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RECENT CHALLENGES IN VACCINE DEVELOPMENT AND AN UPDATE ON USE OF PROMISING ANTIVIRAL TREATMENT FOR COVID-19 THROUGHOUT THE WORLD

Mahadev Tate*, Akshay Gade and Ritesh Fule

Department of Pharmaceutics' H. K. College of Pharmacy, Oshiwara, Jogeshwari (W), Mumbai-400102.

***Corresponding Author: Mahadev Tate**
Department of Pharmaceutics' H. K. College of Pharmacy, Oshiwara, Jogeshwari (W), Mumbai-400102.
Email ID: mahadevtate81@gmail.com,

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ABSTRACT

In recent time numerous viruses have arisen and affected the global health. Millions of people are at higher risk of acquiring Several evolving viral infections. Novel corona virus is a major source of disaster in current 21st century. On 30th Jan 2020, World Health Organization (WHO) officially declared the COVID-19 epidemic as a public health emergency of international concern. Corona virus as known to be a zoonotic virus. During the last 4-5 years, corona viruses have become the most important viruses worldwide because of the occurrence of many recent deaths caused by corona viruses in Saudi Arabia.[1] The first cases of corona virus infection in Saudi Arabia, were reported on June 13, 2012; after this outbreak, corona virus continued to spread to many countries in Asia, Europe, America, Africa. During the research work it is found that there are seven major targets (spike protein, envelop protein, membrane protein, protease, nucleocapsid protein, hemagglutinin esterase and helicase) for which drug design can be considered. There are other 16 nonstructural proteins, which can also consider for drug design. The data reported in current review are based on real time updates. This paper reviews the literature on all available information about risk factors, chance of infection, prevention methods of corona virus (CoVs). The aim is to present current research from the perspective of medicines to allow the rapid discovery of anti-nCoV agent.

KEYWORDS: Zoonotic virus, Non-structural proteins, COVID-19.

1.1. INTRODUCTION

Two novel viruses are implicated to be responsible for severe acute illness in recent times i.e. Middle East Respiratory syndrome corona virus (CoVs) and Severe Acute Respiratory Syndrome corona virus. This newly identified virus is belonging to family Coronavirus, genus Betacoronavirus and subgenus sarbecovirus.[2] The reservoir of this viruses is usually animals, including bats, camels or chimpanzees.[3] This virus is transmitted from animals to human and from infected patient to healthcare professionals as well as other persons. Although no specific treatment has been recommended for their management so far; supportive treatment has shown to improve outcome. Intensive education awareness and multidimensional care does not give satisfactory result. Therefore, an accurate knowledge of their reservoir, transmission ways, presenting symptoms and best possible management together with preventive steps is necessary. There are seven strains of human corona virus which include 2019-corona virus, severe acute respiratory syndrome corona virus, NL63, HKU1, OC43, 229E, Middle East Respiratory corona virus. Among these seven viruses SARS-CoV, 2019-nC and MERS-CoV are highly pathogenic. In past two decades, this is third instance of emergence novel coronavirus, after SARS in 2003 and MERS in 2012.[4] The previous two attacks of corona viruses in china and Saudi Arabia and outbreak from that situation highlighted their adaptive potential to changing environmental conditions and they are classified under emerging viruses.[5]

1.2. Structure of Corona Virus Corona virus is approximately 50-200 nm in diameter with single positive stranded RNA genome, covered by an enveloped structure.[6] Among all RNA viruses, the RNA genome of corona virus is largest which is in crown shape under electron microscopy.[7,8] The genetic material of corona virus is susceptible for frequent recombination processes, which can give rise to new strain with alteration in virulence. It is divided into four groups (α, β, γ, δ) while β-corona virus further subdivided into A, B, C, D lineages.[9] The most important structural proteins of corona virus are membrane protein(M), spike protein (S) which is trimeric, nucleocapsid protein(N) and envelop protein(E). The RNA genome of CoV has seven genes in following order ORF1a, ORF1b, S, OEF3, E, M, N in 5'to 3' direction. The RNA genome is packed in nucleocapsid protein and further covered with envelope. The nonstructural proteins take part in various types viral activities. The remaining genome encodes the mRNA which produces the structural proteins. SARS-CoV-2 is 96% similar at the whole-genome level to a bat corona virus. Mainly, five SARS-CoV-2 targets are known

including Receptor Binding Domain (RBD) protein, 3CLpro, RNA-dependent RNA polymerase (RdRp), E protein and N protein.[10] the spike glycoprotein of corona virus is the main mediator of entry into host cell.[11] The amino acid sequence of receptor binding sites of SARS-CoV-2 is 74% homologous to that of SARS-CoV suggesting similar or even identical cell entry mechanisms for both viruses.[12]

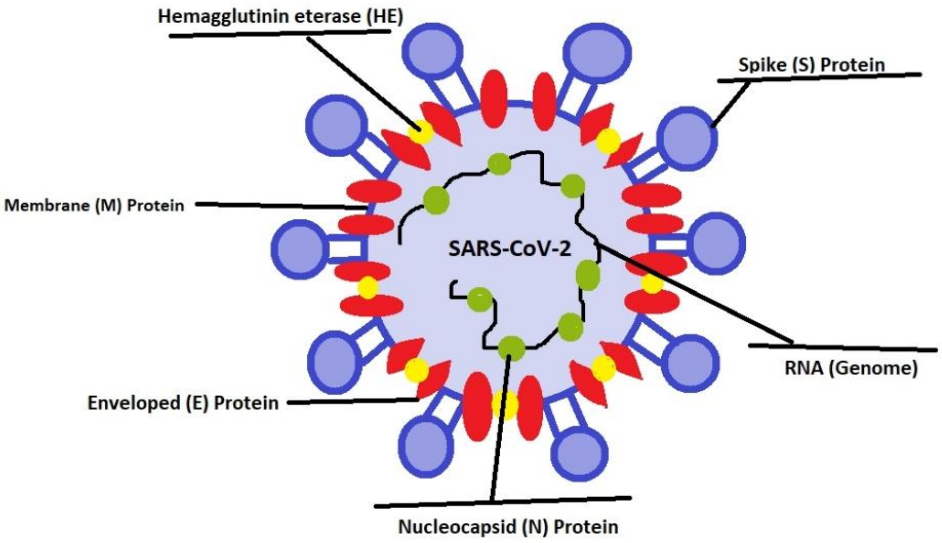

Figure.1 Structure of Corona Virus

1.3. Symptoms

SARS-CoV-2 infection of respiratory epithelial cell activates monocytes, macrophages and dendritic cells, resulting in secretion of range of proinflammatory cytokines including interleukin-6 which is responsible for lymphatic changes.[13] Coronaviruses first replicate in epithelial cells of the respiratory and enteric cells, which leads to cytopathic changes. Infection with upper and lower respiratory tract, pneumonia, common cold, bronchiolitis, rhinitis, pharyngitis, sinusitis and other symptoms such as occasional watery and diarrhea. SARS-CoV, MERS-CoV and 2019-nCoV are highly pathogenic and responsible for pandemic situation. The clinical presentation of corona virus ranges from flu-like symptoms, i.e. cough and fever in 87% of patients, chills, rigor, rhinorrhea, myalgia, and fatigue, to more severe symptoms, including shortness of breath in 48% of patients and respiratory failure, resulting in the requirement for intubation and ventilation.[14] Gastrointestinal symptoms, including nausea, vomiting, diarrhea, and abdominal pain, have also been reported in about 35% of cases, and acute renal failure necessitating hemodialysis has been seen. Sometime hepatic dysfunction also can occurs in severe cases and were associated with fatal outcome.[15] COVID-19 is sever in patients with lung cancer.[16,17] As compared to the normal patients, cancer patients have high risk of infection and poorer outcomes after COVID-19.[18] Recent reports from around the world highlight chance of pernio (superficial inflammatory vascular response) like phenomenon in association with COVID-19.[19]

1.4. Transmission of Corona Virus

Respiratory viruses are most contagious when a patient is symptomatic.[20] presently COVID-19 seems to be spread by the same mechanism as other common cold or influenza virus.[21] Transmission is occur through droplet infection (respiratory secretions) and close person-to-person contact.[22–24] It can also spread through sweat, urine, and respiratory secretions.[25] COVID-19 infection may lead to gastrointestinal infection and may exist in stool also.[26] Nosocomial transmission of SARS-CoV-2 is common pathway.[27] Corona virus spread through touching contaminated surfaces also.[28]

As per WHO guidelines transmission of the corona virus can occur by two ways, either by direct contact with infected people or indirect contact with surfaces within the immediate environment or with objects used on the infected person (e.g. stethoscope, thermometer or personal equipments). Airborne transmission of this virus is different from droplet transmission because it refers to the presence of microbes within droplet nuclei, which are generally considered to be particles size <5µm in diameter, can remain in the air for long periods of time and can be transmitted to others over distances greater than 1 m. This virus can survive for 2 hours to few days in cough and sneezing droplets therefore chance of transmission of this virus is more as compared to other virus.[29]

Salivary gland is the significant reservoir of the virus in saliva. As per research data COVID-19 is spread through contaminated saliva also.[30]

2.1. Cycle of Corona Virus in host cell

Angiotensin converting enzyme act as cellular entry receptor for SARS-CoV-2.[31] When corona virus enters into the body, it binds to the primary target cells such as enterocytes and pneumocytes, thereby establishing a cycle of infection and replication. CoV attaches to the target cells with the help of spike protein–host cell protein interaction. Then receptor reorganization is done and after that virus genome with its nucleocapsid is released into the cytoplasm of the host cells. The viral genome contains ORF1a and ORF1b genes, which produce two PPs that are pp1a and pp1b. These proteins help to take command over host ribosome for their own translation process. NSPs have their own specific functions. All NSPs have an important role in replication and transcription.[13]

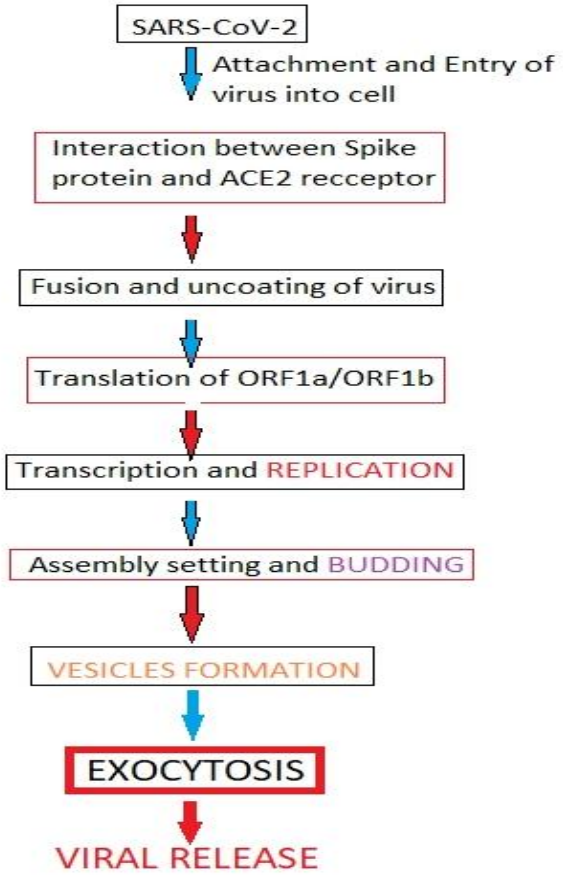

Figure.2 Life cycle of Corona Virus in host cell

2.2. Diagnosis Methods of COVID-19

The first step is to ensure adequate isolation to prevent transmission to other contacts, patients and healthcare workers. The main clinical manifestations are fever, fatigue, myalgia, dry cough, and shortness of breath. Few patients may present with nasal congestion, runny nose, sore throat, hemoptysis, or diarrhea. SARS-COV-2 is the etiologic agent of COVID-19, and its viral nucleic acid detection using real-time polymerase chain reaction (RT-PCR) is considered the reference standard for the diagnosis.[31] Specimens should be obtained from saliva, upper respiratory tract (nasopharyngeal and oropharyngeal swabs), lower respiratory tract (sputum, endotracheal aspirate, or bronchoalveolar lavage), urine and stool if possible.[32] Repeated testing is necessary for confirmation. If the SARS-COV-2 nucleic acid is not detected in respiratory tract samples taken on two consecutive occasions at least 24 hours apart, COVID-19 can be ruled out. According to current diagnostic criteria founded by the China National Health Commission, laboratory examinations, including nasopharyngeal and oropharyngeal swab tests, have become a standard assessment for diagnosis of COVID-19 infection.[33] SARS-CoV-2 infected individuals produce antibodies against the virus and these antibodies may be used for detection of infected person.[34] In case of rapid diagnostic testing, if the target antigen is present in sufficient concentrations within the sample, then it'll bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal, typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, this type of tests are best to identify acute or early infection. The level of C-reactive Protein (CRP) also reflect disease severity and should be use as key indicator for disease monitoring.[35]

RT-PCR test is standard for diagnosis of COVID-19.[36] However RT-PCR test itself also has limitations as like false result because of incorrect sampling location of the swab test and insufficient viral material in specimen. Therefore RT-PCR test for COVID-19 have high specificity but low sensitivity.[37] RT-PCR test is time consuming also. However Chest CT play an important role in diagnosis and management of COVID-19 patients.[38,39] From the current clinical evidences, lung's ultrasound patterns of subject with COVID-19 pneumonia include a patchy distribution of interstitial artifactual signs.[40] in case of ventilated patients, cardiac, vascular and lung ultrasound should always be evaluated together in order to gain a more comprehensive understanding of the relationship between lungs, cardiac and vascular abnormalities.[41]

2.3 Current situation of Covid-19 in India

As per the WHO reports, on 30 Aug 2020 India's Covid-19 recovery rate is 76.61%. Case fatality rate is continuously declining and it falls up to 1.79%. The top five most affected countries in the world are US, Brazil, India, Russia and Peru. India comes on third rank globally in Covid-19 cases but even more worrying is that the infections are fast moving towards rural and semi-urban areas. If situation is not controlled in rural areas in coming days then it may become challenging for government and medical professionals. Still in rural areas, the population density is low as compared to urban parts but the only problem is that health infrastructure is not good as available in cities.

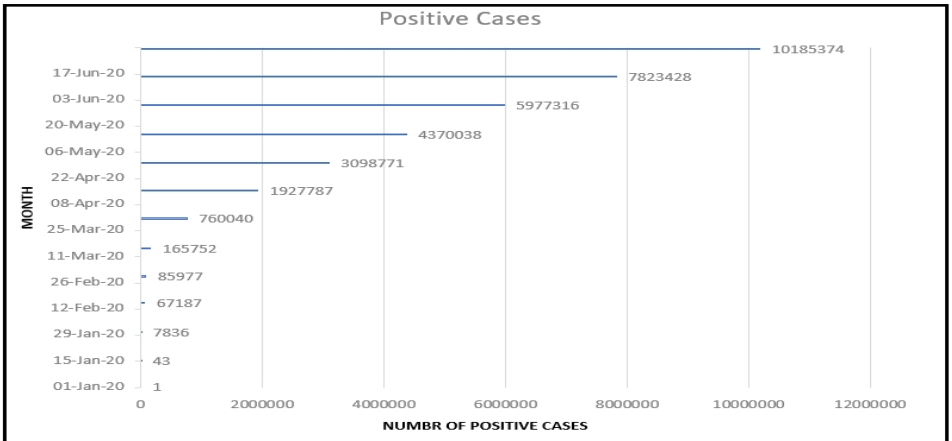

Figure.3 Cummulative Global Corona Positive Cases

3.1. Potential Treatment Options for Symptoms of Corona Virus

According to the guidelines for the treatment of COVID-19, suspected and confirmed cases should be treated in isolated hospitals with effective isolation and protection condition.[42] Some statistical studies have shown that, on average, one infected patient may cause a subsequent 5.7 confirmed cases. Most treatment strategies focus on symptomatic management and supportive therapies only. Medicines to be used in treatment of COVID-19 must be as per model list of essential medicines (MEM) published by WHO.[43] The antimalarial drug Chloroquine phosphate which has already been approved is confirmed to have an anti-SARS-CoV-2 effect and has been included in diagnostic and therapeutic guidelines.[7] It is one of the most prescribed drug in the world to fight against corona virus.[44] Chloroquine seems to be effective in limiting the replication of corona virus causing COVID-19 in vitro.

Ritonavir, Remdesivir, and chloroquine showed efficacy at cellular level in treatment of corona virus.[45] Incubation period of COVID19 is about 2-14 days. In vitro infection of Vero E6 cells by SARS coronavirus (SARS-CoV) is blocked by hexapeptide Tyr-Lys-Tyr-Arg-Tyr-Leu. The peptide also inhibits proliferation of coronavirus. This peptide was shown to be specific against coronaviruses that attach to the ACE-2 receptor.

3.2. Herbal Treatment

Now a days many herbs are also used in treatment of covid-19.[46] According to Chinese report most frequently used herbs included Radix astragali (Huangqi), Radix glycyrrhizae (Gancao), Radix saposhnikoviae (Fangfeng), Rhizoma Atractylodis Macrocephalae (Baizhu), Lonicerae Japonicae Flos (Jinyinhua), and Fructus forsythia (Lianqiao).[47] A Cochrane Review[48] found that Chinese herbs combined with western medicine significantly improved symptoms of SARS, including decreasing body temperature, cough and breathing difficulties, dosages of corticosteroids, improving absorption of pulmonary infiltration, and quality of life. Traditional Chinese medicines like shenmai injectin, xuebijing injection also control oxygen level and inhibit the release of inflammatory factors.[14] Some Indian herbs are also use in fighting against corona virus as immunomodulator, anti-inflammatory. plants indigofera tinctoria, Gymnema sylvestre, Leucas aspera, Sphaeranthus indicus, Clerodendruminerme Gaertn, Evolvulus alsinoides, Vitex trifolia, Abutilon indicum, Cassia alata shows anti-coronaviral activity in mouse.[49] Immunomodulatory drugs/ medicinal plants play an important role in fight against COVID-19.[50] As per the theory of traditional medicines Scutellariae Radix, Artemisiae Annuae Herba and Belamcandae Rhizoma are use frequently together in herbal formulary for it's heat clearing effect. So these herbs are also use in treatment of COVID-19.[51]

Recently researchers work on melatonin as adjuvant in COVID-19. It is mainly use as anti-inflammatory, anti-oxidant and immune response regulator. But still direct evidence about the use of melatonin in COVID-19 is unclear.[52] However, it is clear that finding an effective antiviral and developing a vaccine are still significant challenges. The costs of the epidemic aren't limited to medical aspects, because the virus has led to significant sociological, psychological and economic effects globally. According to the current studies it is proved that N95 respirators can also reuse after it's disinfection by proper methodology.[53] Human coronaviruses can be efficiently inactivated within 1 min using surface disinfection procedures with 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite. The main treatment strategy for typical corona virus infection is supportive therapy, Anti-viral therapy and corticosteroid therapy.[54]

3.3. Anti-viral Therapy

There is no specific antiviral drug for COVID-2019. All drug options are taken from previous experiences and outbreaks.[55] S-protein is the principal antigenic determinant and target of neutralizing antibodies therefore S protein is valuable target in vaccine and antiviral efforts.[56] Mostly combination of antiviral drugs

are used to treat COVID-19 infection. The recommended dose is two capsules of Lopinavir/Ritonavir (200 mg/50 mg per capsule) orally together with nebulized α-interferon inhalation (5 million IU in 2 mL of sterile water for injection) twice a day.[57] Remdesivir, a nucleotide analog, and chloroquine, an antimalarial drug, are promising drugs against COVID-19 as they are known to inhibit SARS-COV-2 virus. Favipiravir, Ribavirin, Penciclovir also use as therapeutic drug by inhibiting RNA dependent polymerase enzyme. As result replication of corona virus stop. Patient preferences should be taken into consideration in all of the scenarios because individual patients need is may be different.[58]

3.4. Corticosteroid Therapy

In general, use of corticosteroids in the treatment of COVID-19 pneumonia is not recommended as it may delay the virus clearance from the body.[59] However, short-term administration of methylprednisolone (1-2 mg/Kg bodyweight per day) has been used frequently. This regimen is also recommended for pregnant women with COVID-19. A recent study performed in china, found that treatment with methylprednisolone reduces the risk of death among the patients with Acute Respiratory Distress Syndrome (ARDS).[60]

3.5. Supportive Therapy

Adequate rest, hydration, nutritional support, and water and electrolyte balance should be ensured. It is essential to monitor vital signs and oxygen saturation closely. Depending on the severity, oxygen is supplied to patient. Other complications may include septic shock, acute kidney injury, and virus-induced cardiac injury. Therefore, it is important to check all these factors. Mental support also plays an important role during treatment time. If patient is mentally strong enough then chances of recovery are increases.

3.6. Plasma Therapy

Plasma Therapy is based on concept of passive immunity, where antibodies corona virus are developed in person are use to treating others. Plasma in the blood contains antibodies, which helps to fight foreign pathogens. The people who fought and recovered from coronavirus might have developed antibodies in their body, which can be a boon for others.[61] It was also used earlier during the Ebola outbreak. Collection of plasma takes more time than blood donation because procedure for plasma collection and it's storage is complex. Although doctors do not recommend it, as storing plasma makes the antibodies present in it less effective. Immune plasma therapy is a promising option in treatment of COVID-19 syndrome.[62]

Monoclonal antibodies can only recognize a single antigen epitope, which limits the use of MAb114 and REGN-EB3 in the treatment of COVID-19.[63] Researchers suggests for combined use of antibodies against corona virus infection.[64] Till today`s date, for treatment of novel corona virus no one monoclonal antibodies are developed.[65]

Off-label drug candidates against COVID-19 disease.

Name of potential agent	Mechanism of Action	Dose for Adult	Trial or Phase
Ivermectin	Act as hydroxychloroquine, Stop the transportation of viral protein through importins into nucleus and diminishes the ability of virus to cause harm to cell.	12 mg/ day	NCT04392713
Remdesivir	Remdesivir Triphosphate act as direct antagonist of ATP and block the function of RdRp.[66,67]	100mg/ day Duration: 9 days	ChiCTR2000029559 (+), NCT04280705, phase 3
Zinc and chloroquine	zinc shut down RNA dependent RNA polymerase/ replicase and chloroquine helps to increase intracellular level of zinc.	15 mg/ day	------
Famotidine	Target papain like protease (PLpro) and stop the virus replication.	136 mg/ day Duration: 6 days	NCT04389567
Nicotine	It is cholinergic agonist and act as important inhibitor of pro-inflammatory cytokines acting through cholinergic anti-inflammatory pathway via α7-nAChRs.	-----	-----
Hydroxychloroquine (Broad spectrum antiviral)	Block viral entry into cell by inhibiting glycosylation of host receptor, endosomal acidification and proteolytic processing.[68–71] Interfere with sialic acid biosynthesis.[72] It has immunomodulatory effect, such as suppression of production and release TNF-α and IL-6.[73]	500 mg/ time Duration: 7 days	NCT04261517, Phase 3 (+)
Tocilizumab	Act on immune system (IL-6 Blocker), Reduce the effect of cytokine release syndrome (CRS).[74]	160 mg/ day	NCT04317092, Phase 2

Umifenovir	Targeting S protein/ACE-2 interaction and inhibiting membrane fusion of the viral envelope.[75]	-----	ChiCTR2003024 Phase 4
Ritonavir/lopinavir	Act as inhibitor of protease inhibitors. Interrupts viral nucleic acid replication via inhibition of 3CLprp.[76,77]	400 mg/100 mg each time, 2 times per day Duration: 10 days	NCT04257656, Phase 3 (-)
Arbidol	Act as broad spectrum anti-viral, Block the fusion of the virus with host cell.[78]	200 mg/ time Duration: 10 days	-----
Favipiravir	Block the function of RdRp.[79]	400 mg/ time Duration: 10 days	ChiCTR200030254
Imatinib	Shows immunomodulatory role in COVID-19 patients. Reduce transcription factor NF-kB signaling pathway.[80]	800 mg/ day Duration: 14 days	Phase 3
Ruxolitinib	Inhibitor of JAK 1/2 which is responsible for multiple cellular signals including IL-6. It also act as immunomodulator.[81]	20 mg/ day Duration: 7 days	Phase 2

4. PREVENTION

A proactive and targeted public health response is fundamental for interrupting human-to-human transmission chains and preventing further spread, thereby reducing the intensity of the epidemic.[82] To stop the transmission of virus social distancing is the best way which break the chain. Till today's date there is no approved treatment for COVID-19 therefore prevention plays crucial role in spreading of this virus. Isolation of confirmed or suspected cases with mild illness at home is recommended. Healthcare workers should be provided with fit tested N95 respirators and protective suits and goggles. Because of the recombination process or changes that occurred in the CoV and changes in protein structural and functional levels, no one vaccine is act specifically against the corona virus. Modern techniques of identification of viruses by PCR and genetic coding techniques may be unable to provide an early and accurate isolation of the virus. Pregnancy is a state of partial immune suppression which makes pregnant women more vulnerable to viral infections.[83] Therefore we have to take more care of pregnant women. A large number of cases requiring hospitalization and intensive care is a serious burden even for affluent countries with well-developed healthcare systems.

Vaccine is the best option for blocking any infectious disease circulation. After the SARS epidemic in 2003, researchers from all over the world got in the efforts of developing a vaccine for corona virus.[84] There are about 43 vaccines that have been developed. But most of them are in preclinical phase.[85]

The use of mouth rinse and local nasal products which include beta-cyclodextrins in conjunction with flavonoid agents, might provide invaluable care to minimize the viral load of saliva and nasopharyngeal microbiota including SARS-CoV-2.[30]

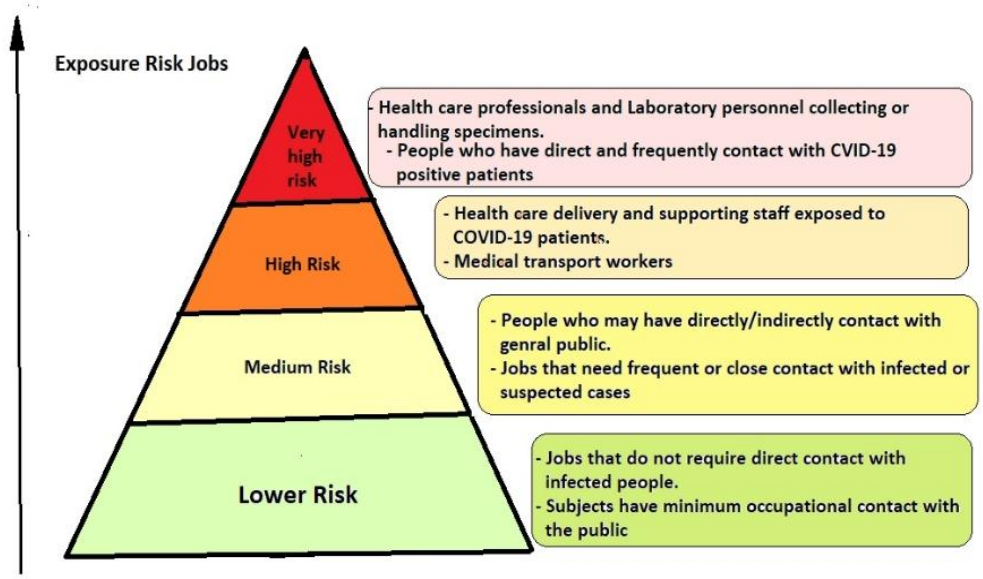

Figure.4 Risk pyramid for COVID-19 based on occupation

5. UPCOMING WAVE IN RESEARCH OF CORONA VIRUS TREATMENT

To meet the urgent need for an effective vaccine against COVID-19 transmission, industries and institutions worldwide have been working on corona vaccine candidates. This rapid approach is based on in-silico informatics.[86] Most promising one of them is mRNA-1273 vaccine, which is developed by National Institute of Allergy and Infectious Disease scientists together with Biotechnology company Moderna.[87] Strong international co-ordination between all countries, collaboration among studies, pharmaceutical companies, governments and regulators are needed to ensure that promising therapies or vaccines can be manufactured and supplied successfully to all over the world in this pandemic.

Vaccine candidates for Covid-19.

Name of Vaccines	Description	Clinical Phase	Developed By
ChAdOx1 nCov-1	Recombinant adenoirus vaccine	Phase 3	University of Oxford
mRNA-1273	mRNA vaccine encodes for stabilized form of virus spike (s) protein	Phase 3	Moderna Inc.
Ad5-nCoV	Recombinant adeno-virus, type-5 vector	Phase 3	Cansino Biologics
CoronaVac	Based on inactivated pathogens.	Phase 3	Sinovac Research and Development Co. Ltd
BCG-live attenuated vaccine	Effective against multiple infectious diseases including Covid-19 by increasing immune response.	Phase 2/3	University of Melbourne and Murdoch Research Institute
AZD1222	Recombinant viral vector vaccine	Phase 2/3	Oxford university
BNT162	RNA based vaccine	Phase 2/3	Pfizer, BioNTech
ZyCoV-D	Two approaches- 1.DNA Vaccine by using viral protein 2. By using live attenuated recombinant measles virus.	Phase 2	Zydus Cadila

6. CONCLUSION

Corona Virus is a lethal zoonotic virus that originated in the Middle East. The main source of transmission, as has been shown in several studies, is through camels, bats. Drug discovery against the CoV is really challenging job for researchers. Therefore, providing appropriate clinical management and support to patients while adequately protecting healthcare professionals should be our goal. Several vaccination trials are underway in different parts of world. For avoiding the transmission of COVID-19, WHO emphasize the importance of frequent hand hygiene, respiratory etiquette, and environmental cleaning and disinfection, as well as the importance of maintaining social distances and avoidance of close, unprotected contact with people with fever or respiratory symptoms. Combination therapies with some of the above-mentioned drug with appropriate immunomodulatory diet, proper mental support will eventually be effective against corona virus.

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