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Autumn 2022, Volume 7, Issue 27 (14-20)

SARS-CoV2: A perspective on genetic and protein structure, function and potent treatments with a comparison with other coronaviruses

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DOI: 10.22034/pmj.2022.700888

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Submitted: 2022-08-02
Accepted: 2022-08-26

Keywords:
SARS-CoV2
MERS
ACE2
Anti-viral drugs

Abstract:
Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV2) leading to COVID-19 has initiated a catastrophe for humans since December 2019. Genetic and protein similarities between SARS-CoV and SARS-CoV2 offer the same treatments for both types of virus. However, there are some sequence or structural differences between SARS-CoV2 and SARS-CoV as well as other coronaviruses that make difficulties in discovering drugs and vaccines against this novel type of virus. Therefore, it is vital to recognize protein and genetic structures of SARS-CoV2 to discover drugs which directly target this strain of coronavirus. This review presents a perspective on SARS-CoV2, it's genetic and protein structures with a brief comparison with other coronaviruses as well as summarizing some immune responses activated against SARS-CoV2. In addition, it introduces the novel strategies to combat with COVID-19 that would be potentially effective on SARS-CoV2.

INTRODUCTION

Coronavirus is one of the most crucial infectious agents causing respiratory, gastrointestinal, liver and central nervous system infections in humans and vertebrates such as birds, bats, mice and other wild animals (1). Coronaviruses as the members of the Coronaviridae family and the Coronavirinae subfamily have a single RNA strand and spike glycoproteins protruded from the virus envelope (2). The Coronaviridae family is classified into four groups, α, β, δ, and γ, also β type is divided into four types A, B, C, and D (Table 1) (1). Previously, Sever acute respiratory syndrome (SARS) and Middle east respiratory syndrome (MERS) caused pandemics from animal to human and from human to human in 2002/2003 and 2012, respectively (3). Recently, a novel Coronavirus, 2019-nCoV, or SARS-CoV2, which leads to acute and severe respiratory symptoms and COVID-19 disease, has broken out firstly in Wuhan, China and then spread out in other provinces/regions of China and many countries in other continents where reported high rates of death (1, 3). Compared to SARS-CoV, SARS-CoV2 is responsible for higher mortality in people with age of over 60 as well as people with diabetes and/or hypertension (4). Recognition of genetic and protein structures of the SARS-CoV2 and identification of the differences between coronaviruses can be effective in discovering novel drugs and strategies against SARS-CoV2. Recently, researchers have attempted to figure out a novel drug or vaccine for treatment of COVID-19. This review presents a perspective on SARS-CoV2, it's genetic and protein structures with a comparison with other coronaviruses as well as detecting immune responses activated during infection and summarizing the novel discoveries on SARS-CoV2 that may be effective for COVID-19 therapy.

Source of SARS-CoV2

Genetic comparison of SARS-CoV2 with coronaviruses derived from five wild animals, Paguma larvata, Paradoxurus Hermaphroditus, Civet, Aselliscus stoliczkanus and Rhinolophus sinicus demonstrated less than 75% homology in terms of total genome sequence, ORF1a, ORF1ab and spike (S) protein. However, compared to Bat-Coronavirus RaTG13, SARS-CoV2 indicated more than 96% similarity in terms of total genome sequence, ORF1ab, Nucleocapsid (N) and S proteins (5-7). It has also been shown that various species of animals, exception of Rat and mice, are involved in the 2019-nCoV infection as intermediate hosts (8). The conserved structure of the SARS-CoV2 receptor in humans and animals such as fish, amphibians, birds, reptiles and mammals suggests that these creatures can be identified as the hosts of the virus (9).

Table 1. Coronaviridae family and several types of coronaviruses in human

Coronaviridae	Types of Coronaviruses		Features
α Coronaviruse		HCOV-229E HCOV-NL63	Mild cold
β Coronaviruse	A	HCOV-OC43 HCOV-HKU1	Mild cold
	B	SARS-COV SARS-COV2	Acute respiratory syndrome
	C	MERS-COV	Acute respiratory syndrome
	D	-	-
δ Coronaviruse		-	-
γ Coronaviruse		-	-

SARS-CoV2 entry to host cell

SARS-CoV2 implicates the specific receptor and several co-receptors for cell entry. It uses angiotensin converting enzyme 2 (ACE2), as a surface receptor and also dipeptidyl peptidase 4 (DPP4) and mammalian glutamyl aminopeptidase (ENPEP) as co-receptors for binding, membrane fusion, and cell entry. S glycoprotein which have been protruded from the virus envelope also mediates binding and membrane fusion (10) whereas MERS tends to bind to DPP4 / CD26 as co-receptors on the surface of respiratory tract cells and lymphoid tissues for binding, membrane fusion, and cell entry (11-13). Totally, various strains of coronaviruses implicate different types of receptors for entry into the host cells as SARS-CoV, SARS-CoV2 and HCoV-NL63 use ACE2, and MERS-CoV and other coronaviruses use aminopeptidase N (ANPEP) and DPP4 (4).

Structure and Function of ACE2

The specific function of ACE2 is defined by it's structure which is consisted of several domains with several amino acid sequences (14). Figure 1A shows the protein structure of ACE2.

The ACE2 receptor and co-receptors are highly expressed in lung, liver, kidney, heart, stomach, intestine, ileum and oral cavity epithelial cells (10, 15)15</style>. In recent studies, the ACE2 receptor has also shown high expression in cholangioyte, myocardial, and bladder cells (4). Despite these tissues expressing ACE2 highly, the primary target of the SARS-CoV2 is AT2 cells in the lung tissues (10). The critical role of ACE2 is angiotensin maturation, a peptide hormone involved in regulating blood pressure. ACE2 also acts as a chaperone for the membrane trafficking of the B0 AT1 amino acid transporter. This transporter is implemented in transferring neutral amino acids to intestinal cells in a sodium-dependent pathway (16). Moreover, ACE2 recruits B0AT1 aminoacid transporter to form ACE2 / B0AT1 complex which binds to the S protein for membrane fusion and virus entry into the host cells (17). Therefore, this proposes that B0AT1 may be considered as a potential therapeutic target to design drugs.

Structure and function of SARS-CoV2 S glycoprotein

S glycoprotein of SARS-CoV2 binds to the various host cell targets such as ACE2, CD26, Ezrin, Cyclophilin, and other adhesion molecules (18). S glycoprotein is a trimer with 1273 amino acid which is consisted of two subunits in each monomer, subunit1 (S1) in N-ter and subunit2 (S2) in protein C-ter, that are

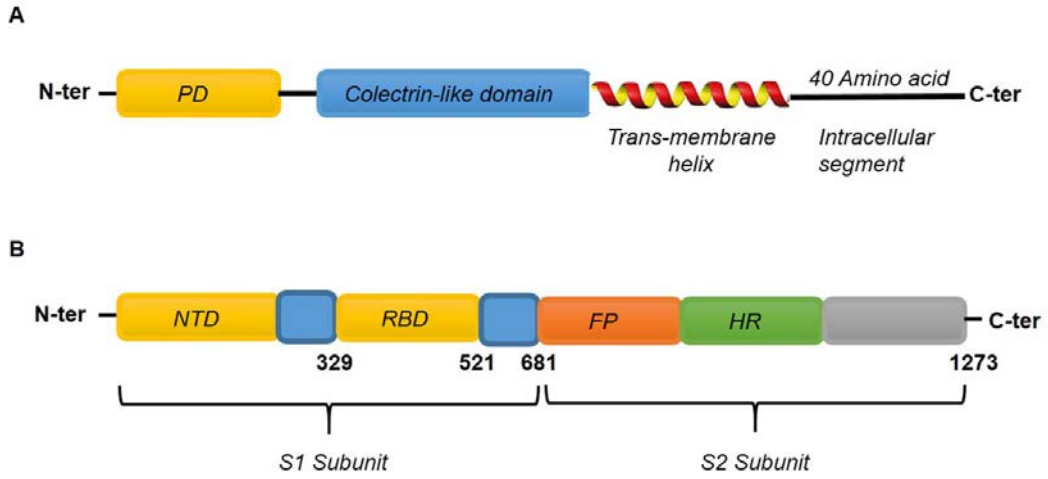

Fig1. The protein structure of ACE2 and Spike protein. A) The protein structure of ACE2, B) The protein structure of Spike protein.

effective in binding and fusing the virus into the host cell, respectively. Each monomer is about 180KD and contains a receptor binding domain (RBD) (Figure 1B) (19, 20). RBD domain of S subunit has two up and down conformations; the up and the down modes are related to the available and unavailable configuration of the receptor, respectively. Initially, subunit S1 is bonded to the ACE2 and thus changing the conformation causes S1 to shed off and S2 to convert to the stable conformation (21, 22).

To better understand, when the S1 subunit is bonded to the ACE2, the Heptad Repetition 1 (HR1) and HR2 domains of S2 subunit interact together and make six-helix bundle which brings the host cell membrane and the virus closer to fuse and enter the host cell. In HR1 domain, there are several mutations that increase the HR1 affinity to interact with HR2. For this reason, the affinity of the SARS-CoV2 is higher than other coronaviruses to interact with the host cell membrane (23).

Activation of S protein occurs in two stages of cleavage. In the first step, the RBD of S1 subunit binds to the peptidase domain (PD) of ACE2, directly. Thus, changing the protein conformation causes S1 and S2 to separate and S2 is then exposed and broken down by peptidase. Following this, protein reaches to a stable conformation which is necessary for membrane fusion and viral infection (16). In the second step, S protein is subjected to more cleavage by one or more host cell proteases such as Furin, trypsin, cathepsins, transmembrane protease serin protease-2 (TMPRSS-2) and TMPRSS-4 or human airway trypsin-like protease (HAT). Studies on SARS-CoV2 indicate that the virus enters the host cell through endocytosis which is mediated by TPC2, PIKfyve, and cathepsin L. Compared to SARS-CoV, S protein of SARS-CoV2 is less stable (24). Research on S protein recommends using spike glycoprotein as a primary target for vaccine and therapeutic antibodies.

SARS-CoV2 Genome and proteins

Coronaviruses have the largest genome (26-32Kb) among RNA viruses that it is almost 29.8 kb to 29.9 kb in SARS-CoV2. SARS-CoV2 has a single RNA strand with a 5' cap and a 3' poly A structure. 5' region of RNA encodes Orf1ab polyprotein which is cleaved into non-structural proteins (nsps 1-16) and 3' region of RNA encodes structural proteins such as spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins as well as encoding accessory proteins such as Orf3a, Orf6, Orf7a, Orf7b, Orf8, HE protein, Orf3a/b and Orf4a/b protein which play a critical role in the virus replication and contagious (1, 7, 25). Majority of the SARS-CoV2 genome encodes more than 20 types of proteins which are required in RNA replication and gene transcription in infected cells. Table 2 presents the several types of proteins encoded by both SARS-CoV2 and SARS-CoV. Once virus enters the host cells, it begins to mutate, generating changes that mostly occur in five major genes including genes encoding N, S, Orf8, Orf3a, and Orf1ab proteins. Furthermore, 42% of the mutations are well-known as a non-synonymous mutation (5). Several 3'-5'-exoribonuclease enzymes which provide proofreading ability for coronaviruses,

Table 2. Types of proteins encoded by both SARS-COV2 and SARS-COV.

Protein name	Function	References
Nsp1-nsp16	Protease, Replicase, Ribonuclease, Ribose methyltransferase	(*57*)
Spike (S) protein	Membrane fusion, entry into host cell	(*25*)
Nucleocapsid (N) protein	Structural protein	(*25*)
Membrane (M) protein	Structural protein	(*25*)
Envelope (E) protein	Structural protein	(*25*)
Orf1a/ab	Polyprotein cleaved into nsp1-nsp16	(*25*)
Orf3a/b	Pathogenicity, Inhibition of IFNβ expression, Genome maintenance, Virus replication	(*17*)
Orf4a/b	Genome maintenance, Virus replication	(*58*)
Orf6	Production of type I IFN and inhibition of signaling	(*59*)
Orf7a	Increase in NF-κβ, JNK, p38 MAP kinase, Inhibition of host translation, Induction of apoptosis and cell cycle arrest	(*59*)
Orf7b	No known function	(*59*)
Orf8a/b	Trigger intracellular stress pathways	(*59*)
HE protein, 3a/b and 4a/b protein	Genome maintenance, Virus replication	(*58*)

distinguish them from other RNA viruses (1).

One of the most crucial conserved proteins in coronaviruses is main protease (M^{pro}) or 3C-like protease ($3CL^{pro}$) with 306 amino acids. M^{pro} is required to replicate and process the viral functional proteins (2). Primary role of the M^{pro} is cleavage and activation of two viral polyproteins called PP1A and PP1AB as well as cleaving 11 sites in Replicase 1ab. The cleavage sites identified by M^{pro} are Leu-Gln (Ser, Ala, Gly) (presents the cleavage site) (26). In addition, research on the three-dimensional structure of SARS-CoV2 protease has shown nearly 96% similarity with SARS-CoV protease. Also, the cleavage site of M^{pro} has been conserved among the proteases in different types of coronaviruses. This suggests that inhibitors of the SARS-CoV protease could have a similar effect on SARS-CoV2 M^{pro} or 3C-like protease. Among well-known protease inhibitors, Ladipasvir and Velpatasvir can be hypothetically effective on SARS-CoV2 without any side effects (27). In addition, creating some alterations in chemical structure of protease inhibitors in order to protect host cell proteases from cleavage, can provide a vital step to modulate a safe anti-SARS-CoV2 drug with the least side effects. For instance, Peptidomimetic α-ketoamide endures some alterations in it's chemical structure through hiding the P2-P3 amide bond in the pyridone ring. Moreover, in this molecule, less hydrophobic groups are replaced by hydrophobic groups to increase solubility and reduce binding ability to plasma proteins (26).

Additionally to the catalytic residues His 41 and Cys 144 of the protease, research on the active site of $3CL^{pro}$ protease in SARS-CoV has found four novel main amino acids (PDEV) for protease activity that mutations and changes in these amino acids can greatly reduce protease activity. This proposes that intra-molecular dynamic changes in protein-substrate interaction may impair protease activity. Discovering novel and important amino acids in the active site of proteases can provide more effective sites for the design and development of protease inhibitors (28).

Comparing SARS-CoV2 genome and proteins with other coronaviruses

SARS-CoV2 has almost 89% and 96% nucleotide identity with SARS-CoV and Bat coronavirus, respectively (16). Genomic analysis has detected that slow codons and slow di-codons (codons without cognate tRNA gene) are lower in both SARS-CoV2 and SARS-CoV, thus the rate of protein synthesis is faster in comparing with other coronaviruses (29). In spite of high homology, comparing SARS-CoV2 and SARS-CoV genetic sequence, identified six regions of difference (RD), RD1, RD2 and RD3 (448nt, 55nt, and 278nt, respectively) in the ORF1ab coding gene, RD4 and RD5 (315nt and 80nt, respectively) in the S protein coding gene, and RD6 (214nt) in ORF8 and ORF7b coding genes (30). These different regions can be considered as diagnostic molecular markers and implicated to develop novel drugs against SARS-CoV2. Research on sequences, genome variations, and comparison of SARS-CoV2 with other β-coronaviruses, MERS, and SARS, identified at least two hyper-variable genomic hotspots in the SARS-CoV2. In spite of the low rate of variation in SARS-CoV2, one of the genome variations (C / U) is Ser / Leu84 in the protein encoded by ORF8. Comparison of ORF8-S with ORF8-L has shown that ORF8-S leads to some structural alterations in protein C-ter. It can also provide a new phosphorylation target for host cell Ser / Thr kinases. The other genome variation is a synonymous variation (U / C) encoding Serin 2839 in a protein encoded by ORF1ab (31). Furthermore, comparing the SARS-CoV2 genome with Bat-coronavirus and SARS-CoV also confirmed the replacement of a Ser to Gly in position 543 of the nsp2 protein encoded by ORF1ab. This variation plays a vital role in increasing the contagious ability of the SARS-CoV2. The other identified protein variation occurring near phosphatase protein and playing a key role in replication of the viral genome, is a prolin located at 192 of the nsp3 in SARS-CoV2 instead of polar and apolar amino acids in Bat-coronavirus and SARS-CoV, respectively. This variation recommends a potent strategy to diagnose and distinguish SARS-CoV2 from SARS-CoV (32). Identification of the amino acid differences in the protein sequences of SARS-CoV2 could help to discover novel antiviral drugs and strategies to combat with COVID-19 (31).

Comparing the sequences of S1 and S2 subunits in SARS-CoV2 S glycoprotein and SARS-CoV shows 91% and 55% similarity in S2 and S1 subunit, respectively. On the one hand, amino acid different regions in the S2 subunit of viruses are included 677-690, 877-884 and 930-943, whereas 570-1278 region is considered as a similar one. On the other hand, the amino acid differences in the S1 subunit of the SARS-CoV2 and SARS-CoV are located at 01-550 region (18). Moreover, four insertion sequences in SARS-CoV2 have been identified in comparing with SARS-CoV that three sequences are common in Bat coronavirus RaTG13 S glycoprotein. These four insertion sequences are GTNGTKR in subunit S1, YYHKNNKS in subunit S2, GDSSSG in subunit S3, and QTNSPRRA in subunit S4 (33)Protein structure and sequence reanalysis of 2019-nCoV genome refutes snakes as its intermediate host and the unique similarity between its spike protein insertions and HIV-1. Also, there are some differences in glycosylation sites in two strains of virus which can lead to diverse responses of SARS-CoV2 to anti-SARS-CoV drugs (18). In spite of these differences, there are several similar sequences

Table 3. Types of variations in SARS-COV2 in comparing with SARS-COV and MERS-COV.

Gene/Protein name	Variation type	Variation	Variation effect	References
ORF8	Non-synonymous	84Ser/Leu	Structural deviation in C-ter Novel phosphorylation site for Ser/Thr kinase of host cells	(*31*)
ORF1ab	synonymous	Serin 2839	-	(*31*)
ORF1ab/Nsp2	Non-synonymous	543Ser/Gly	High ability of contagious	(*32*)
Nsp3	Non-synonymous	192Prolin/Polar, Apolar AA	Possible role in virus replication	(*32*)
S1 subunit	Insertion	GTNGTKR	-	(*33*)
S2 subunit	Insertion	YYHKNNKS	-	(*33*)
S3 subunit	Insertion	GDSSSG	-	(*33*)
S4 subunit	Insertion	QTNSPRRA	-	(*33*)
RBD	Non-synonymous	Glycyl loop/ prolyl loop	-	(*9*)
RBD	Insertion	F486	Strong interaction of RBD with ACE2	(*9*)
N protein	Non-synonymous	37S/P ،215 G/S ،243 G/S 267A/Q	-	(*5*)
S protein	Insertion	680PRRA	Proteolytic cleavage of S protein by cellular protease High ability of contagious	(*5*)

that provide conserved structures between SARS-CoV2 and SARS-CoV, offering the same targets with different molecular interactions for both viruses.

Although comparing RBD domain of SARS-CoV2 and SARS-CoV has shown 28% variations in amino acid sequences, they are structurally too similar. A sequence difference in RBD domain of both viruses is a loop of Glycyl residues in SARS-CoV2 instead of prolyl residues in SARS-CoV. Furthermore, phenylalanine F486 in RBD domain of the SARS-CoV2 leads to strong interaction with ACE2 receptor through interacting phenylalanine with hydrophobic pocket in ACE2. It can be the other reason for greater affinity of SARS-CoV2 to bind to the host cell in comparing with SARS-CoV. Therefore, the usage of antibodies and inhibitors which prevent RBD from binding to the ACE2 can be applied as a combating strategy against SARS-CoV2 (9).

Comparing N proteins of SARS-CoV2 and Bat-coronavirus has shown four different amino acids in 37S / P, 215 G / S, 243 G / S and 267A / Q, respectively. Also, 33 different amino acids are located at the regions of 439-449 and 482-505 in SARS-CoV2 S protein. An insertion peptide (PRRA), which is located at amino acid 680 of S protein in the SARS-CoV2, may be involved in contagious ability of the virus and in proteolytic cleavage of the S protein mediated by cellular proteases. In addition, 103 different amino acids have been known in 919-1227 region of Orf1ab protein (5). Table 3 presents types of variations in SARS-CoV2 in comparing with SARS-CoV and MERS-CoV.

Despite the amino acid differences in the S protein, there are no structural differences in the protein between both strains of virus. This suggests that inhibitors of SARS-CoV S protein could also be used as inhibitors of SARS-CoV2 S protein. Although there are some similar epitopes in both viruses, there are numerous antigenic differences between them (34). These variances could provide an opportunity to develop novel and more effective drugs and vaccines against SARS-CoV2. The similar epitopes are well-known as RISNCVADY, CVADYSVLY, RSFIEDLLF, RVDFCGKGY, MTSCCSCLK, VLKGVKLHY, whereas different epitopes are KTSVDCTMY, STECSNLLL, ECSNLLLLQY, and LTDEM. In addition, diverse glycosylation sites in S glycoprotein of SARS-CoV2 are NGTK, NFTI, NLTT and NTSN, whereas common and conserved glycosylation sites are NITN, NGTI, NFSQ, NESL, NCTF and NNTV in both types of viruses (34).

Immune responses to SARS-CoV2

Once entering the host body, the virus is detected by the innate immune system through C-type-lectin-like receptors, Toll like receptors (TLRs), NOD-like receptor (NLR), and RIG-I-like receptor (RLR). It also activates either inflammatory factors or the synthesis of INFs, however, the viral N protein causes the virus to escape from the host's immune response. The critical role of C3a and C5a complement factors has also been demonstrated in combating against viruses (17). In the next step, CD4+ T and CD8+ T adaptive immune response cells are activated to secrete antibodies and

cytokines and kill the virus directly. However, SARS-CoV2 escapes from the adaptive immune system through inducing apoptosis in T cells. Consequently, high levels of immune responses and high levels of free radicals produced by immune cells can damage lungs and other organs, which thus lead to death (17). Cytokine storm is an over-activated and severe form of inflammatory and immune response in which a large amount and variety of cytokines such as TNFα, IL-1β, IL-2, IL-6, IFNα, IFNβ, IFNγ and monocyte chemoattractant protein-1 (MCP1) are involved in combating against infectious agents like SARS and MERS. In response to the cytokines, immune cells produce large amounts of free radicals, which can lead to severe lung damage (17).Analyzing various cells in particular cells around the viral target also demonstrates the key role of macrophages in immune defense during COVID-19 disease through triggering chemokine signaling pathways and phagocytosis which are mediated by interacting with ACE2-expressing cells (10) Immunoinformatics studies on viral epitopes of SARS-CoV2 have identified five and three epitopes in glycoproteins for detection by cytotoxic T cell (CTL) and sequential B cell, respectively, as well as identifying five epitopes for discontinuous B cell detection. CTL epitopes are able to attach to MHC class I through multiple interactions which mediate activation of the immune responses. Consequently, Some epitopes may be recommended as targets for developing SARS-CoV2 vaccines (35).Additionally, during a virus attack, cell death or apoptosis occurs in order to kill infected cells as an antiviral defense mechanism. It has been shown that the expression level of myeloid cell leukemia 1 (MCL1), a positive apoptotic regulator, which regulates mitochondrial apoptotic responses, greatly increases during coronavirus infection. Therefore, by interacting with Orf7a, MCL1 can trigger apoptosis in infected host cell and induce death in both host cell and virus (11). In addition, there are some defense mechanisms regulated by host cells that help to immune cells indirectly to deal with infectious agents. For instance, eukaryotic translation elongation factor 1 alpha 1 (EEF1A1) playing a critical role in transmitting tRNA to the ribosome, is down-regulated during β-coronaviruses infection (11). The other down-regulated protein, GRAIL, is an E3 ubiquitin ligase that indirectly triggers an antiviral immune response to RNA viruses. TMPRSS2 is the other protein which is down-regulated in SARS-CoV infection as well as in other β-coronaviruses and plays a vital role in interaction with the viral S glycoprotein (11).

Drugs and vaccines against SARS-CoV2

Recently, many efforts have been implemented to develop novel drugs such as remdesivir, GS- 441524, protein inhibitors and vaccines for combating against coronaviruses (36, 37). However, various types of vaccines targeting SARS-CoV2, may have several advantages, disadvantages, and challenges. Overall, antiviral vaccines are included in the whole virus, the recombinant protein, and the nucleic acid vaccines (4).

One major challenge with making coronavirus vaccines is the adverse immune responses such as eosinophilic infiltration and infection which may occur after immunosuppression of the entire virus or complete spike protein. Also, efforts have been made to develop a vaccine against SARS-CoV2, similar to Ebola or Flu vaccines, which use the adenovirus as a vector. Totally, these vaccines consisted mainly of SARS-CoV2 proteins and codon deoptimization technology has also been used to reduce virus activity (4). There are both upsides and downsides in using the whole virus vaccine. One of the most important beneficial aspects is stimulation of TLRs such as TLR3, TLR718 and TLR9, whereas one problem with this vaccine is it's greater infectivity. Protein vaccine is one of the other types of vaccines which either creates immune responses against spike proteins of the virus in order to prevent them from binding to the ACE2 receptor, or is a recombinant S protein in seudo-virus nanoparticles. The other type of protein vaccine which has recently been tested, is subunit vaccine that includes a subunit of S protein with only one RBD. One positive point of subunit vaccine in particular RBD-based vaccine, is minimizing unwanted immune reactions as well as high level of safety. Today, DNA and RNA vaccines with various modifications are being studied to improve the activity of nucleic acid vaccines in humans (4).

A recent study on COVID-19 vaccine confirmed that the usage of vaccine adjuvant toll like receptor agonists, which had previously been an efficient adjuvant in the SARS-CoV vaccine, could be combined with S proteins as a potent effective vaccine for COVID-19 without eosinophilic infiltration with cytokine Th1 / 17 responses, whereas S proteins attached to gold nanoparticles as adjuvants can lead to eosinophilic infiltration as well as creating strong cytokine/ IgG responses (38).

Recently, numerous studies on synthetic vaccines in particular synthetic epitope vaccines have highly been implemented. A highly conserved amino acid sequence among coronaviruses like SARS-CoV2, is KRSFIEDLLFNKVV, which is also attributed to the region around a cleavage site in the SARS virus. This amino acid sequence is critical for the virus entry and can be suggested as a synthetic epitope vaccine (39).

Different studies have reported variety of potent treatments and methods for COVID-19 therapy. The best treatment for patients with severe symptoms is cytokine storm treatment or immunosuppression such as using Corticosteroids, Tocilizumab and Anti-IL-6. Although, usage of steroids may have many side

effects, say, avascular osteonecrosis, a low dose is recommended (17). Furthermore, it has been reported that mesenchymal stem cells (MSC) implantation which are well known as strong immunomodulatory cells, have been resulted in improvement of disease through increasing peripheral lymphocytes, decreasing C reactive protein (CRP), as well as elevating activity of cytokine-secreting immune cells such as CXCR3 + CD4 + T, CXCR3 + CD8 + T CXCR3 + NK cell, increasing CD14 + CD11c + CD11bmid regulatory DC cells and TNFα and decreasing IL-10. MSCs are ACE2- and TMPRSS2^{-}, therefore, they can be considered as safe and effective cells for treatment of COVID-19 pneumonia patients (15, 40). Recently, research has been applied to design and manufacture antibodies to treat COVID-19. Although, various monoclonal antibodies used for SARS-CoV cannot be used and effective for this novel type of virus, recently, an antibody isolated from SARS patients called CR3022 may target either receptor binding sites or conserved epitopes in both SARS-CoV and SARS-CoV2. The function of CR3022 depends on the conformation of RBDs in the S glycoprotein. Once at least two RBDs of trimeric S protein are in the up conformation, epitope will be available for CR3022 (41). SARS-CoV antibodies such as m396 and CR3014 target the various epitopes on RBD of S glycoprotein in ACE2-binding site, however, they cannot be attached to SARS-CoV2 S protein. This suggests that there are some potent sequence or structural differences between RBDs of SARS-CoV and SARS-CoV2 (42). In addition, a recent study on the treatment of COVID-19 has shown that the usage of high-dose intravenous immunoglobulin (IVIG) can be introduced as a satisfactory treatment (43).

On the other hand, it has been reported that HIV-1 protease inhibitors such as Lopinavir, sequinavir, ritonavir can have a strong interaction with activate site of SARS-CoV2 protease. Among the twenty compounds classified in these three groups, five compounds are hypothetically the main compounds that target the SARS-CoV2 main protease. IDs of these compounds are 444745, 444663, ZINC1014061061, ZINC1014061081, 444743 in the ZINC database (44). The other antiretroviral agent, atazanavir has exhibited greater anti-SARS-CoV2 activity in comparing with lopinavir through inhibiting SARS-CoV-2 replication and proinflammatory cytokine production (IL-6 and TNFα), alone or in combination with ritonavir (45). Various studies have been implemented to design and synthesize drugs targeting M^{pro}. Recently, two designed and synthesized components (11a and 11b) have depicted potent anti-SARS-CoV2 and pharmacokinetic activity in vivo (46). In addition, drug designing, virtual drug screening and high-throughput screening recommended ebselen as the other candidate anti-SARS-CoV2 drug which needs to be approved clinically (47).

Moreover, studies have indicated that cyclosporin A inhibits virus replication through binding to cell cyclophilins and inhibiting it's cis-trans peptidyl-prolyl isomerization activity. Cyclophilin A is also involved in binding virus N proteins to cells, thus the usage of cyclophilin A inhibitors such as CSA derivatives including Alisporivir and NIM811 can inhibit protein N interaction with cyclophilin A (48) suggesting cyclophilin A inhibitors can be investigated for treatment of COVID-19 as well as other coronaviruses. Previous studies have exhibited that the Pan-corona virus inhibitor, EK1, targets the HR1 domain of S2 subunit in coronavirus, such as SARS-CoV and MERS-CoV. Recent study on SARS-CoV2 has shown high inhibitory activity (120/240 fold) of EK1 peptide against membrane fusion and viral infections of SARS-CoV2, SARS-CoV and MERS-CoV through binding to cholesterol in order to form EK1C4 lipopeptide (23).

It has recently been reported that protein kinase inhibitors can be recommended as antiviral drugs for the treatment of coronavirus due to the activation of multiple signaling pathways in the host cells during virus's growth. Therefore, protein kinases which are activated by virus or infected cells, may be used as targets for developing anti-COVID-19 drugs. Also, recent studies have exhibited the activation of hydrophobic derivatives of vancomycin, teicoplanin, as well as aglycon or pseudo-aglycon against several viruses such as HIV, HCV, influenza viruses (A / H1N1, A / H3N2), flaviviruses as well as coronaviruses through targeting protein kinases (49).

A number of antiviral drugs are well-known as protease inhibitors, integrase inhibitors, and polymerase inhibitors. Among these drugs, protease inhibitors such as tyrannavir, indinavir, atazanavir, darunavir, ritonavir and amprenavir could, as indicated by in silico virtual screening, inhibit virus replication and are thus suggested as potential effective drugs for COVID-19 (2). Macrolides such as erythromycin, clarithromycin and azithromycin also have anti-inflammatory and antibacterial effects as well as antiviral impacts. Previous studies have confirmed that Macrolides have antiviral effects on Rhinovirus, influenza, Zika and Ebola viruses, all of which are respiratory viruses. Studies on COVID-19 patients have indicated that the usage of azithromycin or macrolides in combination with hydroxychloroquine could represent a potential antiviral drug-combination for the treatment of COVID-1 and that hydroxychloroquine alone could have antiviral effects against SARS-CoV-2 (50). One of the drawbacks of using hydrochloroquine which was previously used as an anti-malarial drug and also

Table 4. Types of drugs and therapy suggested to be used against coronaviruses in particular SARS-COV2

Type of drug	Type of virus	Target	Function	Reference
Corticosteroide Tocilizumab Anti-IL-6	SARS-COV2	Immune response	Suppression of cytokine storm	(*17*)
CR3022	SARS-COV2 SARS-COV	ACE2 binding site	Inhibition of virus entry to cells	(*41*)
m396 CR3014	SARS-COV	RBD epitopes in ACE2-binding site	Inhibition of virus entry to cells	(*42*)
Intravenous immunoglobulin (IVIG)	SARS-COV2	Immune response	Suppression of immune response	(*43*)
Lopinavir Sequinavir Ritonavir	HIV-1 SARS-COV2	Protease	Inhibition of virus replication	(*44*)
Cyclosporin A		cyclophilin	-Suppression of N protein interaction with cyclophilin - Inhibition of virus replication	(*48*)
EK1	SARS-COV MERS-COV	HR1 domain of S2 subunit	Inhibition of membrane fusion	(*23*)
EK1C4 lipopeptide	SARS-COV2 SARS-COV MERS-COV	HR1 domain of S2 subunit	Inhibition of membrane fusion	(*23*)
Vancomycin Teicoplanin Aglycon Pseudo-aglycon	HIV ،HCV A/H1N1 A/H3N2 coronaviruse flavivirus	Protein kinases	Inhibition of virus entry and replication	(*49*)
Tipranavir Indinavir Atazanavir Darunavir Ritonavir Amprenavir	Coronaviruses (SARS-COV2)	Protease	Inhibition of virus replication	(*2*)
Macrolides (Erythromycin Clarithromycin Azithromycin) + Hydroxychloroquine	SARS-COV2	Immune response	Anti-inflammation	(*50*)
Ginkgolic acid (GA)	HIV ،Ebola Influenza EBV coronaviruses	Viral proteins	Inhibition of virus replication	(*52*)

for autoimmune diseases is creating cardiac toxicity that leads to arrhythmia as well as liver and kidney disorders in high-risk individuals. In addition, the risk of overdose is higher in patients with renal and hepatic disorders than in other populations (51). The other suggested drug is Ginkgolic acid (GA) found in the leaves and fruits of Ginkgobiloba. It has shown antiviral impacts against HIV, Ebola, Influenza, and EBV viruses through inhibiting virus's replication. Therefore, GA could be suggested as an effective drug for combating with coronaviruses. Furthermore, GA has previously displayed anti-cancer effects through inhibiting lipogenesis, reducing the expression of proteins involved in metastasis, suppressing sumoylation, and inhibiting fatty acid synthesis (52). Table 4 summarizes several types of drugs and therapies proposed to be used against coronaviruses in particular SARS-CoV2.

Personalized medicine in COVID-19

Personalized medicine in COVID-19 can provide an opportunity for prevention, diagnosis, treatment and management of this disease in the public health. Personalized medicine can detect the susceptibility of everyone to COVID-19 infection with highly variable symptoms. Patients with COVID-19 experience the variable symptoms ranging from mild to severe and progressive infections. Identifying susceptible patients to severe infection can help to better management of COVID-19 pandemic. Personalized medicine in COVID-19 may be lead to design novel therapeutic and preventive strategies according to individual profiles. For this purpose, sequencing of SARS-CoV2 genome along with detection of genome variation of patients can propose the best approach to combat with COVID-19 (53). Individual Factors associated to disease severity are divided to two categorizes,

non-genomics/clinical and genomics factors. Non-genomics or clinical factors include age, gender, BMI, diabetes, hypertension and smoking, whereas genomic factors include variations in chromosomes 1 (1q22.1), 2 (2p21.1), 3 (3p21.1–3), 6 (6p21.1), 8 (8q24.13), 9 (9q34.1–2), 12 (12q24.1–2), 17 (17q21.3), 19 (19p13.1–3) and 21 (21q21–q22) which can be specific for different ethnics or phenotypes. For instance, 3p21 locus variants are present in 30% and 8% of people in South Asia and Europe, respectively that are associated to severe inflammation and infection. In addition, 3p21.31 locus comprises SLC6A20 gene coding for a transporter regulated by ACE2, and other loci may have indirect association with COVID-19 severity (53, 54).

In other hand, variations in SARS-CoV2 are estimated to accumulate at rate of about 1–2 variations per month. Sequencing of viral genome has reported huge variability which affects the severity of infections in patients with COVID-19. For example, D614G variant in spike protein and ORF8 deletion increases and reduces infectivity of the virus as well as mortality of patients, respectively (55, 56). Variations in RNA polymerase can also increase the replication mistakes that could result in resistance to antiviral treatments. Therefore, personalized medicine in COVID-19 may be lead to design novel therapeutic and preventive strategies according to individual profiles in order to better management of COVID-19 pandemic.

CONCLUSION

Recently, SARS-CoV2, the novel strain of coronaviruses has initiated a catastrophe for humans all over the world with sever acute respiratory syndrome and high rate of death. It is essential to study on genetic and protein structure of SARS-CoV2 to discover potent drugs, antibodies and vaccines in order to treat COVID-19. High similarity to SARS-CoV proposes the same treatments, although some differences in genetic and protein structures make difficulties in discovering therapy. SARS-CoV2 uses subunit S1 and S2 for binding and membrane fusion in order to entry to host cells through binding to ACE2. SARS-CoV2 genome encodes several proteins such as nsps, structural proteins and accessory proteins which have been involved in structure, activity, replication and transmission of virus. There are several variations in genetic and protein sequences in SARS-CoV2 in comparing with SARS-CoV and MERS-CoV. These variations recommend a possible mechanism to distinguish SARS-CoV2 from SARS-CoV and MERS-CoV and could help to discover novel antiviral strategies to treat COVID-19. Moreover, recently, variety of publications have proposed several novel vaccines, drugs and antibodies such as anti-inflammatory factors, anti-ACE2, anti-S protein and protease inhibitors to combat with SARS-CoV2.

Acknowledgments

I am sincere to my colleagues at University of Isfahan for their valuable discussions.

Disclosure of potential conflicts of interest

None of the authors has any conflict of interest to disclose, and all authors support submission to this journal.

Research involving human participants and/or animals

This article does not contain any studies with animals and human participants performed by author.

Informed consent

Not applicable.

Funding information

There is no funding for this study to report.

Author Contribution

S.Y.: Conception, Providing the data and design, Manuscript writing.

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