assigned as 20A. The clad 20A with D614G amino acids mutations in S-gene was shown in Table 4. Moreover, 11sequences were clustered as 19B with R102S, A292V, N501T, and H655Y amino acids mutations in S-gene. However, two sequences were clustered as 20B with N501T, D614G, and Q677H amino acids mutations in S-gene, as shown in Table 3.

About 61 sequences were clustered as 20D with S12F, F59I, W64R, D138Y, W152R, A222V, R346S, L452R, E484K, N501T, T572I, Q613H, D614G, Q677H, D796Y, A871S, A899S, D1163Y, M1237I, 1243S, P1263Q, V1264M, and V1268D amino acids mutations in S-gene as shown in Table 2.

Finally, eight sequences were clustered as 20I (Alpha, V1) with N501T, D614G, and Q677H amino acid mutations in S-gene, as shown in Table 1. These eight last sequences were considered the first UK strain detected in Egypt by April 2021 (GISAID, 2021).

4. **Discussion**

According to our study, we found that the viral transmission of the alpha variant is expanding. Moreover, based on hospitalizations and case fatality rates, there is a potential for increasing severity. There was no effect on susceptibility to Emergency Use Authorization (EUA) monoclonal antibody treatments (FDA, 2021). However, there was minimal impact on neutralization by convalescent and post-vaccination sera. Samples have been clustered into the 20D sub clade for the majority of them.

New SARS-CoV-2 variations have emerged in recent months, and their frequency in local populations and even worldwide has shifted fast and repeatedly (Hodcroft et al., 2021; Korber et al., 2021). A number of spike mutations have been found in newer variations, which raises the possibility of immunological escape (Naveca et al., 2021; Tegally et al., 2021).

From the First discovery in England in September 2020, the B.1.1.7 variety has already spread to over 70 countries (Hodcroft et al., 2021). After a mutation was found in the spike protein of a fast-spreading B.1.1.7 strain, it became 56% more transmissible than the earlier SARS-CoV2 strain (Davies et al., 2021a). In South

Africa, a new fast-spreading form of SARS-CoV-2 has been identified as B.1.351 virus. SARS-CoV-2 strains of lineage also were recently found in India: B.1.617.1 and B.1.617.2 (Funk et al., 2021). As a result of this new variation, existing control methods are likely to be less successful, and governments may need to take more aggressive efforts to attain the same level of control (Frank, 1996).

Vaccination was affected by the increase in VOC transmission. A rapid and efficient vaccination delivery system is therefore essential for reducing the effects of the pandemic as soon as possible. VOC will also increase herd immunity threshold, which means that future SARS-CoV-2 burden will be greater and more vaccination coverage will be necessary to obtain herd immunity (Davies et al., 2021a).

A previous study reported that there was no significant difference in the neutralization titers between the B.1.1.7 variant and the combination of three Spike mutations. In the B.1.1.7 variant compared to the wild type, the maximum fold change was approximately 6 and the median fold change was approximately 3.85. (IQR 2.68-5.28) (Collier et al., 2021). Another study included 2,172 nasal/nasopharyngeal swab samples from 44 counties in California came to this conclusion: B.1.1.7, B.1.351, and P.1 all carry the N501Y mutation. Assays of neutralizing antibody titers from convalescent patients and vaccine recipients showed declines of 4.0 to 6.7-fold and 2.0-fold, respectively (Deng et al., 2021).

According to our study, the genetic mutation showed the viral genome, including ORF1a, ORF1b, ORF3a, ORF6, ORF7a, ORF7b, ORF8, S, E, M, and N. Phylogenetic tree analysis showed six clades in Egypt, as shown in. The most common clade was 20D with 61.6%, and the Alpha variant was 8%. There were about seven sequences clustered as 19A with no mutation in S-gene. However, a single mutation with high quality in ten sequences was assigned as 20A. The clad

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Finally, eight sequences were clustered as 20I (Alpha, V1) with N501T, D614G, and Q677H amino acid mutations in S-gene. These eight last sequences are considered the first UK strain detected in Egypt by April 2021

When it comes to B.1.1.7/SGTF, B.1.351 and P.1, there is a higher risk of hospitalization and ICU admission in age groups 60 years in this study. As seen in Germany, B.1.1.7 dominance was associated with an increase in hospitalizations among those under 60 years of age (Funk et al., 2021; Lorbach, 2021). A greater infection rate in younger, school-age age groups was documented in the UK before (Davies et al., 2021b; Gravagnuolo et al., 2021). Denmark has also documented higher hospitalization rates for B.1.1.7 patients (Bager et al., 2021).

When exposed to convalescent sera, variation B.1.1.7 shows only a small reduction in neutralization susceptibility (1.5-fold average) (Muik et al., 2021). When persons receive both doses of both vaccines, neutralization titers have been reported to increase around 10-fold after the second dose, suggesting that a 2-fold decline in neutralization will have no influence on vaccine efficacy (Anderson et al., 2021; Walsh et al., 2021). Recently, it was discovered that the Novavax vaccination was 95,6% effective against the common variant and 85,6% against B.1.1.7 (Callaway et al., 2021). In areas where the B.1.1.7 variety is prevalent,

receiving the second dose as soon as possible is recommended for optimum efficacy (shen et al., 2021).

A cautious strategy should be to experiment with variant sequences and conduct post-licensure investigations to detect changes in efficacy between the preexisting and new variants, according to vaccine developers if a vaccine's strain formulation is altered without any other modifications in its content. Licensing authorities may need to clarify the abbreviated marketing paths for the vaccine (Davies et al., 2021a).

Some of our findings have limitations. We can only assess how well the theories suggested are supported by the data. Still, there may be other viable explanations for the recurrence of cases that we did not investigate. Even though more experimental work could shed light on the biological mechanisms behind our observations, given our projections of a rapid future rise in incidence from the Alpha variant without additional control measures, we must consider what new approaches may be required to sufficiently reduce the ongoing transmission of SARS-CoV-2

5. Conclusion

Viral transmission of the alpha variant is expanding. Moreover, based on hospitalizations and case fatality rates, there is a potential for increasing severity. There was no effect on susceptibility to Emergency Use Authorization monoclonal antibody treatments. However, there was minimal impact on neutralization by convalescent and post-vaccination sera. Samples have been clustered into the 20D sub clade for the majority of them. The eight samples shown in our study are considered the first recorded samples with Alpha variant in Egypt. Therefore, The Egyptian government, represented by the Ministry of Health, must take all

measures to examine the compatibility of the currently used vaccines with this new strain and the feasibility of the treatment protocol presently used with such strains developed in the Arab Republic of Egypt.

6. Funding

Sample CRediT author statement

Mohamed Seadawy: Methodology, Data Analysis and Supervision.

Ahmed Gad: Methodology, Writing Original draft preparation.

Sabah Abo-Elmaaty: Writing and Investigation

Mervat Hassan: Reviewing and Editing

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests

Supplementary data

Supplementary material

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Table 1: Sequences of Alpha variant.

Seq-Name	QC						Clade	Ns Mut	S-gene Mut	non-ACGTN	Ns (missing)	Gaps	Ins.
	N	M	P	C	F	S							
HCoV-19/Egypt/ARMY-239/2021 EPI_ISL_1936108	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30	N501Y-A570D-D614G-P681H-T716I-D1118H	0	0	0	0
HCoV-19/Egypt/ARMY-206/2021 EPI_ISL_1936109	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
HCoV-19/Egypt/ARMY-249/2021. EPI_ISL_1936110	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
HCoV-19/Egypt/ARMY-259/2021 EPI_ISL_1936111	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave354/2021 EPI_ISL_1936284	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave355/2021 EPI_ISL_1936285	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave356/2021 EPI_ISL_1936286	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	30		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave344/2021 EPI_ISL_1936276	✓	✓	✓	✓	✓	✓	20I(Alpha,V1)	34		0	0	0	0

N: Missing Data **M:** Mixed Sites **P:** Private Mutations **C:** Mutation Clusters **F:** Frame shifts **S:** Stop codons

Table 2: Sequences of 20D Clade

Seq-Name	QC						Clade	Ns Mut.	S-gene Mut.	non-ACGTN	Ns (missing)	Gaps	Ins.
	N	M	P	C	F	S							
HCoV-19/Egypt/ARMY-231/2021 EPI_ISL_1936134	✓	✓	✓	✓	✓	✓	20D	27	S12F-F59I-W64R-D138Y-W152R-A222V-R346S-L452R-E484K-N501T-T572I-Q613H-D614G-Q677H-D796Y-A871S-A899S-D1163Y-M1237I-C1243S-P1263Q-V1264M-V1268D	0	0	0	0
HCoV-19/Egypt/ARMY-203/2021 EPI_ISL_1936136	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0
HCoV-19/Egypt/ARMY-204/2021 EPI_ISL_1936137	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0
HCoV-19/Egypt/ARMY-205/2021 EPI_ISL_1936138	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0
HCoV-19/Egypt/ARMY-217/2021 EPI_ISL_1936144	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave361/2021 EPI_ISL_1936291	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0
hCoV-19/Egypt/Egypt-Army-	✓	✓	✓	✓	✓	✓	20D	27		0	0	0	0

Thirdwave362/2021 EPI_ISL_1936292												
hCoV-19/Egypt/Egypt-Army-Thirdwave363/2021 EPI_ISL_1936293	✓	✓	✓	✓	✓	✓	20D	27	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave364/2021 EPI_ISL_1936294	✓	✓	✓	✓	✓	✓	20D	27	0	0	0	0
HCoV-19/Egypt/ARMY-267/2021 EPI_ISL_1936127	✓	✓	✓	✓	✓	✓	20D	28	0	0	0	0
HCoV-19/Egypt/ARMY-250/2021 EPI_ISL_1936112	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-251/2021 EPI_ISL_1936113	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-290/2021 EPI_ISL_1936114	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-291/2021 EPI_ISL_1936115	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-252/2021 EPI_ISL_1936116	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-255/2021 EPI_ISL_1936117	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-256/2021 EPI_ISL_1936118	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-257/2021 EPI_ISL_1936119	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-258/2021 EPI_ISL_1936120	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-260/2021 EPI_ISL_1936121	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-261/2021 EPI_ISL_1936122	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-262/2021 EPI_ISL_1936123	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-264/2021 EPI_ISL_1936124	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-19/Egypt/ARMY-265/2021 EPI_ISL_1936125	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
HCoV-	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0

19/Egypt/ARMY-266/2021 EPI_ISL_1936126												
hCoV-19/Egypt/Egypt-Army-Thirdwave340/2021 EPI_ISL_1936273	✓	✓	✓	✓	✓	✓	20D	29	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave342/2021 EPI_ISL_1936274	✓	✓	✗	✓	✓	✓	20D	29	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave320/2021 EPI_ISL_1936259	✓	✓	✗	✗	✓	✓	20D	30	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave321/2021 EPI_ISL_1936260	✓	✓	✗	✗	✓	✓	20D	30	0	0	0	0
HCoV-19/Egypt/ARMY-208/2021 EPI_ISL_1936102	✓	✓	✓	✓	✓	✓	20D	31	0	0	0	0
HCoV-19/Egypt/ARMY-213/2021 EPI_ISL_1936104	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
HCoV-19/Egypt/ARMY-207/2021 EPI_ISL_1936139	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
HCoV-19/Egypt/ARMY-211/2021 EPI_ISL_1936140	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
HCoV-19/Egypt/ARMY-218/2021 EPI_ISL_1936145	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave305/2021 EPI_ISL_1936244	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave306/2021 EPI_ISL_1936245	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave307/2021 EPI_ISL_1936246	✓	✓	✓	✓	✓	✓	20D	32	0	0	0	0
HCoV-19/Egypt/ARMY-210/2021 EPI_ISL_1936103	✓	✓	✗	✓	✓	✓	20D	34	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave315/2021 EPI_ISL_1936254	✓	✓	✓	✓	✓	✓	20D	34	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave316/2021 EPI_ISL_1936255	✓	✓	✓	✓	✓	✓	20D	34	0	0	0	0
hCoV-19/Egypt/Egypt-	✓	✓	✓	✓	✓	✓	20D	34	0	0	0	0

Army-Thirdwave317/2021 EPI_ISL_1936256												
hCoV-19/Egypt/Egypt-Army-Thirdwave343/2021 EPI_ISL_1936275	✓	✓	✓	✓	✓	✓	20D	36	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave300/2021 EPI_ISL_1936240	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave301/2021 EPI_ISL_1936241	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave303/2021 EPI_ISL_1936242	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave304/2021 EPI_ISL_1936243	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave308/2021 EPI_ISL_1936247	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave309/2021 EPI_ISL_1936248	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
HCoV-19/Egypt/Egypt-Army-Thirdwave327/2021 EPI_ISL_1936264	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave328/2021 EPI_ISL_1936265	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave329/2021 EPI_ISL_1936266	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave330/2021 EPI_ISL_1936267	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave331/2021 EPI_ISL_1936268	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave332/2021 EPI_ISL_1936269	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave333/2021 EPI_ISL_1936270	✓	✓	✓	✓	✓	✓	20D	37	0	1	0	0

Seq-Name	N	M	P	C	F	S	Clade	Ns Mut.	S-gene Mut.	non-ACGTN	Ns (missing)	Gaps	Ins.
hCoV-19/Egypt/Egypt-Army-Thirdwave334/2021 EPI_ISL_1936271	✓	✓	✓	✓	✓	✓	20D	37		0	1	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave335/2021 EPI_ISL_1936272	✓	✓	✓	✓	✓	✓	20D	37		0	1	0	0
HCoV-19/Egypt/ARMY-297/2021 EPI_ISL_1936135	✓	✓	✓	✘	✓	✓	20D	38		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave324/2021 EPI_ISL_1936261	✓	✓	✓	✓	✓	✓	20D	39		0	3	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave325/2021 EPI_ISL_1936262	✓	✓	✓	✓	✓	✓	20D	39		0	3	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave326/2021 EPI_ISL_1936263	✓	✓	✓	✓	✓	✓	20D	39		0	3	0	0

N: Missing Data   **M:** Mixed Sites   **P:** Private Mutations   **C:** Mutation Clusters   **F:** Frame shifts   **S:** Stop codons

Table 3: Sequences of 20B and 19B Clades

Seq-Name	QC						Clade	Ns Mut.	S-gene Mut.	non-ACGTN	Ns (missing)	Gaps	Ins.
	N	M	P	C	F	S							
hCoV-19/Egypt/Egypt-Army-Thirdwave313/2021 EPI_ISL_1936252	✓	✓	✘	✓	✓	✓	20B	23	N501T-D614G-Q677H	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave314/2021 EPI_ISL_1936253	✓	✓	✘	✓	✓	✓	20B	23		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave318/2021 EPI_ISL_1936257	✓	✓	✓	✓	✓	✓	19B	25		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave319/2021 EPI_ISL_1936258	✓	✓	✓	✓	✓	✓	19B	25		0	0	0	0
HCoV-19/Egypt/ARMY-273/2021 EPI_ISL_1936128	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
HCoV-19/Egypt/ARMY-274/2021 EPI_ISL_1936129	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
HCoV-19/Egypt/ARMY-275/2021 EPI_ISL_1936130	✓	✓	✓	✓	✓	✓	19B	26	R102S-A292V-N501T-H655Y	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave347/2021 EPI_ISL_1936277	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave348/2021 EPI_ISL_19362778	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave349/2021 EPI_ISL_1936279	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave310/2021 EPI_ISL_1936249	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave311/2021 EPI_ISL_1936250	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0

Seq-Name	N	M	P	C	F	S	Clade	Ns Mut.	S-gene Mut.	non-ACGTN	Ns (missing)	Gaps	Ins.
hCoV-19/Egypt/Egypt-Army-Thirdwave312/2021 EPI_ISL_1936251	✓	✓	✓	✓	✓	✓	19B	26		0	0	0	0

N: Missing Data   **M:** Mixed Sites   **P:** Private Mutations   **C:** Mutation Clusters   **F:** Frame shifts   **S:** Stop codons

Table 4: Sequences of 20A and 19A Clades.

| Seq-Name | QC | | | | | | Clade | Ns Mut. | S-gene Mut. | non-ACGTN | Ns (missing) | Gaps | Ins. |
	N	M	P	C	F	S							
hCoV-19/Egypt/ARMY-219/2021 EPI_ISL_1936105	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/ARMY-241/2021 EPI_ISL_1936106	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/ARMY-228/2021 EPI_ISL_1936107	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave357/2021 EPI_ISL_1936287	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave358/2021 EPI_ISL_1936288	✓	✓	✓	✓	✓	✓	20A	19	D614G	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave359/2021 EPI_ISL_1936289	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave360/2021 EPI_ISL_1936290	✓	✓	✓	✓	✓	✓	20A	19		0	0	0	0
hCoV-19/Egypt/ARMY-212/2021 EPI_ISL_1936141	✓	✓	✓	✓	✓	✓	20A	21		0	0	0	0
hCoV-19/Egypt/ARMY-215/2021 EPI_ISL_1936142	✓	✓	✓	✓	✓	✓	20A	21		0	0	0	0
hCoV-19/Egypt/ARMY-216/2021 EPI_ISL_1936143	✓	✓	✓	✓	✓	✓	20A	21		0	0	0	0
HCoV-19/Egypt/ARMY-280/2021 EPI_ISL_1936131	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0
HCoV-19/Egypt/ARMY-281/2021 EPI_ISL_1936132	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0
HCoV-19/Egypt/ARMY-282/2021 EPI_ISL_1936133	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave350/2021 EPI_ISL_1936280	✓	✓	✓	✓	✓	✓	19A	0	–	0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave351/2021 EPI_ISL_1936281	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave352/2021 EPI_ISL_1936282	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0
hCoV-19/Egypt/Egypt-Army-Thirdwave353/2021 EPI_ISL_1936283	✓	✓	✓	✓	✓	✓	19A	0		0	0	0	0

N: Missing Data   **M:** Mixed Sites   **P:** Private Mutations   **C:** Mutation Clusters   **F:** Frame shifts   **S:** Stop codons
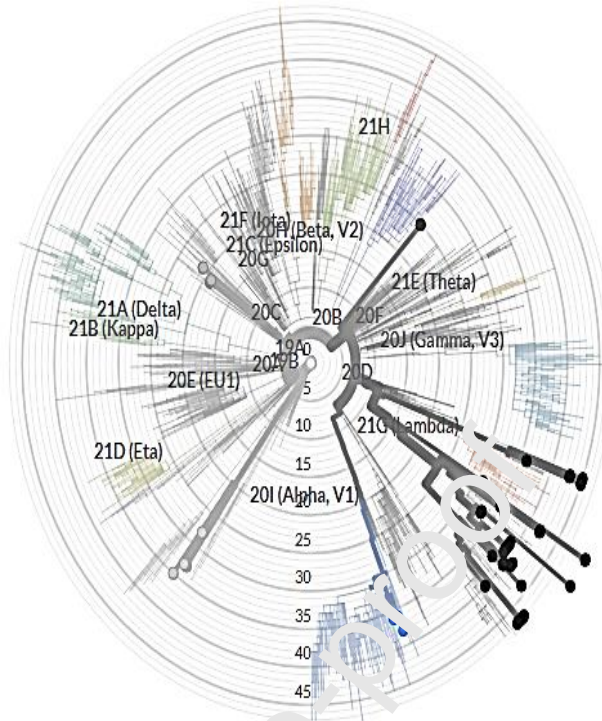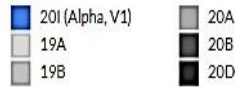
Fig. 1: A and B Rectangular and Radial Phylogeny respectively that showing the distribution of our 99 Sequences according to different clades. Using open access server (https://clades.nextstrain.org/tree).

Fig. 1A
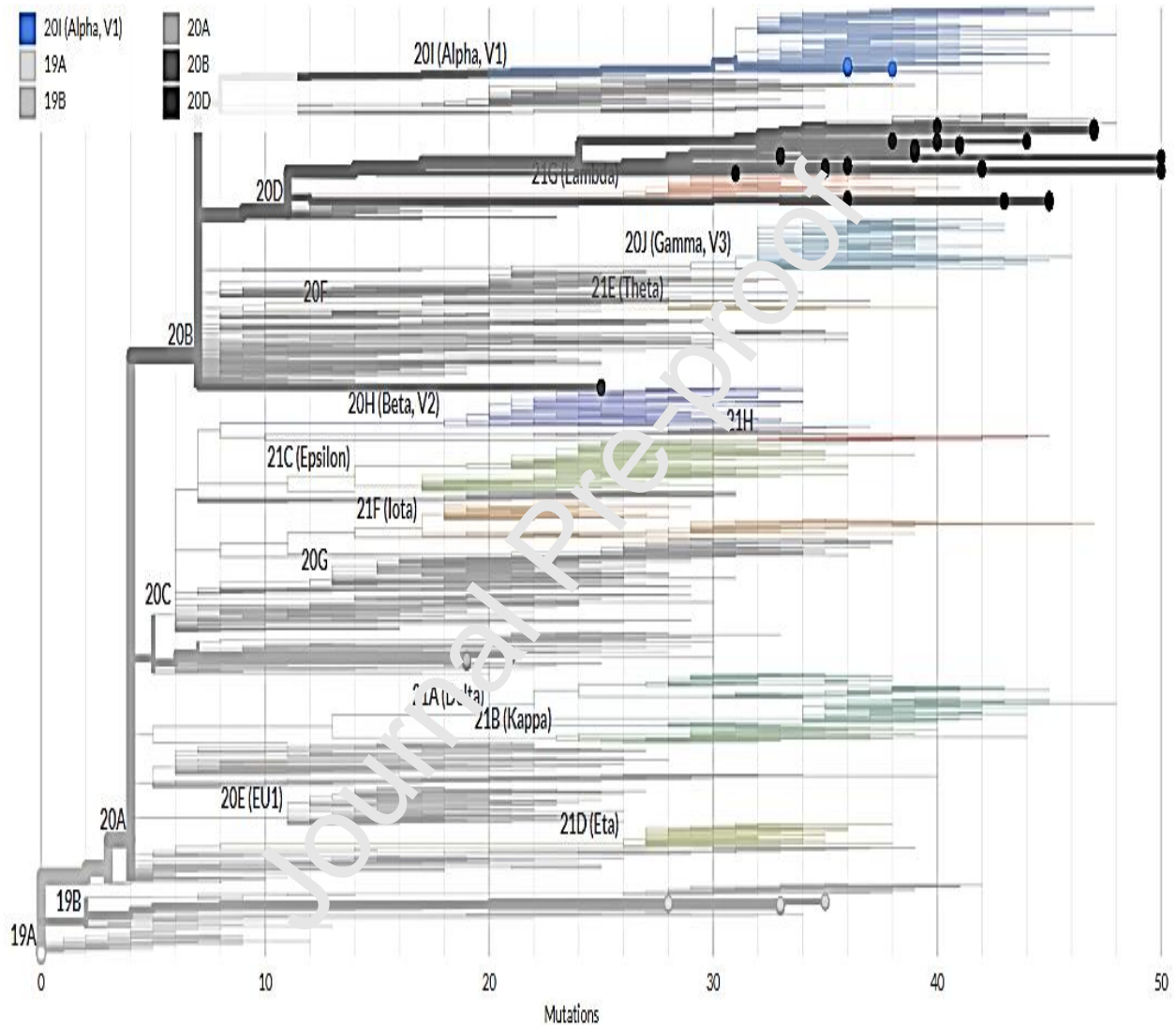
Fig. 1B

Highlights

- Using direct swabs in sequencing to avoid any SNPs resulting from viral culturing.
- The eight samples shown in our study are considered the first recorded samples with the Alpha SARS-CoV2 variant in Egypt.
- Our study recommend that ,The Egyptian government, represented by the Ministry of Health, must take all measures to examine the compatibility of the currently used vaccines with this new strain and the feasibility of the treatment protocol presently used with such strains developed in the Arab Republic of Egypt.