Iranian Journal of Virology 2020;14(2):69-77
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Review Article

SARS-CoV-2: A Review of Present & Future Perspectives

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Abstract

As the dawn of this new decade has begun, we have faced our arch-nemesis right at the start in the form of *Coronavirus* also known as COVID-19. Since its origin from Wuhan, China, it has been declared a pandemic and has affected several countries worldwide. This review article summarizes all the research, techniques, strategies, and treatment methodologies that are taking place internationally in the current frame of time. The road to developing a viable and effective vaccine has many hurdles that we have to pass through. Modern research has identified the receptor ACE2 that is common in both SARS-CoV and SARS-CoV-2. This homogeneity gives us a lead because we have an enormous amount of genomics and proteomics' data related to the original strain and other viruses of the subfamily *beta-coronaviruses* like MERS etc. Furthermore, the organization of the genome of this novel strain, as well as future perspectives of therapeutic procedures like by utilizing stem cells for immunomodulation derived from mesenchymal lining and many other viable options in the treatment of the virus have been briefly enlisted.

Keywords: Coronavirus, SARS-CoV-2, Vaccine, Immunomodulation, Stem Cell Therapy, Genome Organization, Structural Analysis, Phylogenetic Analysis

Introduction

Coronavirus disease or COVID-19 has become one of the major outbreaks since the last Polio and Bubonic plague and thousands of deaths have been reported since its first spread in Wuhan, China. The novel strain that is responsible for this outbreak is SARS-CoV-2 that belongs to the family of viruses known as *Coronaviridae*. Their sub-families contain Alpha, Beta, Gamma, Delta type of coronaviruses. It has become one of the major health concerns globally and the countries all over the world have taken serious measures to prevent its further spread by applying lockdown policies in the places where the number of patients is increasing significantly day by day. The exact point of origin of this novel strain is still unknown to the researchers but most of them believed that the main cause of its spread was the interaction of people living in Wuhan with a rare species of bats known as Horse Shoe bats. These bats are believed to be the major reservoir of this novel SARS-CoV-2. The strains of coronavirus obtained from these *Hipposideros* bat species have a similarity of about 84.3-92.5% with the originally sequences genomes which shows that these bats commonly known as pangolins may be the possible cause of the spread of this virus. But the over research in the area of finding origin is very low and most of the researchers are trying and struggling to find a cure. As of April 2020, there is no vaccine available for this novel strain but the researchers have started to begin animal trials in several countries.

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Transmissions

The way of transmission of this strain is yet poorly understood but most scientists have agreed on the droplet transmission pathway. It means that it can transfer from one human to others by the way of direct contact or by droplet spread in the air through coughing and sneezing. There is also very little evidence of the virus to stay air-borne for about several hours but most of the transmissions take place by droplet pathway. A little amount of research was also made on transmission through common household pets like cats, dogs, and chickens, etc. But still, there is no evidence of transmission of coronavirus through pets to humans. The only effective way to prevent the transmission of is to cover the mouth and nose while coughing and sneezing, using sanitizers and solution that at least have 70% alcohol by volume and cleaning the common surfaces regularly by using phenyl, etc. However, by using all of these interventions, only 80% of transmissions can be blocked because recent studies support the airborne transmission of the virus.

Symptoms and Proliferation Analysis

As we can infer from the name, it is a type of respiratory syndrome called severe acute respiratory syndrome caused by Coronavirus strain number 2, hence the name SARS-CoV-2. From the statistical analysis, it has been observed that babies, kids, and people of older age are most affected as compared to that of adults. The reason behind this has been explained based on the activity difference of the immune system between people of varying ages. The most common symptoms of this syndrome include cough with no sputum or mucus secretions, extreme fatigue, and severe type of fever. These symptoms vary from person to person. Some patients may also experience severe body pains, swollen and painful lymph nodes, nasal area congestion as well as the least common diarrhea. Most of the people show symptoms in 5-6 days after the first contact with the virus but it may take up to 14 days for the threshold of symptoms to reach a specific level (9). One of the major defects that were produced by coronavirus infected patients other than respiratory attack were heart-related like congestion of heart and other heart anomalies and dysfunctions. These defects were confirmed by extensive hormonal analysis of patients that were severely infected with the disease. The major hormones include for the heart analysis are the levels of NT-proBNT in blood following the concentration of cardiac muscle induced troponin. These tests fall in the category of Natriuretic Peptide tests. High levels of NT-proBNT in clinical trials showed that there were severe defects in the blood pumping ability of the heart and most of the patient deaths were eventually due to the heart attack caused by stopping of heart function (4).

Similarly, after further study and examination procedures, the higher levels of troponin were also recorded in patients with severe COVID-19. Troponin hormone is related to cardiac muscles and more than normal levels of troponin may contribute to the congestion of major cardiac muscles that control the pumping ability of the heart.

The major imbalances in hormonal levels were induced when the Human ACE2 constantly fades under the damage induced by the virus to the heart tissue. The major activities that cause these anomalies were the immediate release of inflammatory cytokines that were induced by the infection damage caused by the virus. This inflammation produces blockages in arteries of the heart and the statistics of patients drop as the extent of damage increases and progresses with time. These symptoms produced as a result of elevated levels of this specific hormone were also the major cause of death after the main respiratory infections caused by mostly all the members of the subfamily (15).

Similarly, multiple organ failure is one of the major consequences of coronavirus induced damage. The liver is most affected by his organ failure but in different circumstances, different studies also show the extent of damage caused by the toxic drugs that were used in the therapy of coronavirus. So, at this time, it is uncertain that the damage to the liver and other hepatic pathways is due to coronavirus induced

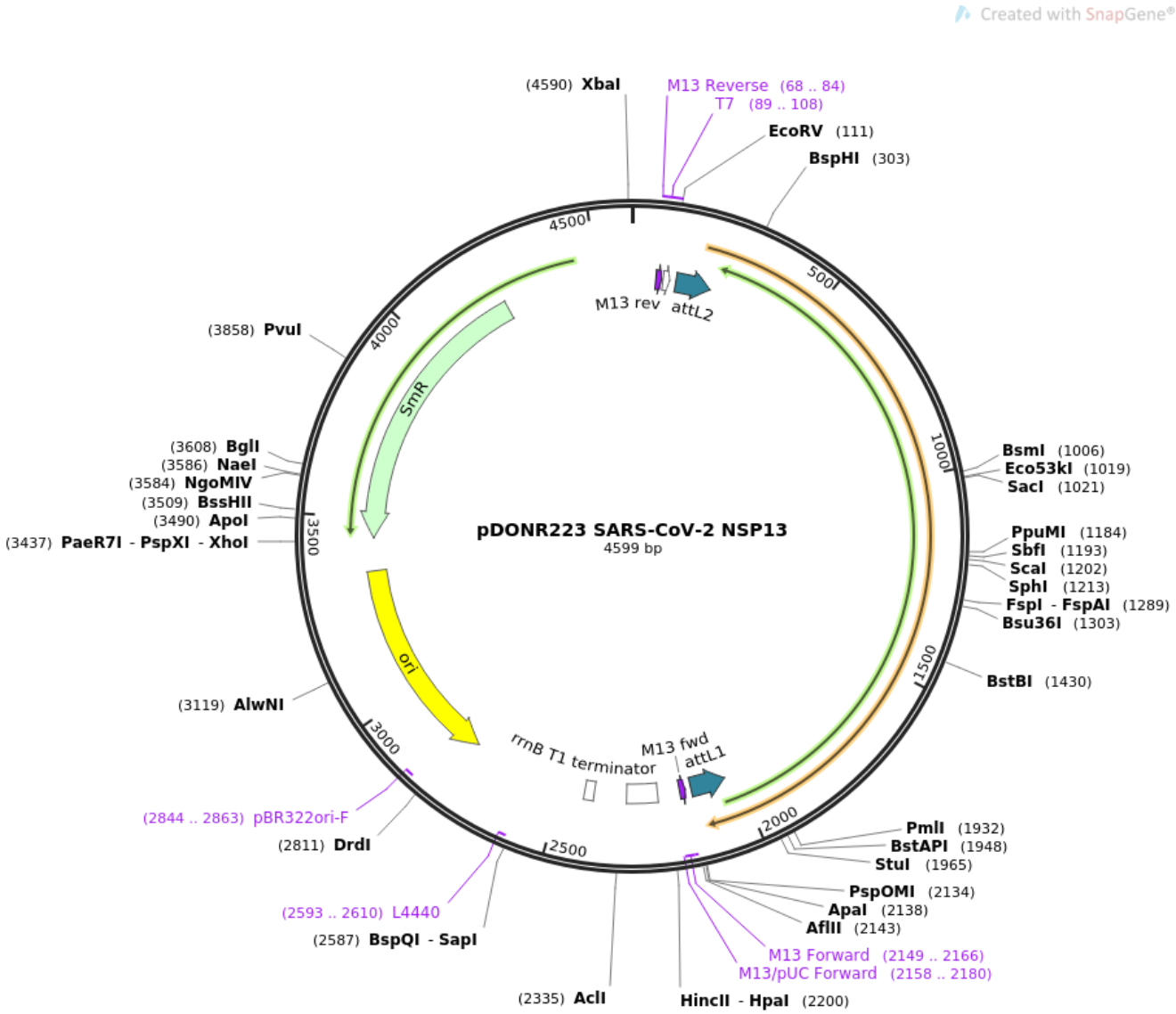


Fig. 1. Gateway-compatible Entry vector, with insert of ORF8 gene's CDS from SARS-CoV-2 isolate Wuhan-Hu-1. Does not contain stop codon (8).

damage or other factors like RDS or cytokines induced inflammatory responses to viral damage of different tissues. These studies emphasize liver-related tests and observations in SARS-CoV-2 infected patients because the statistical data slope for liver dysfunctions on the graph falls in those areas that have most cases of patients as compared to the areas with relatively less number of overall cases (3).

Genome Sequencing

After researchers have understood the basis of Coronavirus, the next major thing is to sequence the whole genome of coronavirus.

Thankfully, due to the feasibility of genome sequencing procedures and with the help of immediate bioinformatics analysis, the whole genome was sequenced by researchers in Nepal (8). The genome's phylogenetic and taxonomical analysis shows the similarity of the virus with its other neighbor strains like MERS (Middle East Coronavirus Disease) etc. The strain was isolated using the oropharyngeal swabs from the patients who have already between affected with the virus for about several days. The overall testing for the virus of all the patients was done by utilizing real-time PCR techniques.

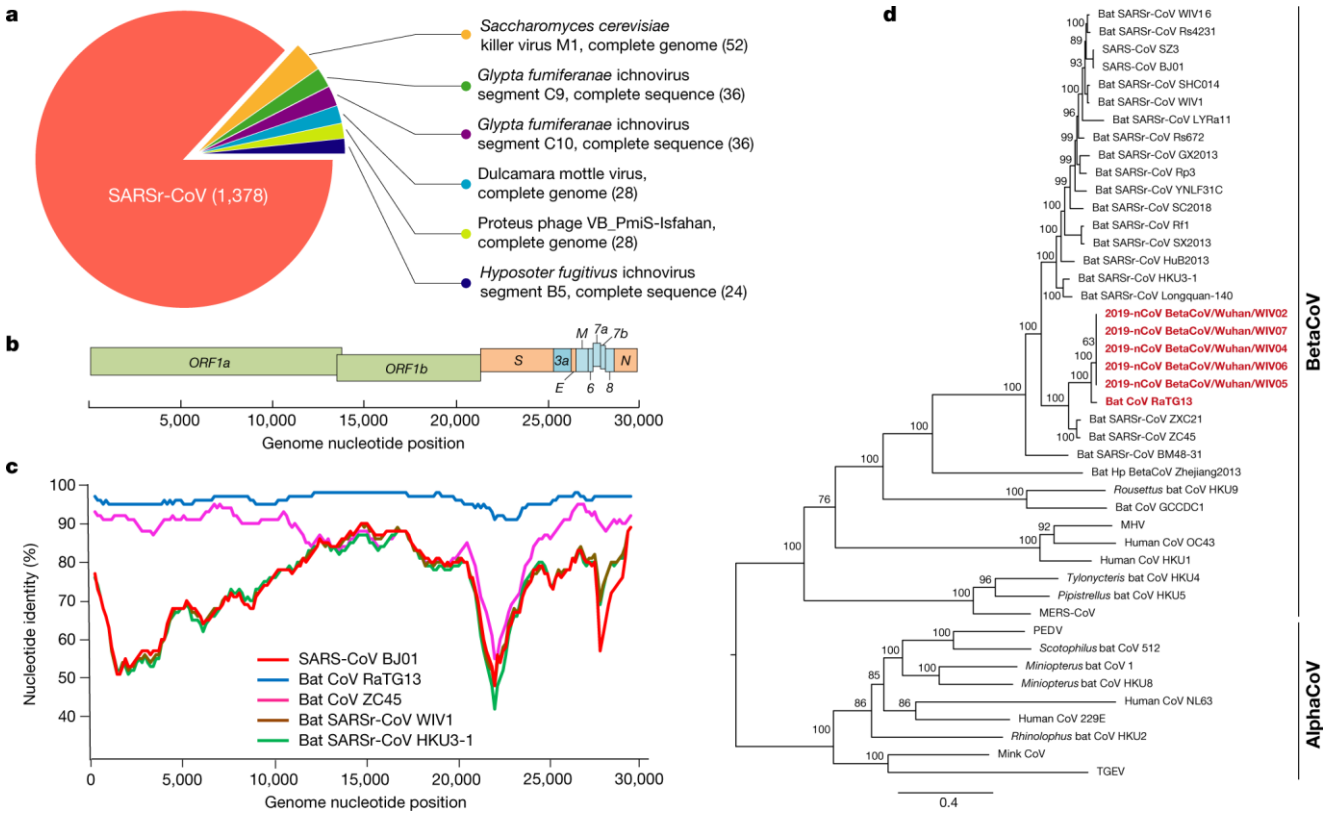


Fig. 2. a, Metagenomics analysis of next-generation sequencing of BALF from patient ICU06. **b**, Genomic organization of 2019-nCoV WIV04. M, membrane. **c**, Similarity plot based on the full-length genome sequence of 2019-nCoV WIV04. Full-length genome sequences of SARS-CoV BJ01, bat SARSr-CoV WIV1, bat coronavirus RaTG13 and ZC45 were used as reference sequences. **d**, Phylogenetic tree based on nucleotide sequences of complete genomes of coronaviruses. MHV, murine hepatitis virus; PEDV, porcine epidemic diarrhoea virus; TGEV, porcine transmissible gastroenteritis virus.The scale bars represent 0.1 substitutions per nucleotide position. Descriptions of the settings and software that was used are included in the Methods. (19).

The whole genome was amplified using the RNA that was extracted previously from the specimens and genome-specific primers were used for sequencing through different sequencing algorithms on a supercomputer.

To read the whole genome, the reference genome map was first modified according to the coherence with the original coronavirus genome. This is a feasible way while dealing with the complex whole genome of several viral species. The terminal for primers that were utilized for sequencing has an exact location between frames ORF1b and N. the overall number of ORF loci are 14 that on final analysis shows the possession of about 27 different kinds of protein that form the building block of the genome. The overall genome alignment and checking the quality of the genome are one of the crucial steps while mapping the genome of a novel species of virus. The extent of the error caused by the misalignments in the similarity analysis can produce faulty results at the end that are avoided by taking every measure possible to increase the stability of the reference genome.

Another major study based on extensive analysis of genome shows the severe accumulation of about hundreds of different mutations in the SARS-CoV-2 genome. This shows the adaptation and immediate survival factor of coronavirus within different hosts.

In this research, the number of nucleotides that were forming the genome is very different from previous researches. The approximation of nucleotides was near to 29,897. These differences were observed in genome models

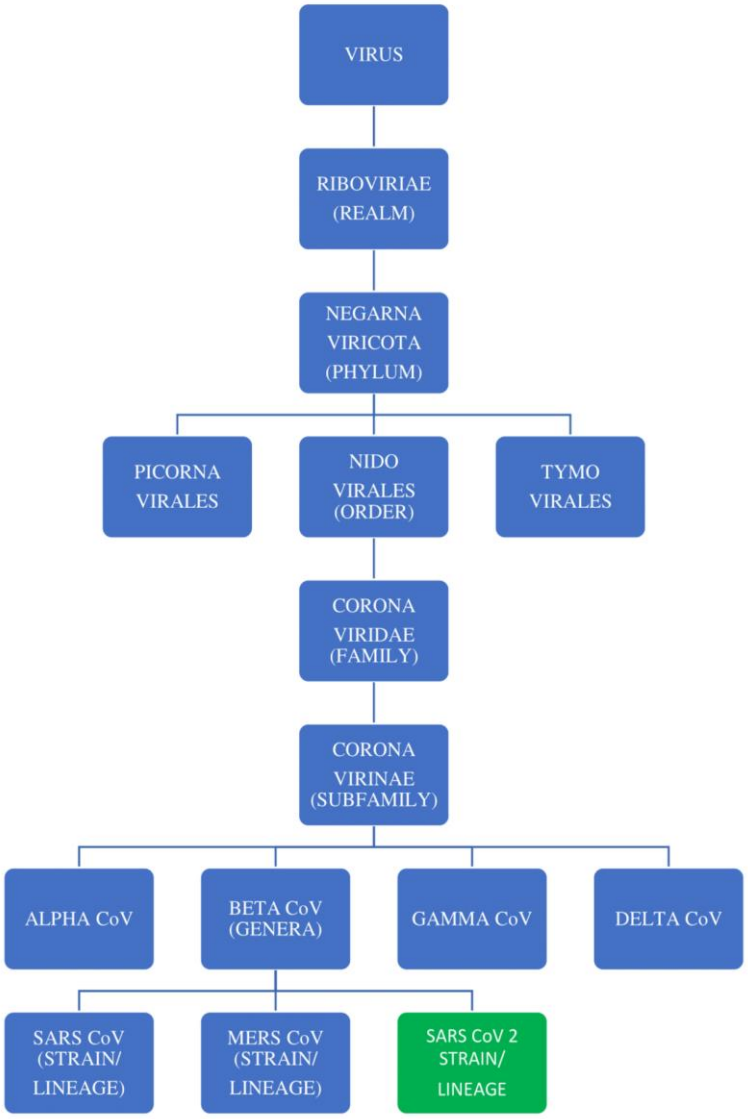


Fig. 3. The classification of the RNA group of viruses and the origin of SARS CoV-2 (12).

created from different specimens at different research centers all over the world (19). These differences were proof of extensive deletion, addition, and overall mutational capability of SARS-CoV-2. Results from this research also showed the overall variations of virus between different human populations so that the necessary measures can be taken before it spreads o other populations and creates more mutated genomes. The more the surviving genomes of a viral specie, the more it is hard to find a suitable therapy due to different case studies that have to be performed crucially in different mutated genomes from different specimens (7). While performing several observations, examinations, analytical and microscopic studies on specimens obtained from different specimens across different countries where the virus has spread. Scientists have isolated a special and novel mutated variant of the virus which is RNA dependent. The basic structure involves the backbone of a specialized and mutated RNA polymerase variant (12). An overall specimen range of 200-250 was analyzed from different parts of the world. After examining with a different reference genome that was available in the databases provided online, it was confirmed

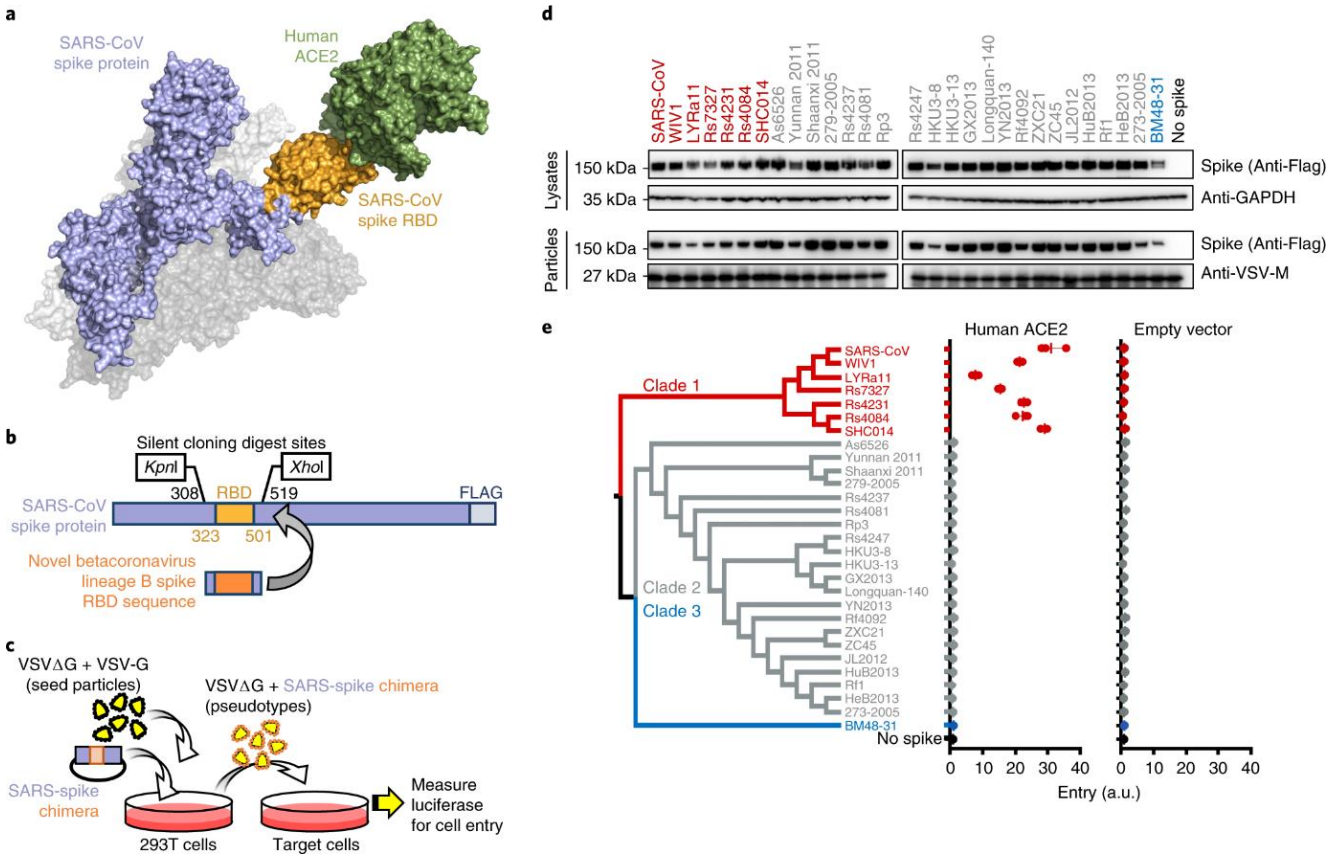


Fig. 4. a, Betacoronaviruses, including SARS-CoV, interact with the host-cell receptor via the RBD in spike (Protein Data Bank ID: 5X5B; 2AJF). **b**, Engineered silent mutations in SARS spike facilitated replacement of the RBD sequence. SARS spike amino acid numbers are indicated in black for the silent cloning sites and orange for the RBD. **c**, Outline of the experimental workflow. **d**, Western blot of producer cell lysates and concentrated reporter particles. The labels along the top show the origin of the RBD in the SARS-CoV spike protein. **e**, Cladogram of the 29 spikes tested. Cells expressing either human ACE2 or empty vector were infected with pseudotyped VSV reporter particles, and luciferase was measured and normalized to no spike as a readout for cell entry. The data are representative of three technical replicates. Vertical bars indicate mean values of all three replicates and horizontal bars indicate s.d. (10).

that this is a novel strain. Furthermore, the statistical analysis by different bioinformatics algorithms proved the overall significance of this RNA polymerase version of the original SARS-CoV-2.

One of the major causes of these types of inconsistencies in statistical data is because of the vulnerable nature of algorithms that depend upon several factors.

The three-point mutational pattern with a statistical probability of 1 to the 1000 part in another evidence in the corresponding mutat-ional analysis (10). As we can infer from the data provided by several research centers that mutation is becoming very severe as the virus is exposing to different genotypes of people of different races. Therefore, the containment strategy should be applied over the areas that are severely infected with this virus. The phenomenon of resistance to anti-viral drugs may be a major concern because of these underlying mutations but the homo-geneity with the genome of the original strain of SARS-CoV has narrowed down the research because of the previously existing enormous amount of data on the original strain (12).

Phylogenetic Analysis

One of the best services for applying phylo-genetic mapping is by using BLAST's sequential algorithms for similarity checking. Researchers have found that the overall simi-larity with the reference genome was more than 99.998%.

An astonishing amount of information about the genome of the corona-virus was revealed after the analysis through BLAST, COBALT, and other similar algori-thms that were present in the database of viral genomes. Surprisingly, all of the analyses through several databases generate the same results. The results obtained show that the overall genome consists up of 9,956,836 records after the reference genome analysis that has a similarity approximation percentiles of about 99.57% with the original standard genome. For reference, the overall genome was short after excluding the inclusion of poly-A tail after analysis with all the similar genomes that were present in the Gen Bank's database. This is proof of the novel nature of the SARS-CoV-2 strain. The overall general analysis shows that 29,856 were deposited on the single strand of RNA genome with bases approxi-mation of about 18.90% for thymine, 31.10% for Adenosine, 29.44% Guanosine as well as 21.56% for Cytosine. The overall length for the original genome was between 25 and 33 kbps (14).

Multi-dimensional analytical methods were used at first to assess the origin of SARS-CoV-2 with an additional 10 fold layering algori-thms but the deformities in graphical interpre-tation don't suggest any viable source of origin due to multi-cluster interference of factors. Though, this proved that the receptor sites on both strains of viruses were different and it is believed that this is the mutated strain of the original strain i.e., SARS-CoV.

In this study, several models were generated based on random forest analysis mechanisms by using thousands of viable strains extracted from infected patients. This study has proved to be very helpful for the prediction of where the next virus outbreak might occur as well as the control over the spread by predicting the possible surveillance zones (13).

Structural Analysis

One of the major structure that is revealed on electron microscope analysis were the huge spikes made up of glycoprotein that are embedded by some kinds of linkages with the outer membrane's surface. The surface of the spikes has some kind of receptors that help them is binding with the cell wall of the host cell and eventually invade the cell. Cell membrane binding is controlled by the S1 type of spikes while the fusion process is initiated by S2 type spikes proteins. Several accessory proteins include orf14, 7a, 7b, 3a, 3b, etc. On the other hand, four different types of major proteins are crucial to maintaining the struc-tural stability of the outer capsid and the inner matrix. These include Matrix protein, major outer nucleocapsid protein, surface glycopro-tein, and SEP.

On extensive analysis, it has been revealed that the overall difference between SARS-CoV and SARS-CoV-2 strains is very small. The main difference comes in the forms of accessory protein arrangement like 3b, 8a, and among several others. This similarity was revealed with the help of extensive phylogenetic analy-sis that was done during the structural mapping of proteins. This analysis also reveals the similarity with other neighboring species of viruses like MERS-CoV. However, most of the coronavirus species have been found in linkage with several other bat species but the research-ers also thought that the spread may be due to the consumption of virus-infected sea-food that was abundantly present in Wuhan's seafood market at the time of the outbreak (16).

The observations also show that the haemagg-lutinin-esterase factor was also missing from the genome. This gene factor performs a very important function in the sub-family of *beta-coronaviruses* but was somehow missing in the SARS-CoV-2 genome. The immune system is mostly affected by the accessory non-structural type of proteins that were present inside the matrix with several other proteins of unknown nature and func-tions. The spike protein modeling was one of the feats of study and it was done to assess the risks related to the

transmission process of coronavirus between animals and humans (1, 17).

Future Interventions

Though recent studies show that the proliferation rate of the virus was very high after the inspection of the different specimens from different regions of the world. For example, when exposing to human Calu3 cells, the overall proliferation time approximation was about 100-140 hours with a half-life of 2 days. It means that the overall population and concentration of virus doubles every two days with a higher graph rate in the end.

Intervention in the treatment of patients is narrow range as compared to the prevention measures that are effective and broad toward an overall population or public. The error ratio is even more mind bogging due to the uncer-tainty in research analyzed statistical data with varying probability. The overall probability range that was used in these researches has a minima value of P = 0.50 and a maxima value of P = 0.54. This is a very narrow range for the analysis of a pandemic and this is the reason why results are very inconsistent. Findings have concluded that ACE2 is one of the major receptors that help the SARS-CoV-2 virus to gain entry into the cell. Researchers have put their concentrations on finding the novel methods and techniques that may help to cure the COVID-19 by inhibiting this main receptor along with several other accessory proteins. Coronavirus has infected millions of people in a matter of months. This shows the exponential increase in the graph of epidemio-logy for coronavirus. After examining the basic viral kinetics and cell necrosis phenomenon it is very surprising to see that the contagious-ness, as well as the overall symptoms that are caused by the SARS-CoV-2 strain, are less severe as compared to the original SARS-CoV. This is the main reason for the good recovery and relapse rates in people after examining the statistical analysis graph related to the pandemic (2, 5).

One of the main hurdles in therapeu-tics' techniques is the basic receptor site data avai-lable for SARS-CoV-2. However, the receptor site for this novel strain is similar to the first strain of coronavirus yet some dissimilarities have been revealed by utilizing Cryo-Electron microscopic procedures. The use of cryogenic temperatures is crucial because, at higher temperatures, the computa-tional algorithms produce very inconsistent results as compared to by achieving cryogenic temperatures using liquid nitrogen. This research involves the overall mapping data that is needed for the Angiotensin-Converting Enzyme type 2 rece-ptor site on the outer surface of cells. The use of protomers is crucial when dealing with a complex viral receptor site and in this case, we utilized two protomers labeled as A and B respectively.

The ABD binding domain was first used as a necessary input but after examining the viral kinetics, the ABD domain was casing hind-rance on the entry site and isn't feasible to examine at such molecular level. So, the naked promoters were used instead and this tier proved to be excellent for the examination of the site. Finally, the 3D structure of the basic site was obtained that was proved useful after bioinformatics' analysis using different valid algorithms. This research provides the basis for understanding the overall viral transfection mechanism and interaction with human cells. This also demonstrates the homo-dimeric nat-ure of the ACE2 receptor site (18). One of the most evolving therapies that are under the focus of researchers is related to stem cells that are derived from the mesenchymal lining. This is a type of modulation of the whole immune system against the virus and has proved to be very effective as seen in the different trials at different research centers from all over the world. There were promising results in treating the major symptoms related to SARS-CoV-2. More intervention will be made in the future according to the promising results that were shown in these studies (6).

Conclusion

Coronavirus has become one of the major world problems since the last world war. Constant interventions and researches are still ongoing in finding a suitable cure. Most of the

researches has shown very promising results in areas related to immunomodulation and stem cells based therapies but still, we don't have a potent antiviral or an effective vaccine that'll eventually treat the virus from inside out.

Researchers can utilize the methods of producing potent antivirals that were previously used for developing antiviral medications for HCV and HIV etc. Though their pharmaco-kinetics would be very different scientists can have some analogy in the proper development of antiviral.

However, we can still rely on the researchers and scientists that are making constant effort and progress in treating this dreadful disease, and soon we'll find a cure as we humans always have.

Acknowledgement

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study was not financially supported by any individual, agency, or institution.

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