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Original article

Whole genome sequencing analysis of SARS-CoV-2 omicron variant isolated from Egypt

Hanaa H. A. Gomaa,[1] Mohamed G. Seadawy[2] , Mohamed Abdel-Razik[1], Ahmed F. Gad[2*], Mervat G. Hassan[3]

1- Botany and Microbiology Department, Faculty of Science, Suez Canal University, Egypt.
2- Biological Prevention Department, Egypt Army, Egypt
3- Botany and Microbiology Department, Faculty of Science, Benha University, Egypt

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ABSTRACT

Background: The omicron variants have been classified as variants of concern by the world health organization due to their initial emergence with a notable mutations that affect on the transmissibility, immune escape and virulence of the SARS-CoV-2 virus. We conducted a cross-sectional analysis study to detect the most common strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Egypt during the 4th wave of the COVID-19 pandemic and used the next genaration sequencing to characterize the SARS CoV-2 omicron variant genome and find the significant mutation influencing the virus's virulence, immune evasion, and transmissibility. **Material and methods**: This study started during the 4th wave of the COVID-19 pandemic by collecting nasopharyngeal swabs (n = 39) from individuals having COVID-19 symptoms. SARS-CoV-2 was molecularly identified using reverse transcription PCR (RT-PCR). For every sample, whole genome sequencing (WGS) was carried out using next generation sequencing Illumina Iseq100 system. Clade assignment, Pangolin lineages, phylogenetic placement, and mutation calling were all carried out using Nextclade tool V3.4.0, Coronavirus Typing Tool V 1.25 and CoVsurver mutation application. **Result:** Sequencing data analysis proved that all the involved samples in the study belong to the Omicron variant with different clade and Pangolin lineage, the most common strain in Egypt during the 4th wave of the COVID-19 pandemic was Omicron clade 22B and BA.5.2 Pango lineage. Mutation analysis showed that the *S* gene of the omicron variant accumulated with significant mutations that affect viral immune escape. **Conclusion:** The omicron variant in Egypt during the 4th wave of the pandemic showed a high ability to escape from the immune system because of newly mutation on the antibodies recognized sites of the *S* gene, so it is very important to perform continuous monitoring for omicron genome mutations using next-generation sequencing to help the scientific community in the development of appropriate vaccine for this variant.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the family coronaviridae, it is responsible for the coronavirus disease 2019 (COVID-19) pandemic [1,2]. It possesses a genome made of single-strand positive-sense RNA that is

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* *Corresponding author:* Ahmed F. Gad
E-mail address: *gad.a.f.science@gmail.com*

roughly 29.9 kb in length. This genome encodes many proteins, one of which is the spike (S) structural protein [3]. The S protein, which consists of 1273 amino acids, binds the virus to the host cell receptor using its receptor binding domain (RBD), specifically the amino acid residues 319 to 529 [4]. The viral genome undergoes genetic changes that are linked to its ability to spread, evade neutralizing antibodies, and cause disease [5-7]. Throughout the pandemic, the SARS-CoV-2 genome has undergone changes [8], resulting in the emergence of several variants that exhibit distinct biological characteristics which are found in different geographical regions. Multiple nomenclature systems are presently employed to monitor the genetic lineages of SARS-CoV-2 on both local and global scales. The mentioned entities include WHO labels, Global Initiative on Sharing All Influenza Data (GISAID), Next Strains, and Pango lineages [9]. Five variations have been designated as variants of concern (VOCs) [10]: alpha (B.1.1.7) [11, 12], beta (B.1.351) [13], gamma (P.1) [14], delta (B.1.617.2) [15 , 16], and omicron (B.1.1.529) [17, 18]. Currently, the only variations that are spreading globally are the omicron variants, which may be categorized into many clades. the variant of concern Omicron has the highest mutation rate, with 50 remarkable mutations detected throughout the genome (32 of which are in the spike protein) [19, 20] that may change the virus's biological properties, making it transmissible and immune-evading [21].

Continuous genomic surveillance is crucial for gaining a deeper understanding and efficiently responding to the possible epidemiological impacts of novel mutations. The objective of this study was to perform a complete analysis of 39 genomes of the SARS-CoV-2 to identify variants, clades, and lineages, enhancing our comprehension of the virus through the identification of mutation behaviors.

Material and method

A statement on the facility and ethics

Biosafety level 3 (BSL3) enhanced facilities were utilized for all procedures involving infectious SARS-CoV-2 in the molecular biology unit at the biological preventive lab/ laboratories in the chemical warfare department of Egyptian Armed Forces. The research ethics committee at Suez Canal University gave its approval to perform this work (Approval number REC662022).

Sampling, extraction of viral RNA, and molecular identification by RT-PCR

At the fever hospital of the Egyptian Army forces, nasopharyngeal swabs (n=39) were taken from patients with suspected COVID-19. The specimens were sent to the molecular biology unit with the BSL3 facility at the laboratories of the chemical warfare department following the biosafety protocols established by the CDC for the handling and processing of coronavirus disease specimens [22]. The transportation was done at a temperature of 2-4 ◦C using a viral transport medium (Hardy Diagnostics Cat. no. R99) and a transport bag (95Kpa).

The extraction of viral RNA for each sample has been performed using QIAamp Viral RNA mini kit 50 (QIAGEN, Hilden, Germany) according to the instructions provided by the manufacturers. Then, the extracted viral RNA for the samples was tested RT-PCR for three distinct SARS-CoV-2 genes (*E, N* and *ORF1ab*) genes using The VIASURE SARS-CoV-2 TRIPLEX real-time PCR detection kit (Catalog number NCO4-1120EN) and AriaMx real-time PCR system (Agilent, Germany). Briefly, we mixed 15 µL of master mix with 5 µL of extracted viral RNA for each sample. Furthermore, we prepared a tube for the positive control by mixing 5 µL of positive control with 15 µL of master mix, and another tube for the negative control by mixing 5 µL of negative control with 15 µL of master mix. Afterward, qPCR was performed using the following parameters: 45°C for 15 minutes was used for the reverse transcription step, 95°C for 2 minutes as an initial heating step, followed by 40 cycles of 95◦C for 10 seconds and 60°C for 50 seconds for the amplification process. By evaluating the fluorescence curves during the exponential phase and making sure the threshold lines were positioned above any background signal, the Ct values were determined.

Library preparation and whole genome sequencing

Libraries were being performed using AviSeq COV19 NGS Library Prep kit (Ref. AVG202096) and Illumina (Iseq 100 System). Briefly, RNA was quantified using the Qubit RNA HS 100 Assay Kit (Cat. No. Q32852) on the Qubit 2.0 Fluorometer, after that, RT Primer Mix DP (BATCH No. 020402C) was added to create cDNA. To amplify the full SARS-CoV-2 genome, a multiplex PCR process was also performed for each

sample by the addition of 2 µL of Nuclease -free water, 2 µL of 5x mPCR Mix and 1 µL of 10x SARS-CoV-2 Primer pool 1/2 to 5 µL of cDNA from previous step. Subsequently, PCR was conducted on thermal cycler FC-96B (Bigfish Bio-tech Co), employing the subsequent parameters: 95°C for 10 minutes for the initial denaturation step, followed by 10 cycles of 98°C for 15 seconds as denaturation step and 60°C for 5 minutes for annealing/extension step, once the PCR program completed, we added 2 µL of stop buffer. To get rid of any non-specific PCR products, digestion was carried out by the addition of 7 µL of nuclease -free water, 2 µL of CP reagent Buffer and 1 µl of CP digestion reagent to each purified PCR product from previous step followed by incubation at 37°C for 10 minutes, once the incubation period completed, we added 2 µL of stop buffer. Following library preparation, high sensitivity D1000 ScreenTape (Cat. No. 5067- 5584) with high sensitivity D1000 reagents (Cat. No. 5067- 5585) and 2200 Tapestation System (Agilent) were used to test the quality of the library.

Data analysis

Fasta files were generated and analyzed using Nextclade tool V3.4.0, coronavirus typing tool V 1.25 and CoVsurver mutation application to determine the clade assignment, mutation calling, pango lineage and phylogenetic analysis.

Results

Molecular identification (RT-PCR**) and measurement of RNA concentration**

The result of RT-PCR showed that all samples (n=39) were positive for SARS CoV-2 (*E, N and ORF1ab*) genes with Ct values from 18.2 to 31.7. The measurement of the concentration of RNA in the samples using a Qubit 2.0 Fluorometer showed that the result ranged from 13.2 ng/uL to 23 ng/uL as shown in (Table 1).

Library preparation and whole genome sequencing

After the libraries were prepared, the amplification of viral RNA was confirmed via electrophoresis, the presence of a peak at ~275 bp indicates successful amplification of the targeted regions (according to AviSeq COV19 NGS library prep kit instruction) as shown in Figure 1. The entire genome sequence for the samples has been completed, resulting in the generation of fasta files. These files have been submitted to the NCBI Virus Genbank with Accession Number (from OP183416.1 to OP183454.1).

Variants calling, lineage and phylogenetic tree analysis

Sequencing data analysis using Nextclade tool V3.4.0 showed that all the samples (n=39) clustered as omicron variants with 3 different clades 21L, 22A and 22B as shown in Table 2. The phylogenetic tree showed that there were 12 samples clustered as omicron clade 21L, 3 samples clustered as omicron clade 22A and 24 samples clustered as omicron clade 22B which represent the most common strain between involved samples as shown in Figure 2. Also, the data analysis showed that the involved sequences in the study have been distributed into different Pango lineages (BA.2, BA.2.40.1, BA.2.5, BA.2.56, BA.4, BA.5.2, BA.5.2.1, BA.5.2.20, BA.5.2.27 and BA.5.3.1), The pango lineage BA.5.2 represent the most common lineage as there was 17 sequences belonged to this lineage as shown in Figure 3.

Sequence homology of the samples with wild-type strains

An investigation of mutations in the genomic sequences was conducted, and the CoVsurver software provided by GISAID was used to construct the 3D structure of the spike protein with amino acid alterations in sequences. The reference strain used was hCoV-19/Wuhan/WIV04/2019. Omicron exhibited greater sequence diversity, particularly in the spike protein region (nucleotides 21,563-25,384; amino acids 1-1273), which includes the receptor-binding motif (RBM; nucleotides 22,869-23,089 and amino acids 438-508). The similarity of the analyzed sequences to the reference strain for the spike protein sequence ranged from 96.30% to 98.19% (23-47 mutations) as showm in Figure 4.

Mutational analysis

The sequences data analysis exhibited numerous clusters of closely grouped mutations, most of these mutations observed in the spike protein region, namely inside its S1 subunit, containing the host RBM. A significant number of mutations found in the omicron variant are also present in the current global variants of concern (VOCs) or variants of interest (VOIs). Table 3 summarizes the reported mutations found in the sequences (n=39) along the *S* gene and their functional characteristics based on the current research (ref), these Mutations were classified into

four main categories: host adaptation, viral reproduction, host receptor binding, and immunological evasion.

Table 1. Ct values and concentration of RNA in the samples.

Sample NO	Ct value			RNA Conc (ng/uL)	Sample NO	Ct value			RNA Conc (ng/uL)
	N	*ORF1ab*	*E*			*N*	*ORF1ab*	*E*	
1	26.5	26.5	23.7	13	21	25.4	24.9	30.5	13
2	20.1	19.5	25.1	19	22	25.4	24.8	25.8	13
3	19.8	19.4	23.8	19	23	24.4	23.7	26.5	14
4	19.1	18.6	23.9	22	24	20.9	20.7	27.9	16
5	24.2	24.1	22.8	13	25	27.2	28.1	29.6	13
6	19.8	19.1	25.5	20	26	29.9	31.6	25.7	13
7	29.1	30.2	24.7	13	27	22.5	21.8	29.6	14
8	23.5	23.5	26.8	14	28	22.8	22.02	27.7	14
9	29.9	31.7	25.6	13	29	18.9	18.9	25.2	21
10	18.7	18.2	24.2	23	30	20.5	21.1	26.7	15
11	24.2	23.8	27.03	13	31	20.7	20.4	29.3	13
12	20.6	20.8	22.8	15	32	23.6	22.8	29.1	13
13	20.6	20.8	26.3	15	33	19.7	19.9	22.1	17
14	20.7	20.7	24.3	15	34	19.4	19.05	23.2	14
15	20.3	20.6	25.2	16	35	22.8	22.3	23.2	18
16	22.0	21.3	25.6	15	36	20.3	20.2	25.5	20
17	22.3	22.4	30.2	14	37	21.01	22.8	24.9	14
18	19.02	18.9	23.1	21	38	23.9	23.5	24.5	18
19	26.8	26.6	33.1	13	39	18.9	20.5	24.7	17
20	18.9	18.7	23.8	22					

Table 2. Distribution of the samples (n=39) according to clade assignment.

ACC. No.	WHO name	Clade	Pango lineage	Mut
OP183416.1	Omicron	21L	BA.2	51
OP183417.1			BA.2	45
OP183418.1			BA.2	49
OP183419.1			BA.2.5	40
OP183420.1			BA.2	53
OP183421.1			BA.2.40.1	47
OP183422.1			BA.2	42
OP183423.1			BA.2	46
OP183424.1			BA.2	44
OP183434.1			BA.2	54
OP183444.1			BA.2.56	55
OP183450.1			BA.2.56	50
OP183451.1		22A	BA.4	48
OP183453.1			BA.4	53
OP183454.1			BA.4	39
OP183425.1		22B	BA.5.2	42
OP183426.1			BA.5.2	44
OP183427.1			BA.5.3.1	52
OP183428.1			BA.5.2.20	43
OP183429.1			BA.5.2	49
OP183430.1			BA.5.2	51
OP183431.1			BA.5.2	47
OP183432.1			BA.5.3.1	44
OP183433.1			BA.5.2.1	42
OP183435.1			BA.5.2	44
OP183436.1			BA.5.2	44
OP183437.1			BA.5.2.20	52
OP183438.1			BA.5.2	48
OP183439.1			BA.5.2	51
OP183440.1			BA.5.2	44
OP183441.1			BA.5.2	44
OP183442.1			BA.5.2	47
OP183443.1			BA.5.3.1	47
OP183445.1			BA.5.2.27	47
OP183446.1			BA.5.2	54
OP183447.1			BA.5.2	47
OP183448.1			BA.5.2	46
OP183449.1			BA.5.2	50
OP183452.1			BA.5.2	46

Table 3. List of mutations found in the sequences (n=39) along the *S* gene and their functional characteristics.

Mutation	Occurrence%	Remarks	Category	Ref.
T19I	39 (100%)	Removes a potential N-glycosylation site at position 17, affecting antigenic drift	immune escape	[23]
L24del	39 (100%)	NEUTRAL	immune escape	[24]
P25del	39 (100%)	NEUTRAL	immune escape	[24]
P26del	39 (100%)	NEUTRAL	immune escape	[24]
A27S	39 (100%)	NEUTRAL	immune escape	[25]
H655Y	39 (100%)	Enhances spike cleavage and viral growth, and is an important fusion inducer, showing increases in syncytia formation	immune escape and virus replication	[26]
N679K	39 (100%)	Reduced virus replication	host adaptation and virus replication	[27]
P681H	39 (100%)	P681H mutation at the S1/S2 site of the SARS-CoV-2 spike protein may increase its cleavability by furin-like proteases	immune escape and host adaptation	[28·29]
N764K	38 (97.4%)	NEUTRAL	virus replication	[30]
Q954H	37 (94.8%)	Increase Host adaptation (cell culture)	host adaptation	[31]
G142D	33 (84.6%)	Antigenic Drift	immune escape	[32]
D405N	32 (82.05%)	Host Change and Antigenic Drift	immune escape and host adaptation	[33]
R408S	32 (82.05%)	Antigenic Drift	immune escape	[33]
K417N	31 (79.4%)	Host Change and Antigenic Drift	immune escape and host adaptation	[33]
G339D	24 (61.5%)	Host Change.	host adaptation	[33]
N440K	18 (46.15%)	Host Change and stabilizes binding with ACE2 and Antigenic Drift	immune escape and host adaptation	[33]
S373P	17 (43.58%)	Host Change.	host adaptation and host receptor binding	[33]
S375F	17 (43.58%)	Antigenic Drift	immune escape	[34]
S371F	17 (43.58%)	Antigenic Drift	immune escape	[35]
T376A	17 (43.58%)	Antigenic Drift	immune escape	[30·33]
L452R	11 (28.2%)	Antigenic Drift	immune escape	[33·36]
V213G	8 (20.5%)	NEUTRAL	immune escape	[24]
N969K	4 (10.25%)	N/A	N/A	N/A
N658S	3 (7.69%)	Increase Stability	Host adaptation	[37]
L452M	2 (5.1%)	Host Change and Antigenic Drift	immune escape and host adaptation	[33·24]
H69del	1 (2.56%)	Antigenic Drift	immune escape	[38]
V70del	1 (2.56%)	Antigenic Drift	immune escape	[38]
Y144del	1 (2.56%)	Antigenic Drift	immune escape	[38]
F157S	1 (2.56%)	NEUTRAL	immune escape	[39]
N188T	1 (2.56%)	N/A	N/A	N/A
S255F	1 (2.56%)	N/A	N/A	N/A
A262V	1 (2.56%)	N/A	N/A	N/A
K417T	1 (2.56%)	N/A	N/A	N/A

Figure 1. Quality check for the libraries (n=39) showing that all libraries gave apeak at ~275 bp indicates successful amplification of the targeted regions. A: Samples (1-14), B: Samples (15-27), C: Samples (28-39).

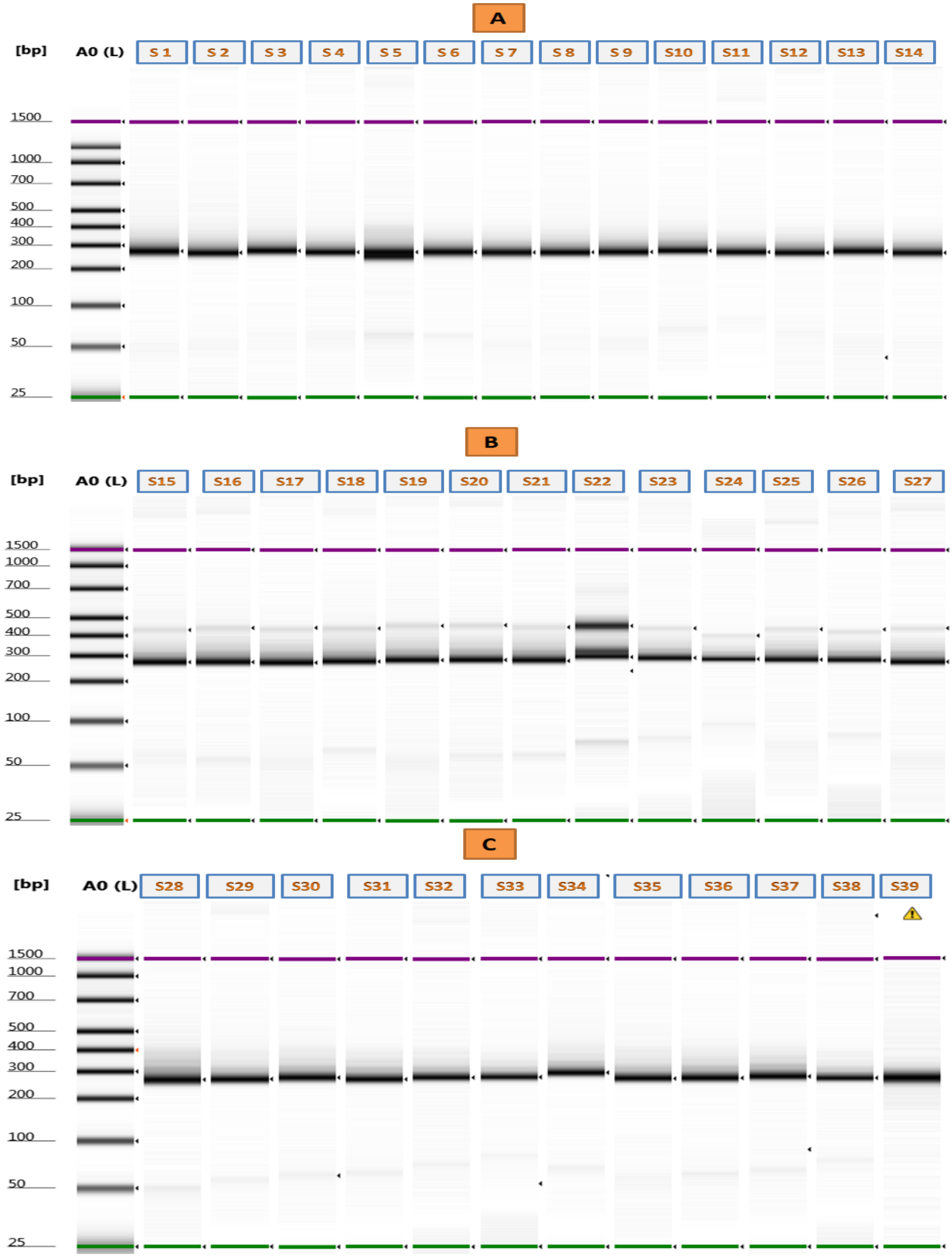


Figure 2. Radial phylogeny showed the distribution of Samples (n=39) according to clade assignment.

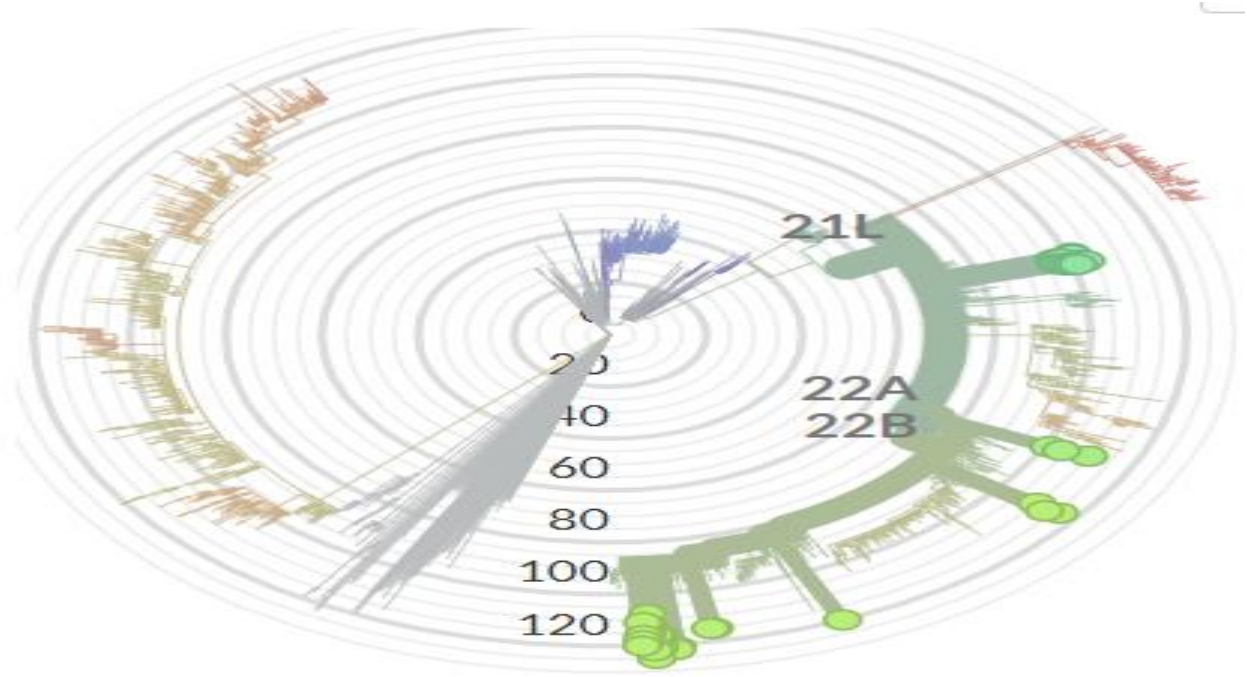


Figure 3. Distribution of Samples (n=39) according to Pango Lineage

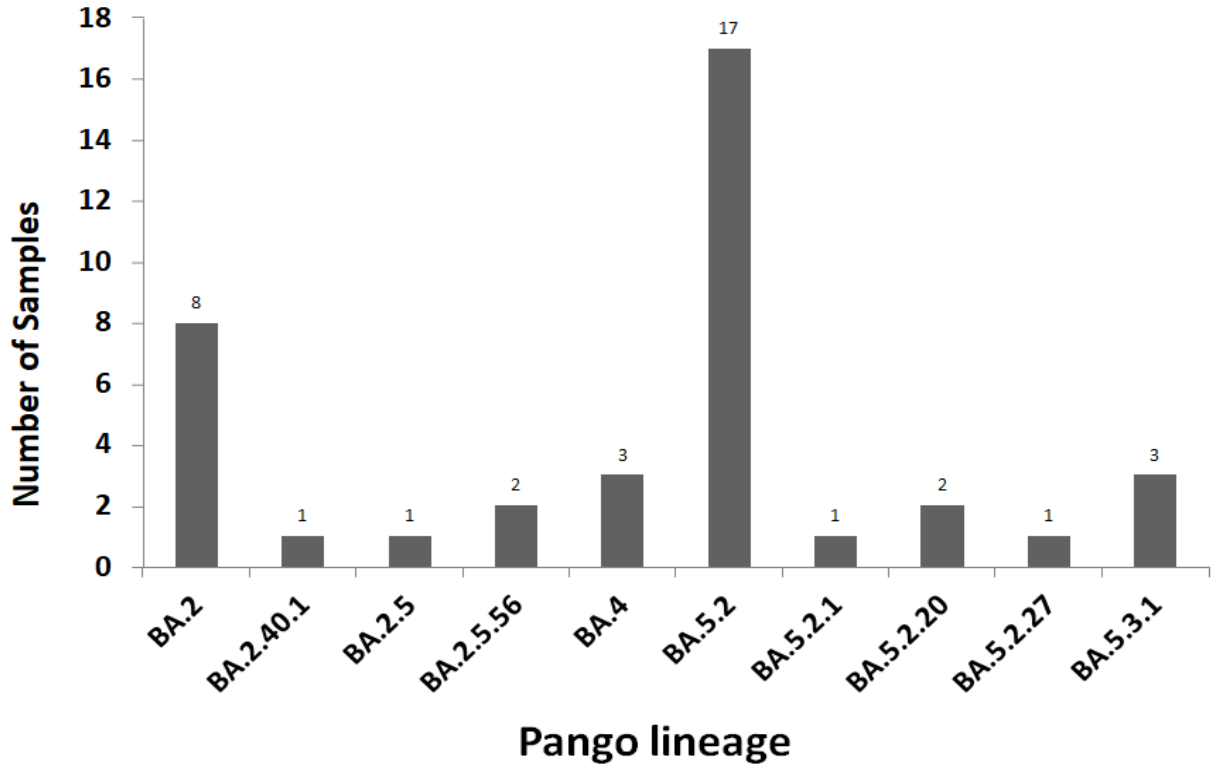
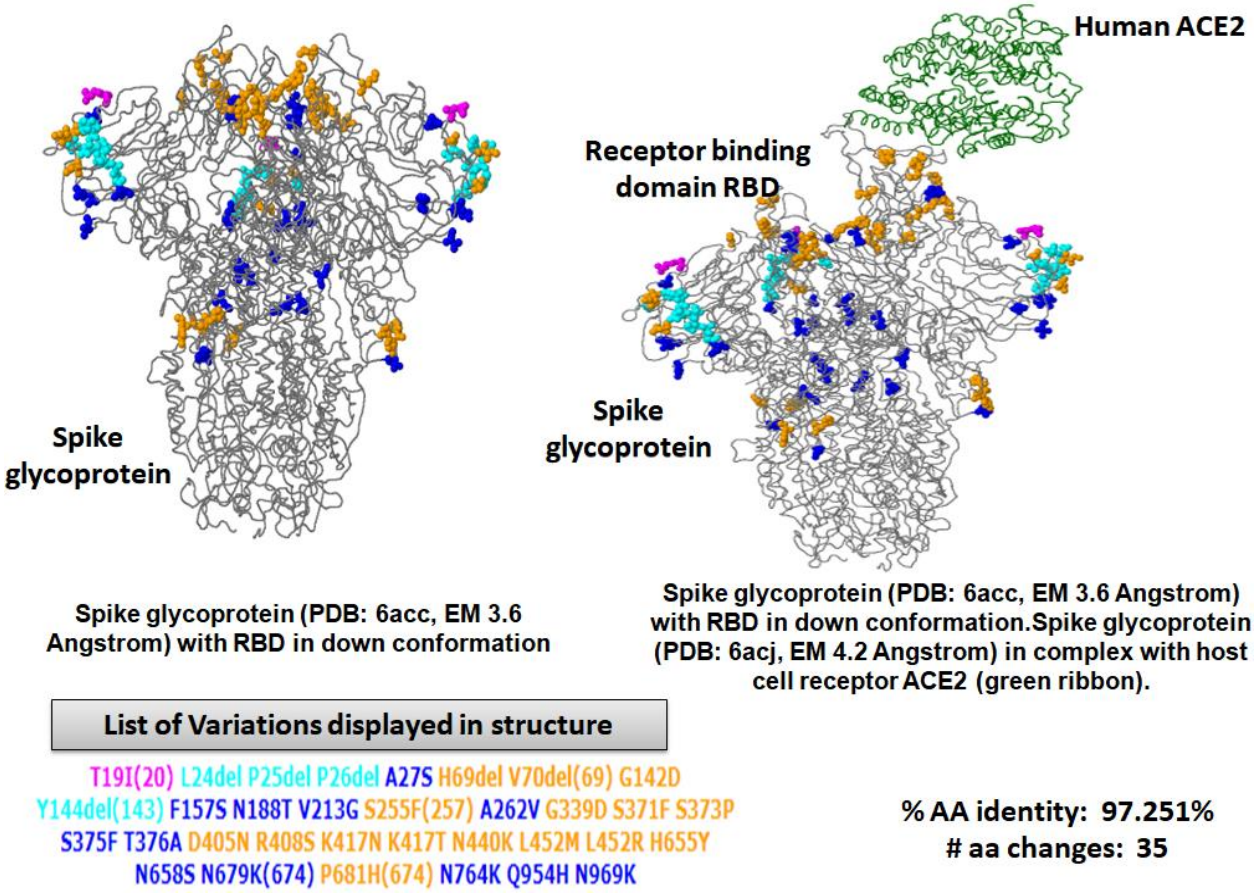


Figure 4. 3D structure of spike glycoprotein in the interaction of human angiotensin-converting enzyme 2 (ACE2), showing key amino acid substitutions in the analyzed sequences. AA/aa: amino acid



Discussion

In Egypt during the fourth wave of the COVID-19 pandemic, despite global attempts to develop treatments and distribute vaccines, the number of infected cases remained high. Our analysis of the SARS-CoV-2 genomic sequences from this particular time frame reveals an omicron variant that was prevalent in Egypt, with different clades and several reports on host-virus interactions. This information may be utilized for predicting the omicron variant's future epidemic potential. We found that omicron had greater sequence variation, particularly in the spike protein and RBM, as compared to the WHO's current list of worldwide VOCs/VOIs. Additionally, the study of mutations revealed enrichment for variants that reduced the expression of RBD protein and ACE2-binding affinity while increasing the possibility of immune escape.

Our study is regarded as the first to use next generation sequencing to identify the different Omicron clades in Egypt during the fourth wave of the COVID-19 pandemic and to provide a comprehensive analysis for mutations that appeared on the omicron variant's *S* gene for use in the development of vaccines and treatment. Some limitations of this study include the limited number of Samples (n=39) involved in the study to give a complete monitoring of the omicron variant in Egypt and its different lineages.

Although next-generation sequencing (NGS) is a technology that many laboratories employ worldwide to study the genetic composition of all living things, its application in the diagnosis of infectious diseases is now somewhat limited. The pathogen's genome sequence is determined through NGS, which provides a wealth of information far beyond what is possible to learn from standard testing procedures. This knowledge can be used to develop new treatments and vaccines, track mutations in the virus as it spreads throughout the population, and gain deeper insights into patterns of transmission over time.

According to our study, phylogenetic tree analysis showed that all the sequences belonged to omicron variants with 3 different clades (21L, 22A and 22B) and 10 different Pango lineage (BA.2, BA.2.40.1, BA.2.5, BA.2.56, BA.4, BA.5.2, BA.5.2.1, BA.5.2.20, BA.5.2.27 and BA.5.3.1), the most common strain was the omicron variant clade 22B and BA.5.2 Pango lineage during the time that the study period.

According to our investigation, omicron acquired several close mutations at the RBM with ACE2, which have an impact on the interaction between the virus and the host. Interestingly, compared to the wild-type strain, this omicron variant shares many of the mutations found in the earlier VOCs, many of which have been demonstrated to improve RBD-ACE2 binding [40]. It is thought that the mutation N440K, which is found at or close to the RBM in the majority of our sequences, stabilizes binding with ACE2 (Table 3). Additionally, the mutations (P681H and H655Y) in the furin cleavage site of SARS-CoV-2 spike protein can increase the proteolytic cleavage of spike protein by a host protease (furin), which is thought to enhance its fusion to the host receptor site [41].

According to our study, the majority of omicron's spike mutations (21/33) occurred at sites that are recognized by antibodies. The function of these mutations in immunological escape against convalescent sera, vaccine-acquired antibodies, and therapeutically utilized monoclonal antibodies has been demonstrated by previous research (Table 3). Data from in situ investigations suggests that omicron may be able to evade vaccination-induced antibodies, convalescent sera, and therapeutically administered monoclonal antibodies [18-34] , K417N mutation which is present in most involved sequences is believed to play an important role in immune escape [40], K417 locus is considered to be an epitope for CB26 that is used as a monoclonal antibody in COVID-19 [40].

According to our investigation, there is a significant mutation (N679K) that has a direct effect on viral replication as it reduces the translation process of the spike protein compared with wild-type [27]. Additionally, a set of mutations (N969K-N188T- S255F- A262V- K417T) that are prevalent in the involved sequences require further investigation to understand their significance to the virus's approach.

Our research indicates that the omicron variant has acquired mutations that may impact the SARS-CoV-2 immune escape strategy. As a result, we need to develop a new vaccine that is appropriate for these novel variants [42].

Conclusion

In silco analysis of omicron genome sequences demonstrate that this variant has accumulated with mutations that provide it with a higher ability to evade the immune system compared to other variants of concern or interest (VOCs/VOIs), Therefore, it is critical for the scientific community to continuously monitor for omicron genome mutations using next-generation sequencing to aid in the development of new vaccinations appropriate for the newly omicron variation in Egypt.

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Conflict of interest

There is no conflict of interest

Authorship contribution statement

Hanaa H. A. Gomaa: Conceptualization, Funding acquisition, Project administration.

Mohamed G. Seadawy: Formal analysis, Writing – review & editing.

Mohamed Abdel-Razik: Formal analysis, Writing – review & editing.

Ahmed F. Gad: Data curation, Formal analysis, Methodology.

Mervat G. Hassan: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing.

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