



Mini Review

The Informative Nature of the Disappeared SARS-CoV-2 Genomic Sequences: A Mini-review with Perspectives

Amgad M. Rabie 

Dr. Amgad Rabie's Research Laboratory for Drug Discovery (DARLD), Mansoura, Egypt
Department of Clinical Research, Dikernis General Hospital, Dikernis, Dakahlia Governorate, Egypt
E-mail: amgadpharmacist1@yahoo.com, dr.amgadrabie@gmail.com

Received: 15 March 2022; **Revised:** 16 April 2022; **Accepted:** 21 April 2022

Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and its mortal coronavirus disease 2019 (COVID-19) are still mysterious in nature. The emergence of the more virulent newer SARS-CoV-2 variants, e.g., Delta and Omicron variants, made the matters worse. CoViTris2020 compound can be considered the most potent investigational anti-COVID-19 agent among others to date. Meanwhile, all countries took very serious steps in vaccination and have started to vaccinate their citizens beginning with the elders. The current data refer that, in the near future, the available vaccines may gradually lose their effectiveness against the newer SARS-CoV-2 strains which have increased transmissibility and infectivity due to the enhanced human angiotensin-converting enzyme 2 (hACE2) affinities and antibodies resistances, respectively. All the problems facing the continuous and successful use of the current vaccines, antiviral agents, and convalescent plasma in their missions against the COVID-19 infection force us to deeply investigate all the updated available data about all the components of the pandemic in a comprehensive way hour by hour to reveal all the secrets and clues behind the SARS-CoV-2. Herein, a brief article about the up-to-date clues, hypotheses, facts, unsolved issues, and informative genetic data of SARS-CoV-2/COVID-19 is introduced.

Keywords: SARS-CoV-2 genome, spike protein, amino acids sequence, Delta/Delta-plus variant, COVID-19 vaccine, antibody, CoViTris2020, vaccine design and biotechnology

1. Introduction

The infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in humans is still rapidly ongoing, with its dangerous coronavirus disease 2019 (COVID-19) being the most prevalent and fatal disease worldwide. Recently, many distinctive variants of the original coronavirus 2, with different genomic sequences, have appeared and spread (of note, the deleted and changed SARS-CoV-2 genome sequences led to the multi mutations and the appearance of the several variants and newer lineages) [1, 2]. The World Health Organization (WHO) classified them using mainly Greek letters, e.g., Alpha variant (lineages B.1.1.7 and B.1.1.7+E484K), Beta variant (lineage B.1.351), Gamma variant (lineage P.1), Delta variant (lineage B.1.617.2), Epsilon variant (lineages B.1.429, B.1.427, and CAL.20C), Zeta variant (lineage P.2), Eta variant (lineage B.1.525), Theta variant (lineage P.3), Iota variant (lineage B.1.526), Kappa variant (lineage B.1.617.1), Lambda variant (lineage C.37), Mu variant (lineage B.1.621), Nu and Xi variants (negligible), and Omicron variant (lineage B.1.1.529) [1, 2]. The series continues to more than 70 SARS-CoV-2 species or variants at the time of submitting this paper for publication (Figure 1) [2]. Studying and investigating the

Copyright ©2022 Amgad M. Rabie
DOI: <https://doi.org/10.37256/acbr.1220221403>
This is an open-access article distributed under a CC BY license
(Creative Commons Attribution 4.0 International License)
<https://creativecommons.org/licenses/by/4.0/>

Indian Delta variant along with the newest variant Omicron is of great importance due to their increased transmissibility/prevalence, virulence/infectiousness, human morbidity/mortality/reinfection, natural antibodies/antiviral drugs/synthetic vaccines resistance, particular demographic/clinical groups' affinity, diagnostic tests detection failure, and contradicted data [2-4]. The coronaviral-2 Delta and Omicron variants are now subrogating almost all the previous SARS-CoV-2 variants (including the ancestral strain) with a much higher reproductive number in comparison to all the original and previous variants of the virus, therefore, significant reductions in the coverage rates of the different COVID-19 vaccines were recently observed [2-4].

On the other hand, drug discoveries for effective broad-spectrum antiviral remedies (able to destruct the several SARS-CoV-2 variants) and/or successful general anti-COVID-19 therapies (able to reverse the severe and mortal actions of the COVID-19) are still insufficient [5, 6]. There are no selective/specific anti-COVID-19 drugs that have been approved by the U.S. Food and Drug Administration (FDA) for the official treatment of SARS-CoV-2 infection and its disease, COVID-19, with 100% tactical efficacy to date [5, 6]. However, there are some promising compounds and agents to do this critical mission that are now undergoing extensive investigations and preclinical/clinical trials, including the RNA-dependent RNA polymerase (RdRp) inhibitors CoViTris2020 (Figure 2) and molnupiravir (now it is FDA approved), which being the most interesting and promising ones in these international studies [5-9].

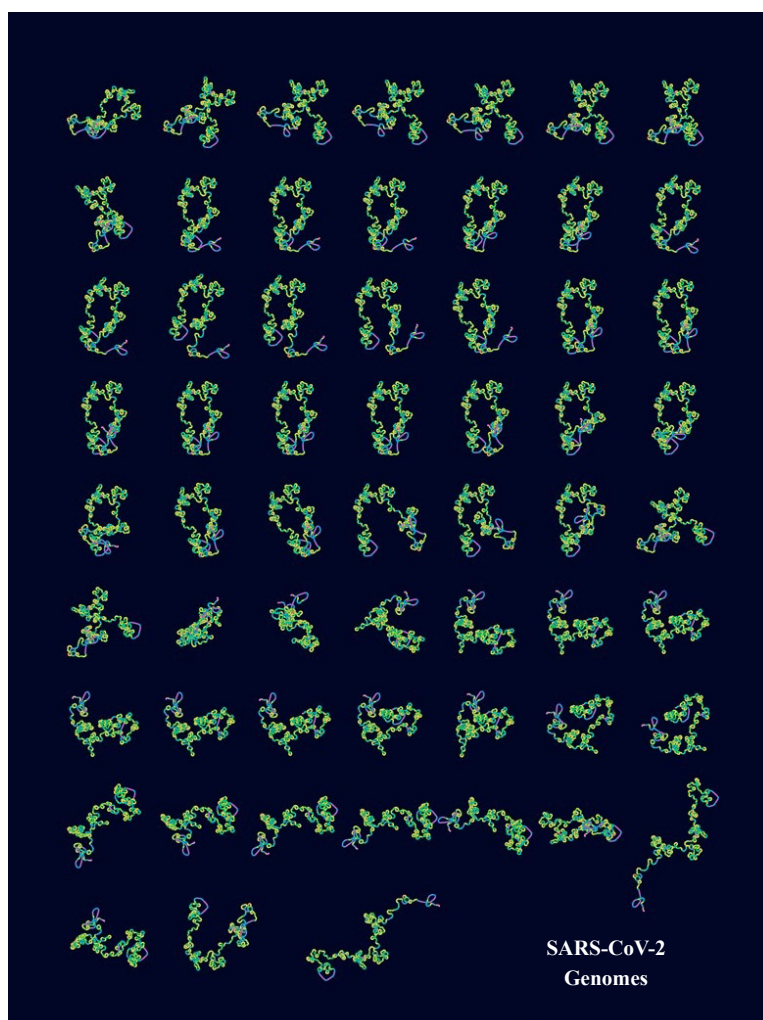


Figure 1. A representative 3D visualization of the most common 59 SARS-CoV-2 genomic sequences (updated image; the previous version was published in February 2021 by Van Noorden [10])

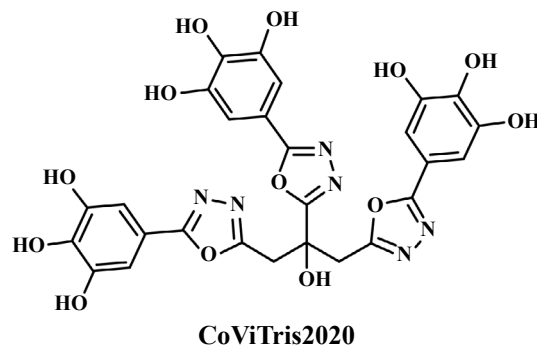


Figure 2. Chemical structure of the CoViTris2020 molecule (a promising under-investigation potential anti-COVID-19 drug)

2. Clues and theories behind SARS-CoV-2 genomic deletions

Scientists have hypothesized some theories for the reasons behind the severe and higher transmission and virulence abilities of the mutated SARS-CoV-2 particles [11-13]. Many researchers support the theory of coronavirus variants origination and the accompanying severe infectivity from the continual infection of the immunocompromised individuals, especially when the selection pressure of antibodies/convalescent plasma therapies renders the coronaviral-2 particle developing urgent escape mutations, with repeated similar deletions in surface antigens recurring in various persons [13]. Then, these new mutations in the different coronaviral-2 variants, in turn, cause modifications in the known electrostatic potential (i.e., electric charge at rest) on the viral spike surface (SARS-CoV-2 spike proteins, which are mainly present on the coronavirus surface, have a major role to enable the viral particle to bind to and invade the human cells). Since the positively-charged moieties in the receptor-binding domain (RBD) and N-terminal domain (NTD) of the spike surface proteins largely increase, while the negatively-charged parts largely decrease, it affects the normal abilities of the antibodies, immunity mediators, and antiviral agents to attach to and neutralize/kill the SARS-CoV-2 particle (Figure 3) [14, 15]. It is worth mentioning that the effects of these spike surface structural changes, which were induced by the mutations, extend to the binding affinities of the RBD to the human angiotensin-converting enzyme 2 (hACE2). As these affinities significantly increased, the new mutant virus strains have relatively higher transmission (and higher virulence) of SARS-CoV-2 infection from human to human compared to the older and original strains [1, 15].

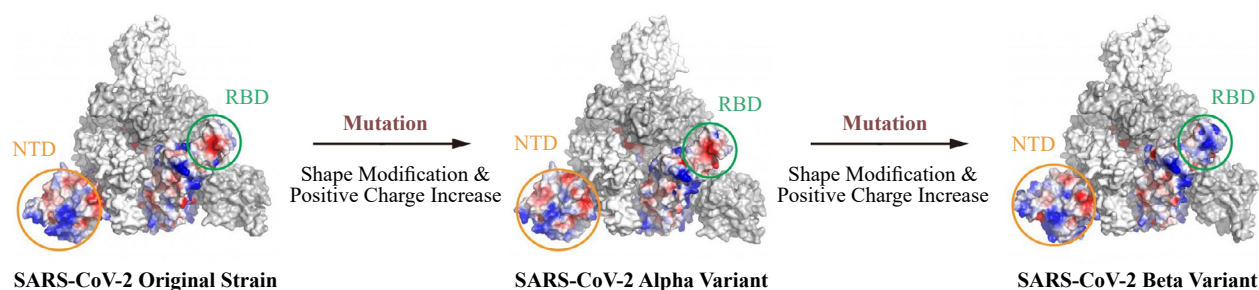


Figure 3. A representation of the gradual changes that occur in the protein shapes and electrostatic potentials on the coronavirus-2 spike surfaces after different mutations of the SARS-CoV-2 (the three examples shown herein are the Wuhan Original, Alpha, and Beta strains; positively-charged positions are represented in blue color while negatively-charged ones are represented in red color; note that the spike surface positive charges increase from an old version of the virus to a newer one with the newest virulent Indian variant being almost the most positively-charged SARS-CoV-2 strain to date; in the SARS-CoV-2 Beta variant, the RBD and NTD have modified substantially, affecting the original effectiveness and ability of the relevant antibodies to attach to and neutralize the viral particles)

The mystery and clue lie here, since the normal original coronaviral-2 spike is majorly positively charged (i.e., the SARS-CoV-2 spike proteins have a positive net charge in almost all positions and areas) and the newer variants or mutated strains are gradually more and more positively charged (i.e., the spikes of each newer SARS-CoV-2 strain

have higher positive charges than those of the preceding one(s), and so on, on a gradual basis with each new mutation in most cases to date) [14]. The great problem is that the present vaccines and antibodies are directed against these spikes and with restricted selective affinity against only these proteins (with their specific amino acids sequences and net charges). Again, the previously-mentioned structural findings indicate that the mutations in both the preceding and the newest SARS-CoV-2 variants, like the Beta, Delta, and Omicron ones, modified the spike protein surface shape and charge at specific areas (recall that SARS-CoV-2 particle uses the specific surface structure/shape of its spikes as the principal entry mean to enter the human cells through binding to the hACE2 receptors). As a consequence, the current vaccines and their stimulated/induced neutralizing antibodies became much less capable of attaching to the resistant newer coronaviruses (i.e., became less effective) (Figure 4). This allows the newest SARS-CoV-2 particles to severely evade the human immune system even when people are full vaccinated several times (i.e., with more than one dose of the vaccine). One of the suggested solutions for this major problematic dilemma is supporting these present vaccines with synthetic boosting agents (i.e., modified vaccines) consisting of proteins/antibodies having the new genetic genome sequences of the newer mutated SARS-CoV-2 versions or of the effective antibodies to these newer versions (which can be used also as a single therapy), respectively. These boosters/adjuvants are expected to be somewhat beneficial for protecting individuals against these resistant coronaviral-2 variants. As one of the clues of the different SARS-CoV-2 variants is present in the electrostatic charge of the coronaviral particle (the dominating positive charges of the spike surface), hence, another proposed therapeutic opinion or option is neutralizing and deactivating the coronaviral particle, and therefore antagonizing/stopping its actions, through ionically/electrostatically interacting with its positively-charged spike proteins using highly negatively-charged moieties or molecules (either single drugs or included in the vaccines).

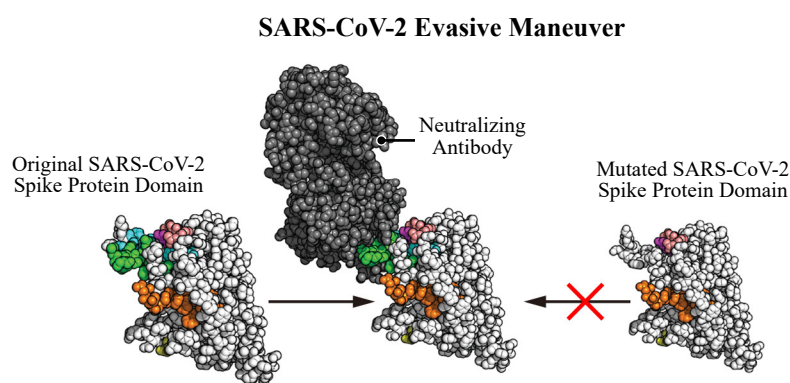


Figure 4. The evasive maneuver defensive mechanism by which the continuous coronaviral-2 mutations on spike protein surfaces hinder the neutralizing antibodies (of all origins) from hitting/deactivating the SARS-CoV-2 particles and inhibiting their biological activities (the present image is modified from the original [3])

COVID-19, an infection belongs to the special group of viral diseases caused by RNA viruses which are lacking DNA. Here, we can suggest another or additional hypothesis concerning the reasons for the emergence of more transmissible/infective SARS-CoV-2 variants and the expected potential of current vaccines to lead to more new virulent SARS-CoV-2 variants. This time we are talking about COVID-19 vaccines of the mRNA type. In order for the coronavirus 2 to generate the surface spike proteins that allow the coronaviral-2 particle to enter the human cells, it uses the metabolic pathway of the host cell for respective protein production. This is attained through transcription by the pivotal coronaviral-2 enzyme reverse transcriptase which mainly transcribes the viral genomic RNA into DNA (although SARS-CoV-2 particles do not extensively code for the reverse transcriptase). This is a very critical process since the produced DNA is directly incorporated into the human/host cell DNA to be translated into mRNA (the genomic translation process) for generating the SARS-CoV-2 spike proteins. The previous pathway is supposed to be bypassed and evaded by the COVID-19 mRNA vaccine which is supplying the mRNA particles. However, surprisingly, the potentials strongly exist for the mRNAs to be assembled and spliced together and then incorporated into the coronaviral-2 RNA for replication (we also cannot ignore that the lack of proofreading of the major coronaviral-2

replicase, RdRp, is one of the possible main causes of the high mutation rate for the SARS-CoV-2 pathogen). The produced coronaviral-2 particles (resulted from replication processes) will certainly infect other human cells but will generate coronaviral-2 particles with different spike proteins with respect to surface shape and charge, i.e., will generate mainly new diverse COVID-19 variants with different and higher transmissibility and infectivity, hence new SARS-CoV-2 variants, which certainly need completely new vaccines in almost all cases, will appear.

As previously mentioned, the main problem and concern associated with the first-generation Wuhan spike protein-based vaccines is the rapid and continuous evolution of new coronaviral-2 strains that are clearly more or less resistant/infectious to Wuhan spike protein-based vaccine-induced immunity in humans. Another unavoidable concern is the severe toxicity of the spike protein of the vaccine since this pathogenic protein alone (even if it is deactivated) can extensively destroy and damage considerable numbers of the vascular endothelial cells in very vital and critical organs of the body, causing very serious and fatal side/adverse effects. A possible solution for these concerns may be the design and synthesis of a multi-epitope COVID-19 vaccine against SARS-CoV-2 particles, excluding the spike protein portions, thus can possibly be much less toxic and much less virulent than the Wuhan spike protein-based vaccines, and also with less dangerous adverse effects, but still sufficiently immunogenic to elicit highly and efficiently protective immune response. Additional important potential benefit and advantage of this multi-epitope vaccine is that, taking into consideration the fact that almost all the new strains of SARS-CoV-2 carry significant new mutations and structural modifications in the pathogenic coronaviral-2 spike protein and less significant mutations in the other structural/functional proteins (either pathogenic or nonpathogenic) of the virus, it can provide more potent and very effective immune responses against the new SARS-CoV-2 lineages and variants (i.e., irrespective of the structure of their spike proteins). A promising under-investigation therapeutic solution to block the severe infectivity, pathogenicity, and toxicity of the different coronaviral-2 spike proteins is the use of the naturally occurring lectins (mammalian carbohydrate-binding proteins) or the design of synthetic lectin analogs [16]. This novel avenue depends on the proven fact that COVID-19 virus (with all its strains) uses a fixed *N*-glycosylation mechanism at specific sites (about 22 *N*-glycan sites of lectin receptors) on the surface of the spike protein to form a protective sugar coat that hides and saves the pathogenic antigenic protein from the human active immune responses (including the vaccine-based ones). Almost all these specific 22 *N*-glycan sites remain highly conserved among all SARS-CoV-2 strains (including the newest variants) [16]. Inhibiting or blocking the lectin receptors of these distinctive sites may be a very effective therapeutic intervention to both lock the coronaviral entry and boost the abilities of the host immune antibodies to invade, neutralize, and destroy the SARS-CoV-2 particles whatever their strain is (pan-variant therapeutic interventions which provide both prophylactic vaccine-like and post-infection antidotal interventions).

The various partial genomic sequences which have recently appeared may solve the current evolutionary enigma concerning the original genetic diversities of the mysterious virus SARS-CoV-2. We are now confident that solving and addressing many puzzles and clues about SARS-CoV-2 and the origin of the pandemic can be achieved by following back all the deleted parts of the sequences of the coronaviral-2 genomes, which will certainly help us to find suitable effective medications for the virus and its disease, COVID-19, and to discover the facts behind the origin(s) of SARS-CoV-2. The obtained data add to a growing body of evidence that the first COVID-19 human cases were not associated with the Wuhan city seafood market as previously thought or claimed (in China). There are still much work and effort to reveal the real origins of the SARS-CoV-2, i.e., where and how the first SARS-CoV-2 particles jumped from animals to people.

The continuous openness of the data of the increasing SARS-CoV-2 genomes sequences is very important and crucial for the fast responses by researchers and relevant authorities against the most dangerous health threat to mankind since an extremely long time. It is very important to put into our consideration that the interaction between the attacked human body and the attacking SARS-CoV-2 particles was previously natural at the beginning of the COVID-19 pandemic but it is now being gradually changed by the availability of the immediate vaccine. The natural emergence of new SARS-CoV-2 variants along with the potential induced appearance of newer SARS-CoV-2 variants that are moderately/fully resistant to the antibodies responses triggered by the present generation of COVID-19 vaccines may inactivate and invalidate all these vaccines and require considerable modification and remodeling of them. These pivotal suggestions are largely supported by the fact that various vaccines generated for the initial coronaviral-2 strain in 2020 have much lower efficacies against many newer variants of the virus and their accompanying forms of the COVID-19 [15, 17, 18]. Designing and developing modified and/or new-generation COVID-19 vaccines (including updated viral

and nonviral vector-based vaccines) is a necessity now to have successful broad-spectrum second- and third-generation vaccines of higher efficacy/effectiveness, better safety profiles, enhanced immunogenicity, and improved stability (for example, nano-based vaccine technology can provide ultrapotent nanoparticle COVID-19 vaccine candidates of highly flexible physicochemical characteristics, rendering these potential new vaccines clinically more successful due to the expected enhanced antigen stability, lower immunotoxicity, elongated sustained release/long action, and improved immunogenicity). Therefore, strategies to generate an effective new-generation COVID-19 vaccine need a daily increase in the comprehensive understanding of the constructure, physicochemistry, and immunopathogenesis of all the new variants/strains of SARS-CoV-2.

3. Final remarks and future perspectives

Finally, we can put some important concluding remarks in the following lines. We cannot ignore that upon injecting any COVID-19 vaccine into our bodies, we, at the same time, inject more or less similar proteins to SARS-CoV-2 particles into our bodies. This similarity can somewhat cause residual dangerous sequelae (including substantial genetic and immunity changes) in the individual's body in the long term. New super transmissible and more virulent COVID-19 strains and variants can appear at any time and reverse falling patients' numbers and hospitalization cases, thus we should not stop following all the genomic changes in each newest SARS-CoV-2 variant day by day. Delta variant (and also Delta-plus variant) needs further investigation and studies since some data refer to the possibility that this strain can infect healthier and younger persons more easily due to its enhanced infectivity and spreadability. It is very important to make all the detailed data of the different SARS-CoV-2 genome sequences available for sharing among all scientists and researchers all over the world without any restrictive barriers, along with establishing strong international genomic monitoring and surveillance systems connecting all world countries together. The expected ineffectiveness (or reduced effectiveness) of the current vaccines and their induced/generated antibodies against the coming and the newer SARS-CoV-2 strains should be always put under the microscope. Furthermore, the increasingly global community distribution of most SARS-CoV-2 strains should be extensively monitored because it may largely boost the possibility of recombination events in the very near future and further speed the development of these virulent coronaviruses. To achieve effective mentoring, we need to use the principal coronaviral-2 mutational fingerprints to recognize and track the SARS-CoV-2 spatiotemporal emergence along with disclosing convergences/divergences of predominant and major variants/strains among the different geographical areas. The evolution and emergence of a much more virulent third edition of the coronaviruses that cause the severe acute respiratory syndrome (SARS), i.e., severe acute respiratory syndrome coronavirus 3 (SARS-CoV-3), in the near future are also possible (regrettably, SARS-CoV-2 is improbable to be the last version or strain of coronaviruses causing a severe pandemic, there are high predictions that a modified coronavirus, i.e., SARS-CoV-3, can be in the way in the 2022-2025 period and also with pandemic potential). Surprisingly, the diverse SARS-CoV-2 genomic sequences with their endless deletions in the various strains are informative rather than transformative, and this certainly will trigger many international scientific tricks and intrigues in the coming weeks.

Acknowledgment

I gratefully thank and deeply acknowledge anyone who gave a hand to make this new work coming out to light.

Conflict of interest

I hereby declare that I totally have no known competing financial interests or personal relationships that could have appeared to influence the data, opinions, and work reported in this new paper.

References

- [1] Shahhosseini N, Babuadze G, Wong G, Kobinger GP. Mutation signatures and in silico docking of novel SARS-CoV-2 variants of concern. *Microorganisms*. 2021; 9(5): 926. Available from: doi:10.3390/microorganisms9050926.
- [2] World Health Organization. *Tracking SARS-CoV-2 variants*. Available from: <https://www.who.int/en/activities/tracking-SARS-CoV-2-variants> [Accessed 16th April 2022].
- [3] Kupferschmidt K, Wadman M. Delta variant triggers new phase in the pandemic. *Science*. 2021; 372(6549): 1375-1376. Available from: doi:10.1126/science.372.6549.1375.
- [4] Liu Y, Rocklöv J. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. *Journal of Travel Medicine*. 2021; 28(7): taab124. Available from: doi:10.1093/jtm/taab124.
- [5] Jiang S, Du L, Shi Z. An emerging coronavirus causing pneumonia outbreak in Wuhan, China: calling for developing therapeutic and prophylactic strategies. *Emerging Microbes & Infections*. 2020; 9(1): 275-277. Available from: doi:10.1080/22221751.2020.1723441.
- [6] Rabie AM. CoViTris2020 and ChloViD2020: a striking new hope in COVID-19 therapy. *Molecular Diversity*. 2021; 25(3): 1839-1854. Available from: doi:10.1007/s11030-020-10169-0.
- [7] Rabie AM. Two antioxidant 2,5-disubstituted-1,3,4-oxadiazoles (CoViTris2020 and ChloViD2020): successful repurposing against COVID-19 as the first potent multitarget anti-SARS-CoV-2 drugs. *New Journal of Chemistry*. 2021; 45(2): 761-771. Available from: doi:10.1039/D0NJ03708G.
- [8] Mahase E. Covid-19: Pfizer's paxlovid is 89% effective in patients at risk of serious illness, company reports. *BMJ*. 2021; 375: n2713. Available from: doi:10.1136/bmj.n2713.
- [9] Imran M, Kumar Arora M, Asdaq SMB, Khan SA, Alaql SI, Alshammari MK, et al. Discovery, development, and patent trends on molnupiravir: a prospective oral treatment for COVID-19. *Molecules*. 2021; 26(19): 5795. Available from: doi:10.3390/molecules26195795.
- [10] Van Noorden R. Scientists call for fully open sharing of coronavirus genome data. *Nature*. 2021; 590(7845): 195-196. Available from: doi:10.1038/d41586-021-00305-7.
- [11] McCarthy KR, Rennick LJ, Nambulli S, Robinson-McCarthy LR, Bain WG, Haidar G, et al. Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. *Science*. 2021; 371(6534): 1139-1142. Available from: doi:10.1126/science.abf6950.
- [12] Mahase E. Covid-19: where are we on vaccines and variants? *BMJ*. 2021; 372: n597. Available from: doi:10.1136/bmj.n597.
- [13] Kupferschmidt K. U.K. variant puts spotlight on immunocompromised patients' role in the COVID-19 pandemic. *Science*. Dec 23 2020. <https://www.science.org/content/article/uk-variant-puts-spotlight-immunocompromised-patients-role-covid-19-pandemic> [Accessed 24th April 2022].
- [14] Cai Y, Zhang J, Xiao T, Lavine CL, Rawson S, Peng H, et al. Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants. *Science*. 2021; 373(6555): 642-648. Available from: doi:10.1126/science.abi9745.
- [15] Gobeil SM-C, Janowska K, McDowell S, Mansouri K, Parks R, Stalls V, et al. Effect of natural mutations of SARS-CoV-2 on spike structure, conformation, and antigenicity. *Science*. 2021; 373(6555): eabi6226. Available from: doi:10.1126/science.abi6226.
- [16] Hoffmann D, Mereiter S, Oh YJ, Monteil V, Elder E, Zhu R, et al. Identification of lectin receptors for conserved SARS-CoV-2 glycosylation sites. *The EMBO Journal*. 2021; 40(19): e108375. Available from: doi:10.15252/embj.2021108375.
- [17] Hoffmann M, Arora P, Groß R, Seidel A, Hörnich BF, Hahn AS, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell*. 2021; 184(9): 2384-2393.e12. Available from: doi:10.1016/j.cell.2021.03.036.
- [18] Mlcochova P, Kemp SA, Dhar MS, Papa G, Meng B, Ferreira IATM, et al. SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*. 2021; 599(7883): 114-119. Available from: doi:10.1038/s41586-021-03944-y.