SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 5.
Issue: 2.
Page N. 307-318
Year: 2021

Review Article www.wjahr.com

CORONAVIRUS REPORT

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Received date: 20 February 2021 **Revised date:** 11 March 2021 **Accepted date:** 31 March 2021

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ABSTRACT

SARS-CoV-2 is the causative agent of potentially fatal COVID-19 disorder i.e. highly transmittable and Pathogenic viral infection that led to severe respiratory disorders and has become a great global public health concern due to increased globalization and adaptation of the virus in environment.The genomic sequence of SARS-CoV-2 showed greater similarity around 89-96.3% with bats viruses phylogenetically, revealed that bats could be primary reservoir of causing COVID-19 infection. Hence, it is an urgency to produce therapeutics effects against SARS-COV-2. Here, we have discussed about the protein structure, genomic sequence, varying symptoms among SARS, MERS, and COVID-19, replication and pathophysiology, diagnostic test along with treatment used clinically. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was the diagnostic technique that can amplify the genetic material in the sample with the help of primers and probes, thus led to identification of SARS-CoV-2 virus. Serological testing played an integral role in vaccine development and determination of epidemiology of SARS-CoV-2. Plasma and antibodies obtained from the convalescent patients have been proposed for use in treatment. There are various drugs that are being evaluated against COVID-19 in clinical trials, resulted in clinical recovery, but till no clinically approved antiviral drug or vaccine available to be used against COVID-19. However some suggestive approaches were taken to minimize the spread of COVID-19 infection that will significantly reduce the strain of health care workers. The aim of this review is to enlighten the corona viruses in detail as it will be the need of an hour. The SARS-CoV-2 emerged in late 2019 and spread globally, prompting an international effort to accelerate development of a vaccine that will focus on spike protein.

KEYWORDS: COVID-19, Diagnostic Kits, Genome, Liquorice, Remdesivir

INTRODUCTION

Coronaviruses (CoVs) belonged to a large family of zoonotic viruses that consequently led to severe respiratory disorders. Coronaviruses belonged to realm-Riboviria, order-Nidovirales, suborder-Cornidovirineae, family-Coronaviridae and subfamily-Orthocorona-virinae,[1] that consisted of four genera which were further classified based on their phylogenetic relationships and their genomic structures(α, β,γ, and δ coronaviruses). Alpha and beta viruses infected only mammals whereas gamma and delta viruses infected birds as well as mammals.[2] How can we identify the causative agent of SARS-CoV-2 and what are physiological effects caused by it on the patient's body? Recently database showed that all human viruses (SARS-CoV, HCoV-NL63, HCoV-229E and MERS-CoV) were originated from animals origin i.e. bats, dromedary camels, raccoon dogs, civets.[3] Based on antigenic and genetic compositions the coronaviruses were broadly classified into three groups. Group 1(animal pathogen such as 229E), group 2 (veterinary pathogens such as OC43, HKU1, and NL63), group 3 (avian corona viruses)[4,5] The SARS, MERS-COV-2, COVID-19 were interrelated to each other in terms of diseases symptoms, receptor occupancy, incubation period, animal host as carrier were shown in table 1.

Epidemiology

SARS-CoV-2 was declared a public health emergency of International concern by World Health Organisation on 30 January 2020. According to WHO statistics, there was a rapid increase in number of reported cases in China, Italy, Spain, France, and Germany at the end of the march. In the reporting countries population of about 54.6% were undergoing local transmission of infection. According to Chinese centre for disease control and prevention (CCDC) major route of infection was human

to human transmission via respiratory droplets and contact.[9] On 31st January, 2020 all citizens returning from Hubei, province, China were quarantine for up to 14 days as announced by US Centre for disease control and prevention and their incubation period information regarding public health activities toward an infectious disease, including active monitoring, control, surveillance and modelling.[10]

Table 1: Showing relationship between SARS, MERS-COV and COVID-19.[3,6-8]

	SARS-CoV	**MERS- COV**	**COVID-19**
Full form	Severe Acute Respiratory Syndrome	Middle East Respiratory Syndrome	Corona Virus Diseases-2019
Year	2002-2003	2012-2013	2019-2020
Country of origin	China	Middle-East	Wuhan, China
Nature of Diseases (as per WHO)	Endemic	Endemic	Pandemic
Receptor	Angiotensin converting enzyme-2(ACE2)	Dipeptidyl peptidase-4(DPP4)	Angiotensin converting enzyme-2(ACE2)
Incubation period	2-10 days	2-14 days	2-7 days
Distinguishing symptoms	Fever, Non-productive Cough, sore throat, muscle pain, lethargy, headache, diarrhoea, Malaise, Myalgia	Fever, cough, Dyspnoea, muscle pain , vomiting, and abdominal pain, Acute renal impairment	Fever, cough, shortness of breath, pneumonia
Mortality	10%	35%	2-3%
Animal host	Himalayan palm, civets and raccoon dog	Dromedary camels	Bat
Reproductive number	3	0.3-0.8	2.0-2.5

Morphology of Coronavirus

The Coronaviruses were microscopic viruses having size (65-125nm in diameter). Coronaviruses possessed a non-segmented, positive sense single stranded RNA as its genetic material, its size range from 26 to 32Kbs in length i.e. related to length of nucleic acid.[11] The virus was named Coronavirus due to crown like surface spike proteins projection on outside surface of virus seen under electron microscopy,[12] and also being compared with solar corona seen during an eclipse by virologist.[13] Coronaviruses contain nucleocapsids in helical symmetry.[14] The morphological feature of coronaviruses was shown in figure 1.

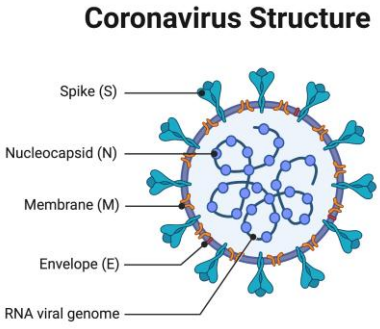

Figure 1:

Structural proteins

Coronavirus specifically composed of a spike, envelope, nucleocapsids and membrane proteins which are responsible for the viral symptoms of infections discussed further in detail.

1. *Spike protein* was responsible for recognising the host cell receptor and found to be critical for binding to host cell receptor that facilitate the entry of virus into host cell. Around 50-100 timers of spikes were enclosed by a Coronavirus particle. An ecto domain elements, transmembrane moiety and short intracellular C fragment were components of Spike protein. An ectodomain contained two subunits i.e. S1 and S2 wherein S1 protein mediate the viral entry into host cell by firstly binding to a host receptor through receptor binding domain and then S2 facilitate the fusion of viral and host cell membrane to penetrate.[15] S2 subunit comprising a single fusion peptide (FP), with an additional proteolytic site, transmembrane domain and a two heptad-repeat were reported to be the novel inserts in S2 subunit of spike proteins, thereby showed high affinity towards Angiotensin Converting Enzyme-2 (ACE-2) receptors.[16] The Type 2 Transmembrane serine protease-2(TMPRSS2) was also co-expressed in type2 pneumocytes simultaneously that accounts for initiating cleavage, membrane fusion and viral entry into host cell and further transmission of infection to neighbouring cells which was specifically high in conditions of low pH.[17]
2. *Envelope protein* (76-109 amino acids) composed of N terminal, C terminal and a hydrophobic domain.[18] Structurally the N terminal expands from 1-9 amino acids; hydrophobic domain from 10- 37 amino acids, C terminal domain extends from 38- 76 amino acids.[19] The amino acid chain till 11th amino acid was present in virion that played an important role in viral assembly and its release whereas the

ahead hydrophobic region oligomerised to form ionic pores across viral membranes.[20,21] Thus the E protein that lies in cytoplasm mechanically target the cis-golgi complex region via proline residues which adheres to endoplasmic reticulum forming an intermediate compartment that leads to exit of virion. The C terminal of E protein possessed a motif named postsynaptic density protein binding motif (PDM), which facilitates the disruption of epithelial cell in lungs associated with Caenorhabditiselegans Lin-7 protein to the PDM. Thus therapeutic benefits against SARS-CoV-2 would be obtained by targeting E protein.

3. *Nucleocapsid protein* genome consisted of serine rich (SR) linker region sandwiched between N terminal domain(NTD) and C terminal domain (CTD). The N protein showed asymmetrical conformation and orthorhombic crystal structure in N terminal domain. It is known to bind RNA genome where sequencing of ordered and disordered region of N protein was done. The amino acid Arginine and tyrosine in N terminal was found to be essential for binding of SARS-CoV RNA. Later on it was proved that C terminal domain was also essential in enhancing the binding capacity of viral RNA. The central linker region rich in serine and arginine residue possessed essential phosphorylation sites that may help in regular functioning of N protein. Higher Alanine substitution reduces the quantity of N protein phosphorylation. The N protein in SARS-CoV promotes the activation of Cyclooxygenase-2(COX-2) while leads to inhibition of phoshorylation of B23 phosphoprotein, type 1 interferon, Cyclin-Dependant Protein Kinase (C-CDK) activity, cytokinesis and protein translation of host cell.[22]
4. *Membrane protein* was most abundant protein among coronaviruses; the M protein (222-260 amino acids) composed of N terminal domain attached to three transmembrane domains further connected to C terminal domain respectively and belonged to N linked glycocylated proteins possessing domain of 12 amino acids.[23] The tyrosine residues were found to be essential in stability of M protein and creating a spherical structure and encircling the ribonucleoprotein. The two dimensional structure of M protein provides a scaffold in viral assembly thereby encouraging the self-association of proteins, improved membrane affinity and retention in golgi apparatus. Our body activated the immune response against pathogens with the help of NFκB (Nuclear factor kappa B). The membrane protein is known to interact with IKKβ(I kappa B Kinase) via inhibition of NFκB factors and also COX-2 reduction thereby enhanced proliferation of viral pathogens.[24]

Genomic organisation

The Global Initiative on Sharing All Influenza Data (GISAD) shared the entire genomic sequence of SARS-CoV-2 on a single platform for the development of vaccination against COVID-19. The genomic sequence of SARS-CoV-2 showed greater similarity around 89-96.3% with two bat coronaviruses i.e. bat-SL-COVZC45 and bat-SL-CoVZXC21[25-27] and strains obtained from bats i.e. RaTG13 indicated 96.2% similarity between two.[28] Therefore SARS-CoV-2 was considered as a new β virus belonging to subgenus sarbecovirus.[2] Recently, genomic sequence of 103 infected patients were analysed and reported that 72 strains was seen in Leucine Codon and 29 strains was seen in Serine Codon at site of 8,728 and 28,144 of sequence. It was believed that the presence of glutamine, serine and proline at various positions in sequence of SARS-CoV-2 affect its property[28]. The genomic sequence was organised into 5'-replicase gene-S-E-M-N-3'flanked by untranslated regions (UTR) on both ends.[29] The genomic compositions of SARS-CoV-2 showed that 14 open reading frames (ORFs) exist and encode for 27 proteins in which longest two ORF1a, ORF1b are present at 5'UTR,[30] constitute two-third of whole genomic length and encode for polypeptide (pp1a, pp1ab). These polypeptides were processed by Chymotrypsin like protease ($3CL^{pro}$), Main protease (M^{pro}), one or two papain like protease to produce a membrane bound replicase transcriptase complex. Upon proteolytic processing, ORF1ab produces 1-16 non-structural proteins (nsps) and ORF1a produces 1-11 non-structural proteins (nsps) which are involved in virus replication and immune evasion.[31-32] The functions of non-structural proteins were shown in table 2.

Thus nsp proteins keep the virus alive inside body of host cells by promoting synthesis, replication and translation. Replicase genes code for non structural proteins, Membrane and Envelope protein that are integral in viral assembly and Nucleocapsid protein are important in RNA synthesis. Replicase gene encode enzyme that use positive stand RNA genome as a template to produce negative stand RNA genome whereas negative strand RNA genome is use as template to produce mRNA molecules that are being translated into structural proteins for production of new viral particle inside host cells.[35] The remaining 12ORFs encode accessory gene (3a, 3b, 6, 7a, 7b, 8a, 8b, 9b) and structural proteins (S, E, M and N) that are present at 3'terminus of genomic sequences,[36] and are translated from sgRNAs of COVs. Different Corona Virus encoded accessory and structural proteins such as Hemagglutinin esterase protein, 3a/b and 4a/b protein which were responsible for genomic maintenance,[32] in which ORF3a, ORF3b and ORF7a proteins induce apoptosis, ORF6 is important for viral pathogenesis.[35]

Entry and replication of Coronavirus

Entry of virus into host cells was an important determinant for pathogenesis and infectivity of virus. To enter into host cells Coronavirus firstly bound to cell membrane surface through RBD (Receptor Binding Domain) for viral attachment, subsequently entered into an endosome and then fusing viral and lysosomal membrane through S2 subunits of Spike. Spike protein

mediated viral entry into host cells as they were present in form of timers. S1 subunit had a receptor binding domain that recognised (ACE2) as its receptor and membrane started to form an envelope around virus until endosome was completed. To fuse membrane, a cell membrane bound serine protease (TMPRSS-2) dissociates S1 from S2 subunit activating them after which virus fused to cell membrane and could be internalised by cell and S2 undergone a series of structural changes, the endosome entered cells where virus was released by acidification or protein cathepsin.[22,37-38]

Table 2: Functions of non-structural proteins.[13,31,33-34]

Non structural proteins	Function
nsp1	IFN antagonist, Inhibition of translation, Degradation of host mRNAs, Cell cycle arrest
nsp2	Unknown; associates with RTCs
nsp3	Papain like proteinase $PL1^{pro}$; PL^{2pro}; polyprotein processing, RNA binding IFN antagonist
nsp4	Unknown; double membrane vesicle (DMV) formation?
nsp5	Three proteases domain, one picoronavirus 3C-like domain
nsp6	Unknown; double membrane vesicle (DMV) formation?
nsp7	ssRNA binding
nsp8	Produces primer with help of primase
nsp9	ssRNA binding; associates with RTCs
nsp10	Dodecameric zinc finger protein, associates with RTCs, stimulates nsp16 methyl transferase activity
nsp11	Unknown
nsp12	RNA dependent RNA polymerase(RdRp) (for utilization of primer)
nsp13	RNA helicase domain and RNA 5'-triphosphatase
nsp14	Exoribonuclease (required for RdRp fidelity) and Guanine-N7-methyl transferase (RNA cap formation)
nsp15	Hexamericuridylate-specific endoribonuclease
nsp16	S-adenosyl methionine-dependant 2'-O-methyl transferase (RNA cap formation)

Many steps involved in life cycle of Corona virus including attachment and entry of virus into host cells, expression of replicates proteins i.e. responsible for RNA replication, transcription, assembly and budding, finally release of matured viral particle through an exocytosis process.[39] S protein mediated the entry of virus into host cell by binding to receptor site ACE2. After binding it induced conformational changes in S protein that facilitated the viral envelop fusion through an endosome pathway. SARS-CoV-2 released its genome RNA (RNA and translated Nucleocapsid proteins started to form an nucleocapsids) which was translated into viral replicase polyproteins pp1a, pp1ab and were cleaved into small products by viral proteinases and these polymerase products undergone a series of sub genomic messenger RNA by discontinuous transcription and finally transmitted into viral proteins which then again assembled into virion in endoplasmic reticulum and Golgi, transported via vesicles and released out of cell through an exocytosis process.[11] The Coronavirus entry and replication were shown in figure 2.

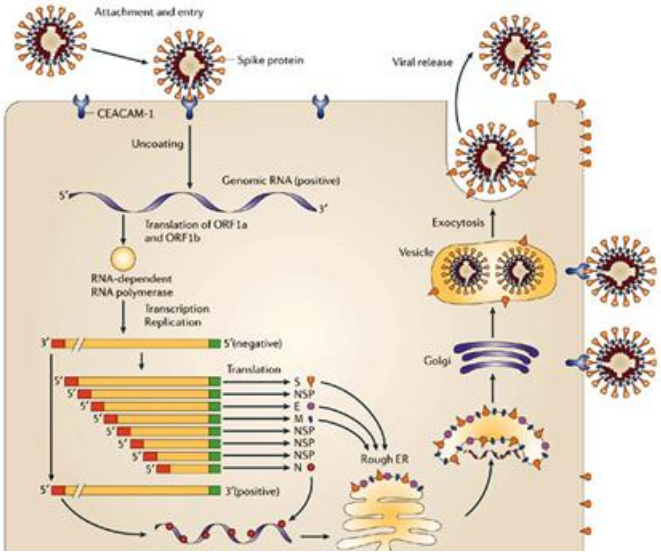

Figure 2: Pathogenesis.

Pathogenesis

The main targeted site of COVID-19 infection was lungs; it caused epithelial cell proliferation and also an increased in macrophages which resulted out in putative Syncytium-like formation.[40] COVID-19 infection induced severe pneumonia cough, RNAaemia and acute cardiac injury patient who were infected with Covid-19 showed higher leukocyte numbers as studied in laboratory around $2.91*10^9$ cells/L of which 70% were neutrophills. Rise in body temperature took place around 39 degree Celsius and increased levels of plasma pro-inflammatory cytokines and chemokines that included interleukin-1-β- (IL-1), interleukin-1-receptor antagonist-(IL-1ra), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), fibroblast growth factor-2 (FGF-2), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (GCSF), interferon gamma (IFN gamma), interferon gamma induced protein (IP-10), monocyte chemo attractant inflammatory protein-1(MCP-1), macrophage inflammatory protein-1-α-(MIP-1 α), macrophage inflammatory protein-1(MIP-1 β), platelet derived growth factor-B-(PDGF-B), tumour necrosis factorα(TNFα), vascular endothelial growth factor-A(VEGF-A). Also blood-C-reaction value 16.16mg/l was noted in infectious patient that was above normal (0-10mg/l) and helped to determine the amounts of inflammation present in the body and also high erythrocyte sedimentation rate was observed.[41-42]

Symptoms

Symptoms that were specifically related to COVID-19 belong to systemic and Respiratory disorder. In systemic fever, cough, fatigue, Sputum production, headache, Haemoptysis, acute cardiac injury, hypoxemia, dyspnoea, lymphopenia, diarrhoea were observed whereas in respiratory disorder Sneezing, Ground glass opacities, acute respiratory distress syndrome sore throat, Rhinorrhoea, RNAaemia.[41,43] After an incubation period of range 5.2 days symptoms of COVID-19 usually appeared. Various risks factors associated with symptoms were age of patient, sex, history of smoking, hypertension, Diabetes. Various factors in Blood were found to be influenced while infected with COVID-19 such as Maximum body temperature, heart rate, White blood cells, Lymphocytes, Neutrophills, Platelets, Erythrocyte sedimentation rate, partial pressure of oxygen, C-reactive protein, Pro-calcitonin (peptide precursor of calcitonin, involved with calcium homeostasis), alanine transaminase (ALT), aspartate aminotransferase (AST).[44]

Molecular assay for detection of COVID-19

1. Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

This diagnostic technique can amplify the genetic material in the sample with the help of primers and probes, thus led to identification of SARS-CoV-2 virus. The sample for COVID-19 was collected from upper respiratory tract by using swabs. Other samples (serum, stool and eye secretions) have also been used in some studies.[45-47] RT-PCR commenced with in-vitro conversion of viral RNA into DNA by RNA dependent DNA polymerase enzyme. This reaction involves small DNA sequences on viral genome and RNA dependent DNA polymerase helped in generation of viral complementary DNA (cDNA). In real time PCR, the reaction is monitored in real tie using fluorescent dye or a sequence-specific DNA probe labelled with fluorescent molecules. This amplification reaction automatically repeated maximum 40 cycles until the viral cDNA that can be detected.[48] RT-PCR is usually carried out as one step or two step procedure in which one step real time RT-PCR involves single tubes containing primers to run entire PCR reaction and two step real time RT-PCR involves multiple tubes to run reverse transcriptase and amplification reactions.[49] The one step procedure is the preferred approach for detection of COVID-19 as it is safe in handling, decrease, pipetting errors; reduce bench time and quick in response. The Diagnostic kits prevalent in market manufactured by various companies were shown in table 3.

Table 3: Available diagnostic kits of RT-PCR in market for detection of COVID-19.[50-54]

S.No.	Diagnostic kits in market	Company	Country
1	COVID-19 RT-PCR	LabCorp	United States
2	2019- Novel Coronavirus Real-Time RT-PCR Diagnostic Panel	U.S. Centers for Disease Control and Prevention	United States
3	TaqPath COVID-19 Combo kit	Thermofisher-Applied Biosystems	United States
4	Allplex 2019-nCoV assay	Seegene	Australia, South Korea, Singapore, United States
5	Cobas SARS-CoV-2	Roche	Australia, Brazil, Canada, Japan, Singapore, United States

Although the RT-PCR test is most widely adapted method for detection of SARS-CoV-2 infections but has a disadvantage of requiring highly skilled personnel, most expensive instruments in laboratory and can take few days to generate result.SARS-CoV-2 is a single stranded, positive sense RNA virus, and since its entire genetic sequence was uploaded to the Global Initiative on Sharing All Influenza Data (GISAD) platform on

January 10, 2020, companies and research groups in a matter of weeks have developed a range of diagnostic kits for COVID-19. The availability of sequence data has facilitated the design of primers and probes needed for the development of SARS-CoV-2-specific testing.[55] Multiple primer and Probes sets were designed from Urbani strain of SARS-CoV polymerase 1b and nucleocapsid gene sequences.[56] with some default settings: the temperature of melting primer set at 60 degree Celsius whereas probe at 70degree. All primer and probes were synthesised by standard phosphoramidite chemistry at the Biotechnology Core facility at the Centre for Disease Control and Prevention (CDC). At 5'- end TaqMan probes were labelled with molecule 6-carboxy-fluorescein (FAM) and at 3'-end with quencher Black hole Quencher 1. Cross titration of serial two fold dilution of each primer gave optimal concentration of primer and probe against a constant amount of purified SARS-CoVRNA.The real time RT-PCR assay was performed by using one step RT-PCR Master Mix (Applied Biosystems). For this purpose 25 µL reaction mixture was prepared by using 12.5 µL of 2X Master Mix, 0.625µL of 40X MultiScribe and RNase Inhibitor mix, 0.25 µL of 10 µM probe, 0.25 µL each of 50 µM forward and reverse primers, 6.125 µL of nuclease-free water and 5 µL of nucleic acid extract. In 96 well plates amplification was carried on an iCycleriQ Real Time Detection System.

Here two targeted genes including ORF1ab and N protein were amplified simultaneously and tested. Forward primer 5'-AGATTTGGACCTGCGAGCG-3'; Reverse Primer 5'- GAGCGGCTGTCTCCACAAGT-3'; Probe 5'-TTCTGACCTGAAGGCTCTGCGCG-3'.This assay was performed using COVID-19 nucleic acid detection kit, fluorescent measurements were taken and the threshold cycle(Ct) value was calculated for each sample.[57-58] A cycle threshold value(Ct-value) less than 35^{o} gave positive test, value of 39.2^{o} or more gave negative test, value intermediate range of 35^{o}-39.2^{o} gave required confirmation by retesting.[59]

2. *Serological assay*

Serological testing are carried out for detection of immunoglobulin M (IgM) and immunoglobulin G (IgG) in blood serum, plasma and biological fluid etc. that played an integral role in vaccine development and determination of epidemiology of SARS-CoV-2 because IgM helped in identifications of early stage infection whereas IgG helped in identification of post infection immunity. Both immunoglobulins are specific for various viral antigens including spike glycoprotein (S1 and S2 subunits, receptor binding domain) and nucleocapsids protein.[60-64] The methodology for the determination includes Enzyme linked Immunosorbent assay (ELISA test), Neutralization assay, Biosensor Test. ELISA test are designed for purpose of detection of antigen and quantifying substances in blood stream such as peptides, proteins, antibodies and hormones. The test provides result within 1-5 hours. ELISA is a microwell, plate based technique that can be used qualitatively and quantitatively. Methodology of ELISA included-polystyrene microtitre plate coated with viral proteins so that antigen will be attached on surface. Microtitre plate was washed by using Tri Phosphate buffer having pH around 7.4 and Detergent used Tween 20 (0.001%-0.005%). Now Enzyme linked specified antibody are added to microtitre plate, again washed with buffer, finally specified substrate are added which on reacting with enzyme linked antibody to form product which can be analysed under colorimeter or fluorescent to get quantity of particular antigen and also to know magnitude of infection in patient. ELISA test has an advantage to execute multiple test samples with high speed and is adaptable to automation for increased throughout.[65] Neutralization Assay Technique was carried out to check the capacity of an antibody to inhibit the infection of cultured virus cells and cytopathic effects of replication of virus. For this purpose, patient blood samples, serum, plasma are collected, diluted and added in decreasing concentration to cell cultures and their level of neutralising antibodies are measured by determining their threshold. The test provides result within 3-5 days.[66-67] Biosensor Tests include the conversion of specific interaction of biomolecules into a measurable read out through optical, electrical and enzymatic methods. For instance, Surface plasmon resonance (SPR) was developed for the diagnosis of SARS as it measures interference with incident light at a solid boundary due to adsorption of antibody and antigen.[68] The test names of various serological assays were developed by various manufacturer to detect COVID-19 among various patients were shown in table 4.

Table 4: Serological test assay kits prevalent for detection of COVID-19.[65,69]

Test name	Manufacture name	Country
DEIASL019/020 SARS-CoV-2 IgG ELISA Kit	Creative diagnostics	United States
KT-1033 EDI Novel Coronavirus COVID-19 ELISA Kit	Epitope diagnostics	United States
VITROS-Immuno diagnostics products Anti-SARS-CoV-2 total reagent pack	Ortho-Clinical diagnostics	United States
qSARS-CoV-2 IgM/IgG Rapid test	Cellex Inc.	Australia, United States

Treatment for COVID-19

Various drugs are being used for the treatment of COVID-19 based on their mechanism of action; category and mode of delivery are being shown in table 5.

Table 5: Clinically used drugs, their category, mechanism of action and mode of delivery for treatment of COVID-19.[70-75]

S. No.	Treatment	Category	Mechanism of action	Mode of Delivery
1	Remdesivir	Antiviral	Speed up the recovery of hospitalized patient by inhibition of viral replication and patient gets discharged within 11 days	I.V.
2	Dexamethasone and N Glucocorticoids	Corticosteroids	Calm down the acute inflammatory responses by decrease responsiveness to inflammatory chemicals	I.V./ orally
3	Favipravir	Antiviral	Selective inhibition of viral RNA dependent RNA polymerase which is needed for replication of Sars-CoV-2 inside human body to cause severe disease	Orally
4	Tocilizumab	Monoclonal antibody	Calm down the hyper immune response called cytokine storm by acting against inflammatory mediators to fight against infection	I.V. drip
5	Hydroxychloroquine	Antimalarial	Found to inhibit the activity of SARS COVID 2 in lab studies by decreasing the acidity in endosomes which are believed to follow pathway for viral infection	Orally
6	Convalescent plasma therapy	Plasma therapy	Given to ill patient to boost their immunity	Transfusion

Convalescent plasma therapy was approved by United States of Food Drug and Administration for patients with severe COVID-19 infectionon March 24, 2020 as this convalescent plasma therapy included the collection of plasma from a recovered individual who had COVID-19 infection and then transfusing into an infected patient as a post exposure prophylaxis. CP(convalescent plasma) was a passive antibody therapy that showed success by neutralizing antigens and preventing replication of virus inside host cells.[75] Plasma was collected through process of Aphaeresis (medical procedure involved removing whole blood from a donor and separating plasma from blood and remaining blood were introduced back into bloodstream of donor). The effectiveness of therapy depended on treatment protocols including timing, volume and dosing of administration that were shown in table 6. Hence, therapy should focus on enhancement of immune system of patient, by inhibiting formation of inflammatory cytokines.

Table 6: Various protocols showing effectiveness of plasma therapy treatment on COVID-19 patients.[76,77]

Patient condition	Number of patient	Timing	Volume	Antibody titer	Outcomes
Critical case series	5	10-22days	200ml	>1:1000	Normal body temperature, PAO^2 increases within 12days
Severely case series	10	16.5 days	200ml	>1:160	Improved oxygenation and reduce inflammation and viral load

Role of Unani medicine

Unani medicines are non-toxic plant based medicines that played an integral role in the prevention of many diseases with no side effects. Various parts of plants are being used to achieve therapeutic action, an aqueous extract of various part of plants such as roots and stolons of *Glycyrrhiza glabra*, dried bulbs of Allium cepa, dried bulbs of Allium sativum, leaves, stem, flower, roots,

seeds and even whole plant of Ocimum sanctum, leaves and barks of *Cinnamomum verum*, the rhizome of Curcuma longa were found to be more effective in the treatment of flu and cold virus infections with lemon juice and honey.[78-80] The roots and rhizome of liquorice had good antiviral potential and antimicrobial activities due to presence of active chemical constituents such as Gycyrrhizin(GL), liquiritigenin (LTG), licochalone A (LCA), Licochalcone E(LCE) and glabridin (GLD), 18β-glycyrrhetinic acid(GA).[81] Several drugs are being used for the sanitization of the environment as it provides a medium for the dissemination of the virus. For instance Resin part of Styraxbenzoides W.G. Craib used in the reduction of airborne bacteria, the root part of Saussureacostus used in insect repellent and toxicity, Wood part of Diospyrosebenum j. Koenig used for fumigation as it contained an essential oil, the whole part of vinegar used for spray due to antimicrobial action. These drugs are mostly aromatic and provide a clearing effect on microbes and a relaxing aroma.[82]

Role of Public in health practices

When the World Health Organisation declared COVID-19 as a pandemic[7] some public health practices were adopted towards the improvement of immunity, preventive the spread of infection, antiseptic measures and promotion of general health practices such as quarantine strategy, sanitization of surroundings, implementation of curfew, treatment of suspected patients.[83] Staying hygiene by washing your hand or cover mouth and nose with tissue during coughing and sneezing was the best protective measure. Single-use surgical masks were the best way to protect ourselves. CPR masks were reusable masks used to deliver positive pressure ventilation in emergency medical response systems and hospital settings so for use this mask filter is created by using the filter provided by the surgical mask.[84] Medical masks and respirators were used to protect the wearer from droplets, airborne particles and body fluids potentially contaminating the face as medical masks were loose-fitting and disposable masks. To design filter airborne droplet filtering facepieces respirators were used which were tight-fitting and disposable protective devices.[85]

DISCUSSION

We have discussed about the composition of virus including structural proteins that are responsible for viral symptoms of infection and genomic sequence that revealed its similarity with strains of bats that could be primary reservoir of spreading infections. In addition we also understood about the viral entry and its replication inside host cell that help in determining the pathogenesis and infectivity of virus. Pathophysiological changes induced among COVID-19 patients showed various symptoms that belong to respiratory disorder and the effects of these symptoms can be mild, moderate and severe based on immunity of patients. Various drug treatments also suggested how to minimize the effect of COVID-19 disorder. Still there is no perfectly remedy to treat COVID-19 infections. But we can follow some preventive measures such as Quarantine strategy, sanitization of surroundings, and treatment therapy such as unani medicine that can minimize the effect of COVID-19 to some extent.

CONCLUSION

Origin of COVID-19 diseases from Wuhan city in China had become pandemic as per World Health Organisation. This disease had spread more than 200 countries and territories with about around 7.2 million patients and more than 0.22 million death globally. The patients showed flu-like symptoms with fever and breathing problems. Still, there are no particular treatment, symptoms and side effects of this disease. Scientists from various countries and many pharmaceutical companies such as Moderna Therapeutics, Inovio Pharmaceuticals, Novavax, Stermirna Therapeutics, Johnson and Johnson, Clover biopharmaceuticals, Codagenix, Vir Bio-technology, etc are practicing a lot to discover a vaccine for this hazardous disease.Till Now, India's 1st indigenous COVID-19 vaccine candidate COVAXIN™ developed by Bharat Biotech India in collaboration with the Indian Council of Medical Research and National Institute for Virology (NIV) gets DCGI (Drug Controller General of India) approval of Phase I and II Human Clinical trials that would be initiated in July at PGI Rohtak. The DCGI has also approved pharmaceutical firm Zydus Cadila to start Phase I and II human clinical trials of its COVID-19 vaccine. This is the second vaccine after Hyderabad-based Bharat Biotech's COVAXIN to get the approval. The Central Drug Research Institute (CDRI) has received permission for carrying out phase III clinical trial of drug Umifenovir against COVID-19. So there is a great need to ban on utilizing wild animals and birds as a source of food. So it is mandatory to follow the strict rules until the vaccine is not developed, prevention and following practices such as Unani therapy can be adopted, Quarantine strategy is one of the best options to break the chain of this infectious disease otherwise the situation can be worst. Till Now, National and International health care agencies have shown appropriate co-ordination in handling of this outbreak up but still there is a great need of hour for further co-operation.

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