ISSN No:-2456-2165

Targeting Surface Glycoproteins of SARS-CoV-2 for Drug Repurposing: State of the Art and Future Opportunities

Tooba Qamar, Ruchi Yadav, Prachi Srivastava*
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, India

Abstract:- SARS (Severe Acute Respiratory Syndrome) virus is analogous to SARS-CoV-2. Both viruses share the same beta corona virus genus (lineage B). One of the crucial step for disease progression caused by novel SARS-CoV-2 involves the entry of virus into the cell. The virus entry inside the host cell is facilitated by the release of spike fusion peptide that is formed by cleavage of spike protein by the host protease. The receptor binding domain similarity of both SARS-CoV-2, delineates that ACE2 receptor is shared by both these viruses. In this research we have studied the structural similarity of COVID-19 surface glycoprotein. Protein sequence of COVID-19 surface glycoprotein was retrieved from NCBI database with Accession number: YP_009724390.1. Protein BLAST was done against PDB database to identify similar proteins. Similar proteins were identified fromMiddle East respiratory syndrome-related coronavirus, Severe acute respiratory syndrome coronavirus 2 (SARS),& other viruses PDB structures were downloaded. Homology modeling of COVID-19 surface glycoprotein was done using modeller tool and structural similarity was done using FATCAT tool. Result shows that spike protein of COVID-19 have 99% structural similarity with SARS spike protein with PDB ID:6VSB_A. Further binding sites were predicted using CASTp tool and binding sites were also compared with SARS spike protein, result shows that both proteins have similar binding sites. This study exemplifies structural similarity of COVID-19 spike protein (surface glycoprotein) with SARS virus spike protein and therefore can be considered conducive in drug repurposing of SARS inhibitors for COVID-19.

***Keywords*:-** *COVID-19; SARS; Structural Similarity; Drug Repurposing; Homology Modeling.*

I. INTRODUCTION

The viral family Coronaviridae which causes symptoms similar to pneumonia has been considered global and massive threat since its initial outbreak in 2002. The Severe Acute Respiratory Disease (SARS) and Middle Eastern Respiratory Syndrome (MERS) were unfolded in 2002 and 2013 respectively. Both of them caused gastrointestinal and pulmonary dysfunctioning maladies (1). The third Coronavirus outbreak which is SARS-COV-2 emerged in 2019, was considered responsible for COVID-19 disease. It's symptoms ranges from the common cold to respiratory infections. The Coronaviruses (CoVs) possess RNA genome with single-positive strand having size that ranges between 26.3 and 31.8 kb (2). Under an electron microscope, they exhibit the crown-like appearance on their surface (3). As they possess such morphology, thus named as Coronaviruses. These 'crown-like appearances' are actually glycoprotein in nature which are present on their envelope that facilitates their entry into the host cells (4).

One of their hallmarks of their transcription is that they produce many sub-genomic RNAs which are having sequences linked to both their genomic ends. They generate mRNAs by utilizing the RNA-dependent RNA synthesis by the genome of host. Amongst all RNA viruses the genome of CoV is largest (5). There are six to ten ORFs in the CoV genome. The infection or spread of these viruses takes place through droplet infection, direct contact, respiratory secretions, sweat etc. The initiation of infection cycle of these viruses starts with the binding to the primary target cells such as enterocytes and pneumocytes (6). Some of the other target cells of infection includes immune cells, tubular epithelial cells of kidney and cerebral neuronal cells(7).According to WHO, COVID-19 has been declared pandemic and it infected approximately 20 million people of all age groups (8).

A. Proteome of SARS-Cov-2

The entire genomes of SARS-CoV-2 have approximately 29,822 nucleotides which encodes for about 29 kinds of proteins (9).The SARS-CoV-2 proteome is divided into the following sections as depicted in table 1.

Table 1. Proteome of SARS-Cov-2 and its Features (10)

S. No	SARS-CoV-2 Proteome	Proteome size (No. of amino acids)	Position / location in sequence of genome
1	Spike (S) protein	1256	Orf2 (21492–25259)
2	Envelope (E)protein	76	Orf4 (26117–26347)
3	Membrane (M) protein	221	Orf5 (26398–27063)
5	Nucleocapsid(N) protein	422	Orf9a (28120–29388)
6	Non-structural proteins (Nsp1)	180	Orf1a (265–804)
7	Nsp2	637	Orf1a (805–2718)
8	Nsp3	1922	Orf1a (2719–8484)
9	Nsp4	500	Orf1a (8485–9984)
10	Nsp5	306	Orf1a (9985–10902)
11	Nsp6	290	Orf1a (10903–11772)
12	Nsp7	83	Orf1a (11773–12021)
13	Nsp8	198	Orf1a (12022–12615)
14	Nsp9	113	Orf1a (12616–12954)
15	Nsp10	138	Orf1a (12955–13371)
16	Nsp11	14	Orf1a (13372–13410)
17	Nsp12	932	Orf1b (13398–16166)
18	Nsp13	601	Orf1b (16167–17969)
19	Nsp14	527	Orf1b (17970–19550)
20	Nsp15	346	Orf1b (19551–20588)
21	Nsp16	298	Orf1b (20589–21482)
22	Orf3a	274	Orf3a (25268–26092)
23	Orf3b	154	Orf3b (25689–26153)
24	Orf6	63	Orf6 (26913–27265)
25	Orf7a	122	Orf7a (27273–27641)
26	Orf7b	44	Orf7b (27638–27772)
27	Orf8a	39	Orf8a (27779–27898)
28	Orf8b	84	Orf8b (27864–28118)
29	Orf9b	98	Orf9b (28130–28426)

➢ *Structural proteins of SARS-COV-2*

- *Spike (S) protein:* It is a transmembrane glycoprotein comprising of 1,255 amino acids and it is further cleaved into segments S1 and S2. They are cleaved as to easily bind to ACE2 receptors. Any mutational change in spike proteome of SARS-CoV-2 facilitates the Furin enzyme to perform the proteolytic cleavage (11).
- *Nucleocapsid (N) protein:* It is a dimer having 422 amino acids that tightly bounds the genome of a virus to membrane forming a protective sheath or shell. It shows non-specific nucleic acid binding capability.
- *Membrane (M) protein:* It is most copious structural matrix protein of the virion having 221 amino acids. It is responsible for defining the shape of an envelope. It possess a triple helix bundle forming a single 3-transmembrane domain. It helps the spike protein during attachment of cell and entry into the host (12).
- *Envelope (E) protein*: It's a small membrane protein comprising of 76 amino acids that are engaged in viral particle assembly, budding and pathogenicity.

➢ *Non-Structural Proteins of SARS-COV-2*

They are usually expressed as two long polyproteins i.e. pp1a and pp1ab, which are further cleaved into 16 smaller mature proteins by the action papain-like protease and 3-chymotrypsinlike protease(13).

- *Nsp1 :* It is having 180 amino acids in which there are total 29 insertions that are observed along its amino-acid sequence. It mediates the suppression of type I Interferon (IFN) expression.
- *Nsp2 :* It is having 637 amino acids and interacts with host protein complex (PHB1 and PHB2) which are implicated mitochondrial biogenesis. The methyltransferase sequence in position 21 has polar amino acid while SARS like coronavirus of bat has non-polar amino acid. Due to their polarity and tendency to form Hydrogen bonds they are highly stable (14).
- *Nsp3 :* It is having 1,922 amino acids . This protein is responsible for downregulating the enzymes of host which are known as poly ADP ribose polymerase (PARPs). They prevent the viruses from replication.
- *Nsp4 :* It is having 500 amino acids and it helps nsp3 in rearrangement of membrane of host cell .They work synergistically .
- *Nsp5 :* It is having 306 amino acids and it is cleaved at 11 sites. It is highly homological to SARS NSP5 with 95% identity and 97% similarity.
- *Nsp6:* It is having 290 amino acids and helps nsp3 and nsp4. Like in other coronaviruses, It represents 7 trans-membrane helices According to the recent studies, There were two mutations detected which is involved in pathogenesis of covid-19 (15).

- *Nsp7 and Nsp8* : They are having 83 and 198 amino acids respectively. The primase complex of SARS-coronavirus is found to be involved in de novo initiation along with the amplification of primer. They even trigger RNA-synthesizing activity of nsp12. Among 2019-nCoV, Beta CoV-RaTG, NS7b and NS8, were exclusively conserved. Any kind of change in their function can act as a biomarker for the viral prognosis (16).
- *Nsp9* : It is having 113 amino acids and is engrossed in reproduction of genomic RNA of virus. The structural analysis of the SARS-CoV-2 Nsp9 unveiled the presence of high level of structural conservation within it.
- *Nsp10:* It is having 138 amino acids that forms a complex in association with NSP16. Such complexes basically capped transcript of viral mRNA in order to undergo efficient translation. It is also involved in evading immune system surveillance.
- *Nsp11:* It is having 13 amino acids and is having overlapping sequence with nsp10.The function of its short peptide is not discovered.
- *Nsp12 :* It is having 932 amino acids. The RNA polymerase complex comprises of viral RdRp which is nsp12 along with corresponding cofactors like nsp7 and nsp8. The study of their alignment shows that they are analogous SARS-CoV with having 96% identity and 98% similarity index (17).
- *Nsp13* : It is having 601 amino acids. They have pyramidal shape with 5 domain like SARS & MERS-Nsp13. It acts as a helicase that helps in unpacking of genome of virus to make it easily accessible.
- *Nsp14:* It is having 527 amino acids and is having the proofreading activity as compared to normal CoV recombination. Thus, an exon of nsp14 has indispensable role in high fidelity of replication and recombination, therefore representing a much conserved target to inhibit viral replication and attenuation (18).
- *Nsp15:* It is having 346 amino acids. According to one of the latest research earlier these proteins were thought to only participate in replication of virus but recently they are associated in producing the innate immune response, they also possess the capability to degrade the RNA of a virus inorder to protect them from host.
- *Nsp16:* It is having 298 amino acids and is involved in forming a NSP10 complex, so as to cap the transcript of viral mRNA for translational phenomenon. Such process helps in evading immune surveillance (19).

➢ *Accessory Proteins of SARS-COV-2*

- *ORF3a:* It is having 275 amino acids. The presence of protein 3a is distinctive feature of SARS-CoV and is a major component for causing pathogenesis, infectivity & viral release.

- *ORF3b:* It is having 154 amino acids and the short peptide named 3b is a potent IFN-1 antagonist. The ORF3b sequences of SARS-CoV-2 are considerably shorter than SARS-CoV orthologs (153.2 ± 0.47 amino acids on average) (20).
- *ORF6 :* It is having 63 amino acids and is an antagonist of IFN-1. It is involved in disrupting transportation of karyopherin of STAT 1 which is one of the transcription factors.
- *ORF7a:* It is having 122 amino acids. They possess the capability to directly bind with BST-2 inorder to inhibit its activity. They worked by blocking the process of glycosylation of BST-2.
- *ORF7b:* It is having 44 amino acids and possesses overlapping sequence with ORF7a. It's not only an accessory protein but also they are structural component of virion of SARS-CoV (21).
- *ORF8:* It is having total 123(ORF8a-39 and ORF8b-84) amino acids. It is the most unique protein as compared to SARS-CoV having only 30% homology. It has been recently reported that a deletion of nucleotide stretch of 382 amino acids deletes the transcription-regulatory sequence (TRS) also. Such activity escalates the transcription of N gene at downstream position (22).
- *ORF9b :* It is having 98 amino acids and is encoded within the N gene. These proteins directly interact with translocase of the outer membrane (Tom 70) which is a type of mitochondrial import receptor Tom70. It acts as an important linking adaptor that connects the MAVS to TBK1/IRF3. Such kind of association results into IRF-3 activation.
- *ORF9c:* It is coded within the N gene and it is reported that they are hijacked by the receptors of sigma 2 (23). They have the capability to interact with many protein moieties that help in modulating the signaling pathway of Inhibitory kappa B kinase (IkB kinase) and Nuclear Factor kappa B .
- *ORF10 :* It is having 38 amino acids and doesn't possess alike proteins in the NCBI repository for SARS-CoV . The stop codon with prematurity was found in both SARS-CoV & BM48-31 which were considered unique to SARS-CoV-2 (24).

B. Current Drug Target Proteins For COVID- 19

The envelope (E), membrane (M) and Nucleocapsid (N) protein of SARS-CoV-2 are important for survival and propagation of the virus, and therefore these structure based proteins are regarded best for targeting drugs (25). It is summarized in Table2. As these structural proteins possess a different structure as compared to proteins of the host, therefore the drug targets on such proteins will have nil side effects. These proteins not only protect the genome of virus but also suppressed the immune system of the host machinery.

Table 2. Recent Drug Targets with their Structure and Function (26):

S.No	Drug targets	Structure	Functions	Advantages
1	Spike protein (S)	Type-I transmembrane protein with amino acids ranging from 1,161-1,403 amino acids. Contains 20 to 36 N-glycosylation regions and ectodomain have N-terminal domain (S1) and a C-terminal (S2) domains.	Facilitates receptor recognition and fusion of cell membrane and thus mediates easy entry of virus into the host.	Most preferred immunological target for vaccines production. Induces potent neutralizing-antibodies.
2	Envelope protein (E)	Main domains this protein includes the charged cytoplasmic tail and hydrophobic domain.	Helps in morphogenesis of virus during the assembly of viral particle . Acts as a virulence factor.	Shows promising in vitro . Associated with low toxicity.
3	Membrane protein (M)	Have three transmembrane domains which are having the long C-terminal present in the inner side and short N-terminal present outside.	Helps in sensitization of the host by the viral particles. Maintains the shape of an envelope of a virus.	Broad spectrum drug target.
4	Nucleocapsid protein (N)	Have three main intrinsic characteristic intrinsically disordered regions (IDRs) that are N-arm, central linker (CL), and C-tail. The structure of RNA binding domain of N-protein, comprises of five-stranded β-sheet.	Helps in genomic RNA binding by leader sequence, leading to the helical ribonucleoprotein complex formation.	Exhibits effective outcomes in *in vitro* and *in vivo* studies.
5	Protease	Genome of coronaviruses having replicase gene that encodes for 16 NSPs in further large Protein Phosphatases (PP1a and PP1ab).	Cysteine proteases acts on these two phosphatases for releasing the NSPs.	Shows active results against MERS-CoV.
6	Hemagglutinin esterase	Are viral envelope glycoproteins. Have oligosaccharide chains in the E, F, and R domains .	Acts as lectins and facilitates the attachment O-acetylated-sialic-acids .	Acts as an excellent viral evolution marker.
7	Helicase	Belongs to the family of steroidogenic factor 1. Possess ring-shaped structure.	Plays crucial role in replication of virus and thus maintains its life cycle.	Recognized as an efficient drug target with better efficacy in anti-SARS therapy.

C. Drug Development and Discovery Methods for COVID 19

The antiviral drugs which targets the SARS-CoV-2 are subdivided into two further classes. First class focus on targeting upon the positive interactions between the virus and host and they even inhibit the viral particles orientation and assembly. The second class consists of those drugs that are engaged in impeding an innate immune response of the host or they can directly interfere and inhibit the signaling pathways of replication of viruses (27). These drugs even engage many receptors of host for the easy viral entry. Fundamentally, screening of antiviral compounds can be achieved by three approaches that possess the capability of hampering COVID-19 resolution(figure 1). These approaches are as follows-

- *Antiviral Compounds Repurposing:*

Initial steps involves the examination of antiviral compounds that are pre-existing along with their estimation of potent effect on viral packaging & replication machinery. The antiviral activities of many potent and efficient moieties like interferon alpha, ribavirin and chemical inhibitors of cyclophilin can be helpful for analysing their effects against coronaviruses (28).These drug molecules possess an advantage as their pharmacodynamic and pharmacokinetic properties are known by active clinical uses.

- *Insilico Approach:*

These approaches regarding repurposing of drugs are solely depends on generation of data. It involves a analysis of chemical structures, expression of genes , proteomics data along with records of health care (29). The computational molecular docking, Signature matching , analysis of genomic association and network or pathway mapping are the most important widely used computational or Insilco approaches . It is depicted in Figure1.

- *Computational Molecular Docking:*

The computational molecular docking is one of the crucial tool for repurposing of activities of the drugs. The structural -computational based strategies are used for calculating the binding efficiency of the target molecule and the drug. With the help of this approach, screening can be

done for many large and small drug molecules that were pre-existed against a particular disease (30). Apart from having many advantages like generation of large number of docked compounds with favourable confirmations having maximum complementarity between them , they possess certain disadvantages like maintenance of the rigid target molecule which becomes difficult for mode of binding due to lack of flexibility and therefore reducing the accuracy (31).

- *Inhibition of SARS-Cov-2 Replication Interceded by Sirna:*

This method involves the development of novel targets that are actually the results of genome and biophysical based study of SARS -CoV-2 cycle. The inhibitors of siRNA moieties have been used to inhibit the activities of specific viral enzymes that are engaged in replication of viral cycle. It directly targets ACE-2 host receptor (32). This approach can acts as a stepping step in designing more specific anti-viral drugs in future against SARS-CoV-2. For development of such an efficient therapies , one should have proper knowledge of siRNA based therapies (33).

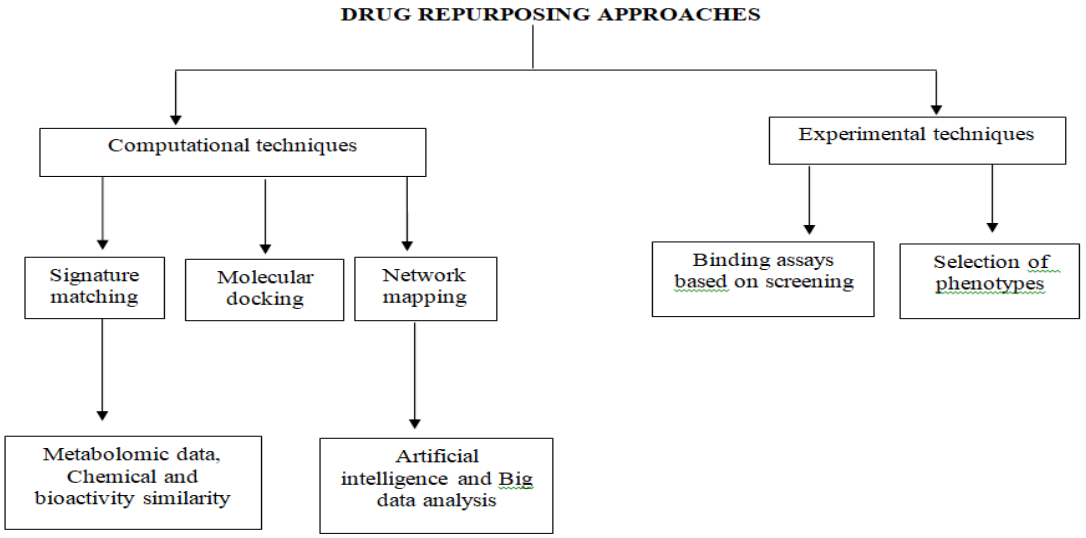


Fig 1. Current Drug Target Approaches Against SARS-Cov-2

II. MATERIALS AND METHODS

A. Structural Analysis Of COVID-19 Surface Glycoprotein

The methodology for the structural evaluation of surface glycoprotein of covid-19 is shown in figure 2 and stepwise detail is mentioned below: -

- *Protein sequence of COVID-19 surface glycoprotein was retrieved from NCBI database with Accession number: YP_009724390.1.*
- *Protein BLAST was done against PDB database to identify similar proteins. Similar proteins were identified from Middle East respiratory syndrome-related coronavirus, Severe acute respiratory syndrome coronavirus 2 (SARS) , and other viruses and PDB structures were downloaded.*
- *The Homology modeling of COVID-19 surface glycoprotein was done using modeller tool*
- *The structural similarity was also done using FATCAT tool. Result shows that spike protein of COVID-19 have 99% structural similarity with SARS spike protein with PDB ID:6VSB_A.(30)*
- *Further binding sites were predicted using CASTp tool and binding sites were also compared with SARS spike protein, result shows that both proteins have similar binding sites.*

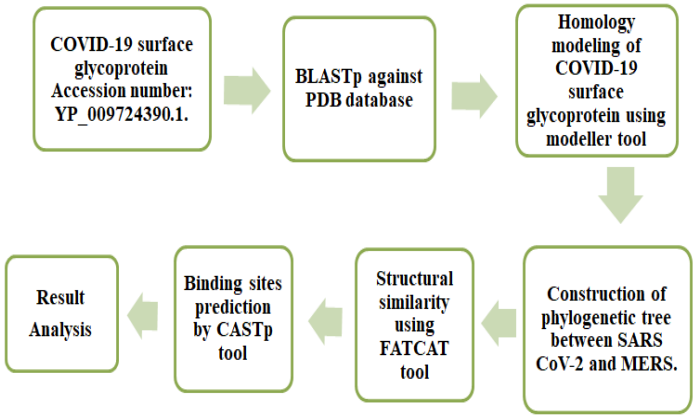
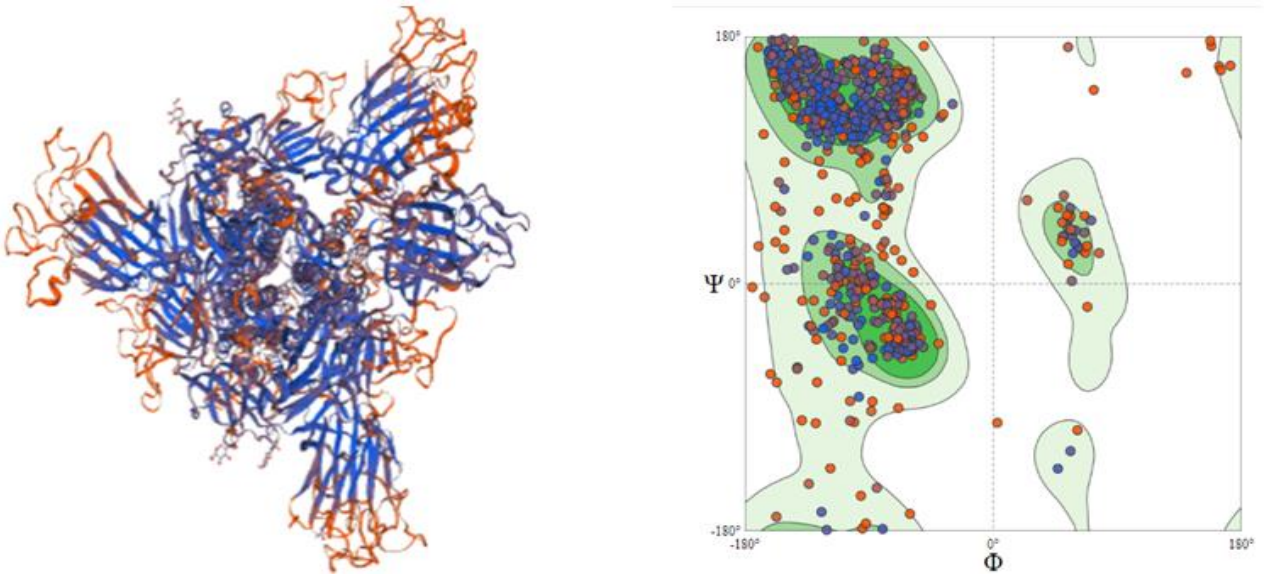


Fig 2. Methodology for Structural and Functional Analysis of COVID-19 Surface Glycoprotein

III. RESULTS AND DISCUSSION

A. Homology Modeling of Surface Glycoprotein SARS Co V

The 3D model of Surface Glycoprotein SARS CoV receptor was generated using the online modeller tool and SWISS-Model was used to generate Ramachandran plot of SARS CoV. The selection of best model was done on the basis of lowest z- score and valid q-mean score (34). Modeled structure of Surface Glycoprotein SARS Co V protein is shown in figure 3A.



A **B**

Fig 3. Structure Prediction & Validation of Surface Glycoprotein SARS Co V. (A) Modeled 3D Structure of Surface Glycoprotein SARS Cov by Modeller Tool (B) Ramachandran Plot of SARS Cov Obtained by SWISS Model

Evaluation of amino acid residues in the allowed and disallowed region along with the overall stereochemical property of the modeled structure of protein was done by the Ramachandran plot (34). It depicts 91.1% in the favored region (figure. 3B), illustrating that dihedral angles, psi and phi of backbone in the modeled structures were in reasonably accurate position, thus indicating the high reliability of the predicted SARS CoV model.

B. Phylogenetic Tree Showing SARS Cov-2 & MERS Evolution

As per the data received by genomic sequence of closely related species, the characteristics of genome and evolution of SARS-CoV-2 were taken into consideration for further studies. For phylogenetic analysis the entire genomic sequences were used.

The results of the phylogenetic analysis revealed that SARS-CoV-2 genome have the nucleotide identity with the genome of MERS. We found that SARS CoV-2 are somewhat closely related to MERS. Here, trees are based on amino acid sequences and are mid-point rooted (Figure. 4).

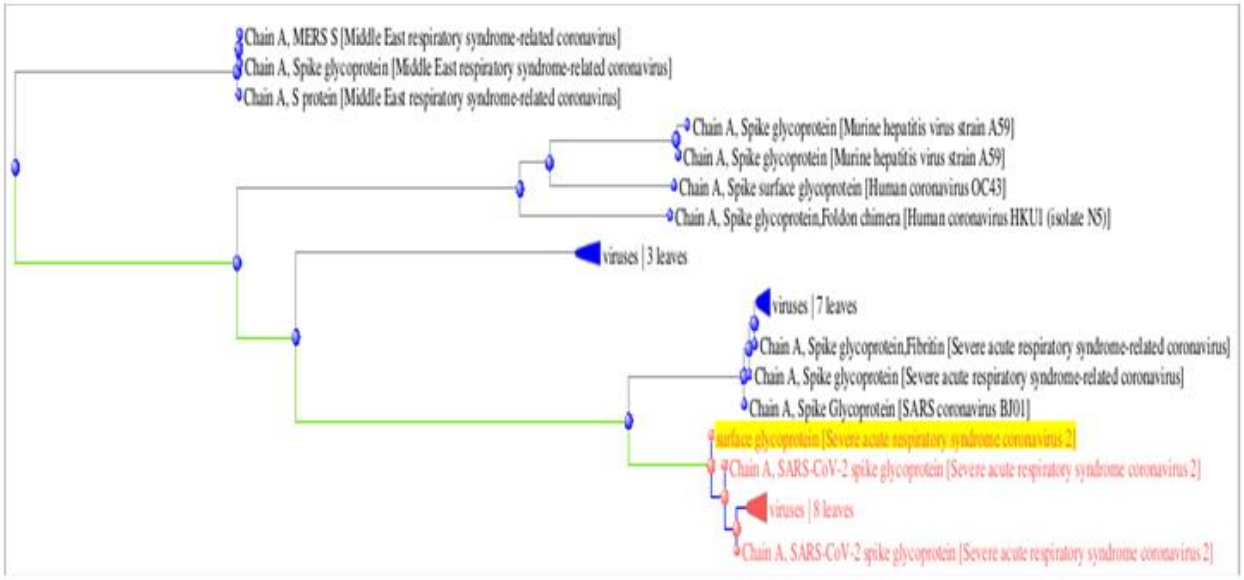


Fig 4. The Construction of Phylogenetic Tree to Depict the Evolutionary Relationship Between SARS Cov-2 and MERS

C. Structural Comparison Of Spike Glycoprotein Of SARS Cov-2 And MERS Virus Using FATCAT Tool

The result shows that the spike protein of COVID-19 have 99% structural similarity with SARS spike protein with PDB ID:6VSB_A. It helps them to enter the host cells by mediating the process of binding to host cell surface receptor and ultimately leading to the viral and host membranes fusion (35).

ISSN No:-2456-2165

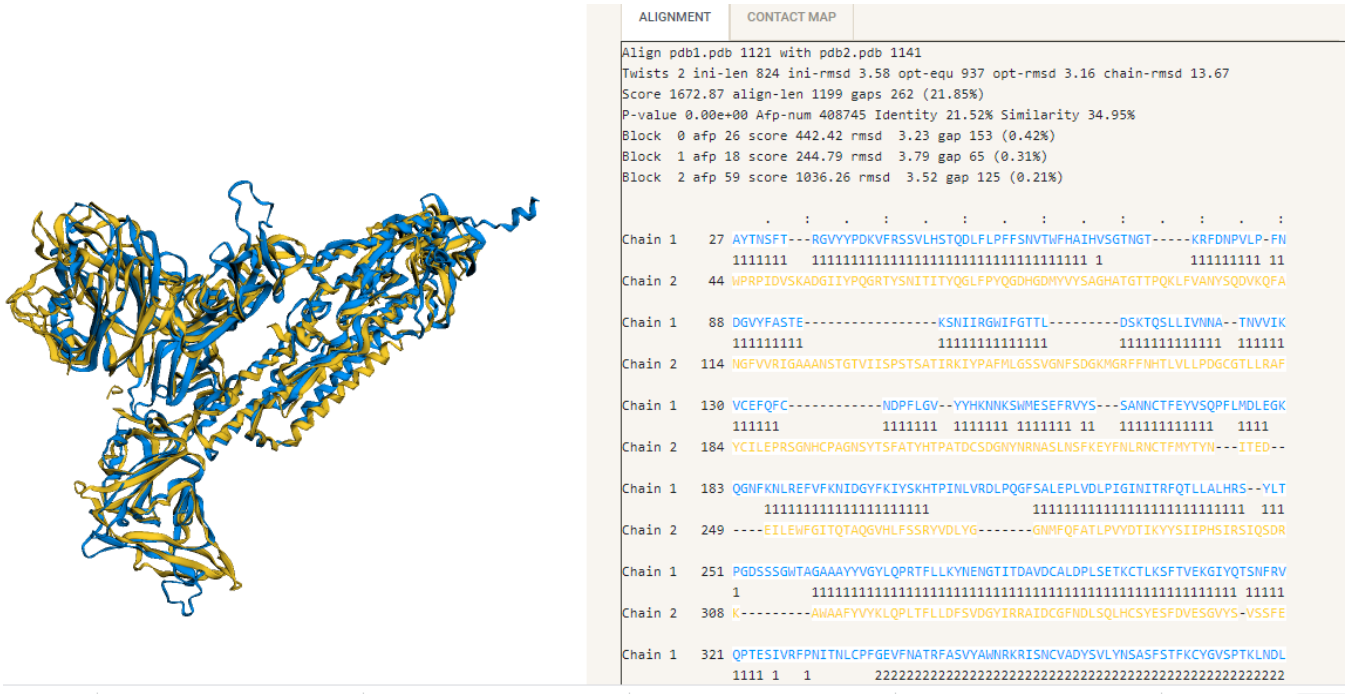


Fig 5. Structural Similarity was Also done Using FATCAT Tool

The structure determination of coronavirus S1 domains help in delineating a better insight into the evolutionary track of coronavirus S1 with that of SARS spike domain (36). The structural and functional similarities between coronavirus S2 with that of spike domain of SARS was reported (figure. 5).

D. Prediction of SARS Cov-2 Binding Sites Using Castp

The result shows that both proteins SARS and SARS-CoV-2 share similar binding sites. Both utilizes the human ACE-2 as an entry receptor and entry activators as human proteases.

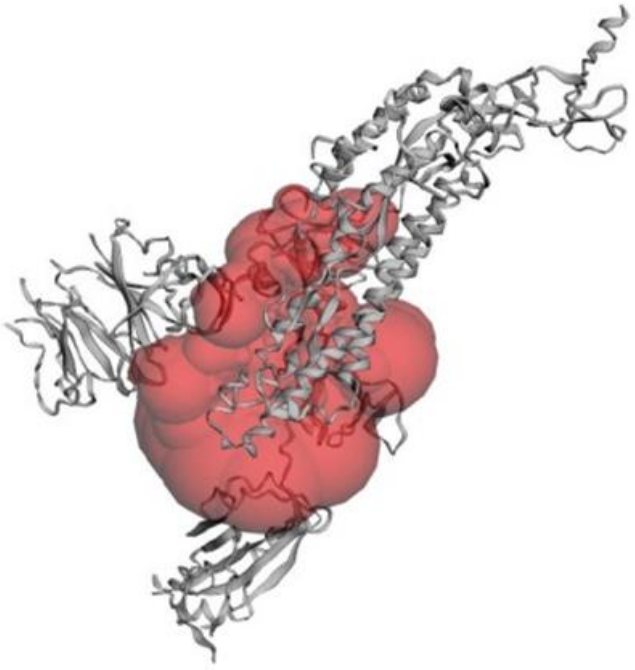


Fig 6. Binding Sites were Predicted Using Castp Tool and Binding Sites were Also Compared with Sars Spike Protein

The surface spike protein of SARS-CoV-2 helps in interceding their entry into various cells (37). For this fulfillment, spike protein of SARS-CoV-2 binds to its human receptor ACE2 (also known as hACE2) by its receptor-binding domain (RBD) that specifically recognizes it (figure. 6).

IV. CONCLUSION

The discovery of drugs and vaccines against the Coronaviruses is most challenging step. There is also a need of considering the details of structural biology dealing with the life cycle of the Coronaviruses, such parameters can proved to be helpful in developing more efficient drug and vaccine in future (38) .The infectious diseases that originates and spreads from the animals are having viruses that mutates itself making it difficult to inhibit it's progression. Even though the world suffered a lot from two outbreaks caused by coronaviruses which is SARS and MERS, still we are unprepared to deal with it because of high mutation rate of these viruses, the scientist cannot develop a potent inhibitor against it (39). Many countries are currently working on the concept of drug repurposing , so as to develop more efficient antiviral drugs against coronaviruses that further block the replication cycle effectively (40). There is also an urgent need to pay more emphasis on prognosis and diagnosis of these viruses so as to check its further progression and community transmission. The results of the comparative genomic data analysis can proved to be useful for the identification of more potent drug target that can block the binding site of these viruses. Homology modeling of COVID-19 surface glycoprotein was done using modeller tool and Ramachandran plot was obtained by SWISS model. The Ramachandran plot depicts 91.1% in the favored region . This indicates that modeled structure of SARS CoV is good with structural stability and reliability and thus can be taken into consideration for further studies. The structural

similarity was also done using FATCAT tool. Result shows that spike protein of COVID-19 have 99% structural similarity with SARS spike protein with PDB ID:6VSB_A. The results of the phylogenetic analysis revealed that genome of SARS-CoV-2 shares the nucleotide identity with that of MERS genome. Further binding sites were predicted using CASTp tool and binding sites were also compared with SARS spike protein, result shows that both proteins have similar binding sites. Both the viruses SARS-CoV-2 and SARS utilizes the ACE-2 (human) as an entry receptor and activators. This study shows that COVID-19 spike protein also called as surface glycoprotein have structural similarity with SARS virus spike protein. This research can be helpful in drug repurposing of SARS inhibitors for COVID-19.

REFERENCES

[1]. Al-Osail AM, Al-Wazzah MJ. The history and epidemiology of Middle East respiratory syndrome corona virus. Multidiscip Respir Med. 2017;12:20.
[2]. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012;4:1011–33.
[3]. Hilgenfeld R. From SARS to MERS: Crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J. 2014;281:4085–96.
[4]. Chang CK, Lo SC, Wang YS, Hou MH. Recent insights into the development of therapeutics against coronavirus diseases by targeting N protein. Drug Discovery Today. 2016;21:562–72.
[5]. Paules CI, Marston HD, Fauci AS. Coronavirus infections-More than just the common cold. JAMA. 2020;323:707.
[6]. Saif LJ. Animal coronaviruses: What can they teach us about the severe acute respiratory syndrome? Rev Sci Tech. 2004;23:643–60.
[7]. Yang H, Bartlam M, Rao Z. Drug design targeting the main protease, the Achilles' heel of coronaviruses. Curr Pharm Des. 2006;12:4573–90.
[8]. Alsaadi JE, Jones IM. Membrane binding proteins of coronaviruses. Future Virology. 2019;14:275–86.
[9]. McBride R, van Zyl M, Fielding BC. The coronavirus nucleocapsid is a multifunctional protein. Viruses. 2014;6:2991–3018.
[10]. Li F. Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol. 2016;3:237–61.
[11]. Tegally H, Wilkinson E, Giovanetti M, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv* 2020;2020.12.21.20248640.
[12]. Guo Y, Korteweg C, McNutt MA, Gu J. Pathogenetic mechanisms of severe acute respiratory syndrome. Virus Res. 2008;133:4–12.
[13]. Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: Implications for pathogenesis and virus transmission pathways. J Pathol. 2004;203:622–30.
[14]. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426:450–4.
[15]. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 2005 ; 69:635–64.
[16]. Mubarak A, Alturaiki W, Hemida MG. Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Infection, Immunological Response, and Vaccine Development. J Immunol Res. 2019;2019:1–11.
[17]. teVelthuis AJ, van den Worm SH, Snijder EJ. The SARS-coronavirus nsp7+nsp8 complex is a unique multimeric RNA polymerase capable of both *de novo* initiation and primer extension. Nucleic Acids Res. 2012;40:1737–47.
[18]. Stobart CC, Sexton NR, Munjal H, Lu X, Molland KL, Tomar S, et al. Chimeric exchange of coronavirus nsp5 proteases (3CLpro) identifies common and divergent regulatory determinants of protease activity. J Virol. 2013;87:12611–8.
[19]. Wang H, Xue S, Yang H, Chen C. Recent progress in the discovery of inhibitors targeting coronavirus proteases. Virol Sin. 2016;31:24–30.
[20]. Egloff MP, Ferron F, Campanacci V, Longhi S, Rancurel C, Dutartre H, et al. The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. Proc Natl Acad Sci U S A. 2004;101:3792–6.
[21]. Hu T, Chen C, Li H, Dou Y, Zhou M, Lu D, et al. Structural basis for dimerization and RNA binding of avian infectious bronchitis virus nsp9. Protein Sci. 2017;26:1037–48.
[22]. Bouvet M, Lugari A, Posthuma CC, Zevenhoven JC, Bernard S, Betzi S, et al. Coronavirus Nsp10, a critical co-factor for activation of multiple replicative enzymes. J Biol Chem. 2014; 289:25783–96.
[23]. Harrison C. Coronavirus puts drug repurposing on the fast track. Nat Biotechnol. 2020;38(4):379–381.
[24]. Narayanan K, Maeda A, Maeda J, Makino S. Characterization of the coronavirus M protein and nucleocapsid interaction in infected cells. J Virol. 2000;74:8127–34.
[25]. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: Recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14:523–34.
[26]. Nieto-Torres JL, Dediego ML, Alvarez E, Jiménez-Guardeño JM, Regla-Nava JA, Llorente M, et al. Subcellular location and topology of severe acute respiratory syndrome coronavirus envelope protein. Virology. 2011;415:69–82.

[27]. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV-a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009;7:226–36.
[28]. Lin SY, Liu CL, Chang YM, Zhao J, Perlman S, Hou MH. Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target. J Med Chem. 2014;57:2247–57.
[29]. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses-drug discovery and therapeutic options. Nat Rev Drug *Discov*. 2016;15:327–347.
[30]. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. Ne Engl J Med. (2020) 382:1199–207.
[31]. Shirato K, Kawase M, Matsuyama S. Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMPRSS2. J Virol. (2013) 87:12552–61.
[32]. Shimamoto Y, Hattori Y, Kobayashi K, Teruya K, Sanjoh A, Nakagawa A, et al. Fused-ring structure of decahydroisoquinolin as a novel scaffold for SARS 3CLprotease inhibitors. Bioorg Med Chem. 2015;23:876–90.
[33]. Three Drugs Fairly Effective on Novel Coronavirus at Cellular Level-Xinhua. [Last accessed on 2020 Feb 03]. Available from: http://www.xinhuanet.com/english/2020-01/30/c_138742163.htm .
[34]. Coronavirus Outbreak: Cocktail of Flu, HIV Drugs Appears to Help Fight Virus, Say Thai Doctors. India Today. 2020. Feb 03, [Last accessed on 2020 Feb 03]. Available from: https://www.indiatoday.in/world/story/coronavirus-outbreak-cocktail-flu-hiv-drugs-treatment-thaidoctors-1642783-2020-02-03.
[35]. Báez-Santos YM, Barraza SJ, Wilson MW, Agius MP, Mielech AM, Davis NM, et al. X-ray structural and biological evaluation of a series of potent and highly selective inhibitors of human coronavirus papain-like proteases. J Med Chem. 2014; 57:2393–412.
[36]. Ghosh AK, Takayama J, Rao KV, Ratia K, Chaudhuri R, Mulhearn DC, et al. Severe acute respiratory syndrome coronavirus papain-like novel protease inhibitors: Design, synthesis, protein-ligand X-ray structure and biological evaluation. J Med Chem. 2010;53:4968–79.
[37]. Ratia K, Saikatendu KS, Santarsiero BD, Barretto N, Baker SC, Stevens RC, et al. Severe acute respiratory syndrome coronavirus papain-like protease: Structure of a viral deubiquitinating enzyme. Proc Natl Acad Sci U S A. 2006; 103:5717–22.
[38]. Yang H, Yang M, Ding Y, Liu Y, Lou Z, Zhou Z, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc Natl Acad Sci U S A. 2003; 100:13190–5.
[39]. Chuck CP, Chen C, Ke Z, Wan DC, Chow HF, Wong KB. Design, synthesis and crystallographic analysis of nitrile-based broad-spectrum peptidomimetic inhibitors for coronavirus 3C-like proteases. Eur J Med Chem. 2013;59:1–6.
[40]. Chuck CP, Wong KB. Crystal Structure of Human Coronavirus NL63 3C-like Protease.Available from: https://www.rcsb.org/structure/3TLO .