PalArch's Journal of Archaeology of Egypt / Egyptology

CONTRIBUTING FACTORS, EXPERIMENTAL MODELS AND CLINICAL TRIAL IN CORONAVIRUS PANDEMIC

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Sangita Saini[1], Sunil Kumar[2], Beena Kumari[3*]: Contributing Factors, Experimental models and Clinical trial in Coronavirus Pandemic-- Palarch's Journal Of Archaeology Of Egypt/Egyptology 17(7). ISSN 1567-214x
KEYWORDS: Corona Virus, factors, Phase, Clinical Trials, Models.

ABSTRACT:
SARSCOV-2 causes Epidemics or pandemics in Human being starting from Wuhan, China in December 2019 that have claimed the lives of millions. The emergences of Novel strains or variants of coronavirus have a deep economic impact due to overburden of healthcare system. Thus, SARSCOV-2 infection considered as a Global health emergency. This international emergency prompts global efforts to accelerate development of vaccine or antiviral drugs. Like other coronaviruses (SARSCOV MERS), SARSCOV-2 belongs to coronavirdae family, a family of large positive RNA virus which mutates rapidly and highly pathogenic in both human and animals. SARSCOV-2 is approximately 70% identical to SARSCOV and MERS. Nevertheless, prior knowledge of coronaviruses, the research on SARSCOV-2 is still in its juvenile stage due to mutation in some genes. Since there is no effective treatment or epidemiological control available for SARSCOV-2 infections, significant efforts have been done to develop vaccines and therapeutic drugs. Several drugs such as Ramdesivir, Azithromycin, chloroquinine, lipnovir etc are repositioning of already approved drugs for treatment of SARSCOV-2 infected patient. On other hand, several SARSCOV-2 vaccines and therapeutics antibodies are under clinical trials. This is a long term process and requires thorough testing of safety. Here, we review the contributing factors in SARSCOV-2 transmission and experimental models used in study of pathogenesis and drug development for SARSCOV-2. In addition, we also discuss the clinical ongoing trials. It is hoped that this review will help in research related to drug development.

INTRODUCTION

SARSCOV-2 is a coronavirus which belongs to subfamily orthoCoronavirnae of family Coronaviridae (order Nidovirales)[1] which are classified on the basis of antigenicity and genomic sequence into four genera: Alpha coronavirus (alphaCOV), Beta coronavirus (Beta COV), Gamma coronavirus (Gamma COV) and Delta Coronavirus (Delta COV)[2]. Genomic characterization suggested that Alpha (HCoV-NL63 and 293E) and Beta coronaviruses (HKU1) are found in many species including wildlife, rodents, livestock and humans, therefore, have ability to cross barrier from one species to another[3]. On the contradictory, Birds are main reservoirs of Gamma and Delta Coronaviruses (HKU 11), but it can infect mammals also[4]. The genus (alpha and beta coronaviruses) is highly pathogenic and has efficient human to human transmission which is essential property for generation of pandemic of virus[5]. The alpha and Beta coronavirus genera cause different type of symptoms in respiratory, gastric, hepatic and genital organs from asymptomatic to severe[3]. The results of comparative genome and phylogenetic analysis suggested that bats and snakes are ancestors of these viruses but still there is no evidence that they had spread from animal market in china[6]. Coronavirus was first recognized in chicken in 1931 and after 30 years, a group of virologist cultivated two strains of Human coronavirus (229E, OC43) from respiratory tract of a person suffering from common cold[7,8]. These strains were found close resemblance with the virus responsible for bronchitis in chicken. Electron microscopy revealed that viruses are medium sized, pleomorphic and club like projections on the surface. Due to its morphological structure, it was named coronavirus (crown like)[7]. A detailed Research on coronavirus revealed that despite of having respiratory illness, it causes low pathogenesis in immunocompetent persons due to changes in immunity during and after coronavirus infection[9]. However, during winter seasons some adults and infants had lower respiratory tract infection of Coronavirus strains (OC38, OC43, MHV) at less extent[10]. After several investigations, it was found that it can cause a number of diseases such as gastroenteritis, encephalitis, pneumonitis in Human and livestock[11].

Based on genomic sequence similarities and replication strategy, these viral genes are segregated into different groups: HCoV-229E and HCoV-NL63 belongs to Group I, whereas HCoV-OC43 and HCoV-HKU1, SARSCoV, MERS comes under Group II, Group III contains Avian infectious bronchitis virus (IBV)[12-16]. Phylogentically, Group I and II are closely related to each other and drift from one species to another i.e. from bats to human and crosses species barrier. The members of first group generally associated with acute respiratory illness, occasionally responsible for more severe Infections before identification of three human coronaviruses in 21st century (SARSCOV, MERS and SARSCOV-2)[17.18]. The pandemic of severe acute respiratory Syndrome (SARSCOV) in human begin in 2002-2003, Middle East Respiratory Syndrome (MERS) in 2012 and SARSCOV-2, another coronavirus causes a massive outbreak in late 2019 in China[19,20]. SARSCOV, MERS, SARSCOV-2

which belongs to a group of Beta COV of B and C lineage are associated with fatal respiratory or extra respiratory illness[21]. Death rate among confirmed cases from concerning SARSCOV and MERS was up to 10% and 35% respectively whereas number of confirmed cases from SARSCOV-2 has surpassed the cases of SARSCOV and MERS.

A new coronavirus (SARSCOV-2) emerged in beginning of December 2019 in Wuhan (Republic of China) which has Pneumonia like symptoms. From genetic analysis, this virus is seventh member of Beta COV and has 70% similarity with SARSCOV. This outbreak starts from Wuhan, spillover almost all countries. The governments of almost all countries are under pressure due to extra burden on healthcare and economy rate[22]. Therefore, World Health Organization declared this as Global Health Emergency.

In this review, we mainly focus Contributing factors for SARSCOV-2 transmission and various model used in the study related to SARSCOV-2. In addition we focus on ongoing clinical trials in Coronavirus Pandemic.

Mode of transmission of SARSCOV-2

There exist several classes for virus transmission includes human human transmission, airborne transmission and other mean of transmission endogenous infection, common vehicle, and vector spread. In this airborne transmission are most important pathways. Airborne transmissions are those in which microorganism spread through coughing, sneezing, speech, vomiting or atomization of faeces in faecal matter in infected person[23] (Amirian 2020)[24,25]. When an infected person speaks loudly, cough or sneeze; the virus will be exerted from the body in environment and become bio-aerosol. The size of bio-aerosol is 0.3 to 100 um. Smaller size particles remain in air and larger particles deposited on the surface[26,27]. Frequent hand washing and face covering are protective measures to reduce the number of infection[28,29]. SARSCOV-2 virus remains viable in aerosol for duration of 3 hours. Noteworthy, it is more stable on plastic and stainless steel than copper and cardboard. On plastic, it is viable for 72 hrs whereas copper measured no viability after 2 hrs. SARSCOV-2 virus remains infectious in aerosol for hours and on surface for days[30,31]. Therefore, this is main reason for super spreading of infection. It is also reported that the SARSCOV-2 infection can occur in third trimester with less likely to death and undergo preterm labor and pose risk to neonates[32,33].

CONTRIBUTING FACTORS IN SARSCOV-2 TRANSMISSION

Age

There is limited knowledge available on burden of disease in pediatrics and geriatrics. SARSCOV-2 causes a large number of infections which involves children more frequently than the young one. The etiological prevalence of these virus in pediatric is 5-30% in which 2.2% was infants[34,35]. In 2002-2003,

SARSCOV has infected more severely to the person above 60 and very mildly to individual below 20 years[36]. Elder patient are more susceptible to severe illness and easily hospitalized due to less rigorous immune response[37]. A major shift in epidemiology of SARSCOV-2 towards those over age 60 has been recognized. Some studies revealed that severity of illness and death among older patient are higher due to multi co-morbidities such as heart disease, diabetes and Acute Respiratory Distress Syndrome[38,39]. Italy, Spain, France and Germany have a large number of confirmed case in which 90% of confirmed cases pass away because these countries have large percentage of older people. More than 95% of all deaths are people over 65 years. But this never means that young people are resistant to infection and they can move freely.

Gender

The data on SARSCOV-2 showed that there is not any specific difference between cases of men and women. Both are equally infected but there is significant difference in mortality and susceptibility. Males are at higher risk compared to female because of difference in their immunological response. In particular may be due to different life style of male such as prevalence of cigarette smoking, alcohol intake etc[40]. Some studies showed that female have robust immune response due to specific steroid hormones in their body and Toll like receptors (TLR7) gene encoded on X choromosome. TLR7 causes higher production of interferon alpha on exposure peripheral blood mononuclear cells (PBMC), makes women's immune system stronger. The production of cytokines and chemokines by immune system are also sex specific[41]. In addition, Estrogen hormone in female promotes T cell by neutrophil accumulation while testosterone suppresses innate immune response[42].

Life style

In modern days, smoking is status symbol and very common cause of mortality in males and female. Tobacco industry gives million of revenue in almost all countries but it is also a major burden on healthcare system. The smoke composed of acrolein, acetaldehyde, formaldehyde and free radicle which are habit forming and difficult to quit. Smoking can cause heart disease, respiratory illness and other cancers. In pregnant women, it can cause complication at the time of delivery or have underweight infant[43]. Cigarette smoking, whether active or passive can cause structural changes in respiratory tracts such as pulmonary tissue damage, inflammation, mucus overflow and air flow obstruction. These changes make smokers more susceptible to viral infection. Cigarette smoking has a significant effect on immune system also. Smokers have more than 30% elevated level of all major blood cells than non-smokers[44]. Cell mediated and humoral immunity affected by prevalence of smoking. Some studies showed a significant increase in CD3+ and CD4 count. CD4 cells cause B cell proliferation and differentiation, reduce level of circulating immunoglogulin and phagocytic activity, hence decrease in response for certain antigen[45]. A systemic review by Vardavas et al[46] involved five studies

on effects of smoking on infection of SARSCOV-2 . The studies revealed that smokers have 1.4 times more severe symptoms (RR=1.4 95% clearance=0.98-2.00) of SARSCOV-2 and need of hospitalization and ventilation as compared to no smokers. A meta-analysis performed on 460592 patients suggested that smokers are more vulnerable to lung infection. SARSCOV-2 is also a respiratory illness which causes damage more likely to lungs. Therefore, smokers are at higher risk of SARSCOV-2 infections[47]. Water pipe smokers are conduit of this infection because they use café for inhalation and increased the risk of transmission of disease in their family also[48]. In current smokers, there is increased gene expression related to ACE-2 receptors. Hence smokers are more prone to viral infection[49].

Alcohol consumption is also a part of modern lifestyle but it put a burden on healthcare system. Excessive alcohol consumption can compromise with immunological response of a person. The main site of alcohol absorption is gut which contains a number of microbial floras. These microbes get destroyed in the presence of alcohol. These gut bacteria helps in promoting immune response and health. The cilliary function of upper respiratory tract also disturbs by consumption of alcohol which impairs the function of immune system and makes lungs prone to viral infection**.** There is limited data available which show relationship between alcohol and SARSCOV-2 infection[50].

Climatic factors

Studies on SARSCoV-2 suggested that virus remain viable for 3 weeks at room temperature while on higher temperature, it lose its viability within a few minutes. Therefore, higher temperature results in low survival of virus and low temperature make prolonged survival[51]. Wang et al[52] investigated the effect of high humidity and temperature on intensity of SARSCOV-2 infection in US and China. Temperature and humidity plays an important role in virus transmission. Another Study from contaminant zone of US with varying temperature demonstrated that summer season with high humidity have very little effects on incidence of virus infection[53]. Furthermore, experimental studies have shown that virus transmission is consistent with latitude, temperature and humidity. Weather modeling can be used to find out the high risk area of contamination in next week[54]. Although, higher temperature will reduce the SARSCOV-2 at a certain level but no evidence was found that makes a clear association between temperature and spreading of virus[55].

MODELS FOR SARSCOV-2 RESEARCH

The progress of any research and drug discovery depends on faithfulness of in vitro and in-vivo model. It is very difficult to elucidate the mysterious interplays between virus and host cell without cell lines, organoids and animal model. Realistic models are essential for fundamental research on SARSCOV-2 to develop anti-viral drugs and vaccines[56]. Needless to say that model is a tool to find the viral infection and pathogenesis. SARSCOV-2 is also a respiratory disease which affects upper respiratory tracts. For SARSCOV-2,

various animal species were used as model for human disease including Mice, rats, ferrets, Hamsters and guinea pigs. Sometimes mice obtained by gene manipulation (transgenic and gene knockout mice) are also used to study human respiratory virus. Therefore, experimental model are important for both fundamental research and drug discovery related to SARSCOV-2[57].

Cell lines

Vero cells are most common mammalian cell used for production of live and attenuated viral vaccine throughout the world[58]. Commercially, there are many cells available which are derived from kidney of green monkey[59]. Ng et al[60] cultured Vero E6 cell lines to find out mechanism of SARSCOV-2 replication and postulated that inflammation occurs due to cytokines. Vero-h-SLAM cell line was used to investigate effect of Ivermectin antiviral drug for infection against SARSCOV-2[61]. Human bronchial epithelial cell lines Calu-3 can be inoculated by viruses as monolayer of cells and cell layer with tight junction and microvilli. Calu-3 cell lines are isolated from human pulmonary bronchial epithelial cells. This study suggested the entry of virus through ACE-2 receptors in calu-3 cell line. Care must be taken in interpreting results when using epithelial cell lines[62]. Chou et al[63] inoculated 25 cell lines from different tissue and organ of different natural reservoir of coronavirus including nine from human origin using RT PCR techniques. Five human cell lines showed SARSCOV-2 viral replication in duration of 120 hr. Calu-3 cell line, Caco-2, Huh-7 and 293 T are more susceptible for infection. Non-human primates' cell line showed same type of replication as in human cell. Substantial Cell damage such as rounding, detachment and degeneration was found only in Vero E6 and FRhK4 cell. This study gives new insight to viral replication and cytopathic effects however we should pay attention that results from cell culture might not summarize the results in whole organism[64]. Human airway epithelium (HAE) cell cultures resemble to human airway represents and can be used to study viral entry[65].

Animal model

In preclinical trials, animal model should reflect the same clinical symptoms, pathology and replication mechanism as in the human. In previous studies, there were a large number of animal models which can be used to study SARCOV and MERS. These include non-human primates, mice, Hamsters and ferrets. Selection of Animal models depends on purpose of study either for pathology or drug development (Vaccine, immunotherapy and antiviral drugs). The ideal animal model is one that reflects same level of human disease such as route of infection, severity of disease and comparable levels of mortality/morbidity. The presence of receptors and viral replication pattern in selected animal species should be same as in human. The model should be selected carefully to achieve experimental goals[66,67]. Some mice species are infected with SARSCOV and showed high titre value in upper and lower respiratory tracts. Knockout mouse, Golden Syrian hamsters can be used for evaluation of vaccines and antiviral drugs[68]. Despite of ferret and cats are

vulnerable to SARSCOV infection, more studies have not found. Based on ACE-2 receptors interaction in SARSCoV-2, some studies are performed using hACE-2 transgenic mice. Viral replication, neuroinvasion, use of interferons 1 alpha and weight loss was observed in transgenic mice rather than wild type. Viral infection was observed in bronchial epithelial cells, alveolar epithelia and macrophages[69,70]. The mouse model may facilitate in drug and vaccines development against SARSCOV-2. Severe or chronic cases of SARSCOV-2 cannot mimic by transgenic mice but transduction BALB/c mice gives better results. Earlier studies related to animal model could not satisfy the criteria related to vaccine development. There is need of robust model for development of therapeutic agents for SARSCoV-2[71]. SARSCOV-2 infection was inoculated in rhesus Macque via ocular route. Mild symptoms of pneumonia observed. No lethal pneumonia or death was observed by SARSCOV-2 in rhesus Macque. Studies were performed on cynomoglus Macque but rhesus was more susceptible[72]. Soldatove et al reviewed the novel approach for creation of SARSCOV-2 sensitive mice. ACE-2 and TMPRSS-2 gene are placed on LOX p sites and make mouse SARSCOV-2 sensitive. The breeding will be done in mice with expressing Cre-recombinase which is safe and representative of SARSCOV-2[73].

Ex-vivo

Human lung tissue was used for SARSCOV-2 infection. Three male and females with average age of 53 years. SARSCoV-2 showed triggered amount of low level interferon and cytokines. Lung tissues are limited and cannot represent the effects of inflammatory response and immune system[74]. Conjunctival, lungs and Bronchus tissues were infected with SARSCOV-2 and compared with AEC and Caco-2 cells. The ciliated, club cells of lung tissues showed similar viral replication as in SARSCoV-2 and CoV and in conjunctiva it has higher for SARSCoV. This study provides important insights to transmissibility and pathogenesis[75].

ONGOING CLINICAL TRIALS

There is no specific antiviral or vaccines are available for prevention and control of SARSCOV-2. The global community is not prepared for this pandemic and development of vaccine takes few years during which it is spread globally and creates burden on health care system. Treatment option depends on symptoms of patient. The symptoms can vary from mild flu to multi-organ failure. Self-quarantine is recommended in mild to moderate condition. Seriously ill patients need hospitalization and Intensive Care due to hypoxic respiratory failure. Some traditional and alternative antiviral agents are used to reduce the symptoms of this disease. According to Chinese government guideline, patients with SARSCoV-2 are treated with conventional medicine and traditional Chinese medicine (TCM)[76,77]. Some studies are done on Ayurvedic treatment of covid positive patient. Various herbs are used to treat symptoms of covid 19. However, there are no supportive clinical trial data available till now[78-80]. There is urgent need of safe and effective vaccine which

immunizes the whole community and gives a solution of this international pandemic. Vaccines development process is often a long and complex process that takes several years or decades that require collaboration of academic, government and manufacturing companies[81]. The vaccines are developing at noteworthy speed and hope that effective vaccine might come in few months. Rapid progress of vaccine is due to prior knowledge of similar virus (SARSCOV) pathogenesis and role of neutralizing antibody against the spike protein in immunity[82]. WHO landscape database have 28 candidates vaccine are under clinical trial (Table 1) till 10th August, 2020. Approximately 139 candidates are under preclinical data. mRNA-1273 from Moderna was first candidate to enter in the clinical trial. Interim results of Phase 1 trial showed that antibody response was dependent on dose and found to be an effective candidate against SARSCOV-2 infection[83,84].

Table 1. On-going Clinical Trial for Coronavirus Vaccine

S. No.	Type of vaccine	Sponsor/Developer	Number and route of administration	Country	Current status	Clinical trial. registration number
1.	Inactivated Vaccine	Sinovac Biotech Co.Ltd	2 IM	China	Phase 3	NCT04456595
		Wuhan institute of Biological Products China National Pharmaceutical Group/Sinopharm	2, IM	China	Phase 3	ChiCTR 2000034780
		Beijing Institute of Biological Products/Sinopharm	2, IM	China	Phase 3	ChiCTR2000034780
		Bharat Biotech	2, IM	India	Phase 2	NCT04471519
		Institute of Medical Biology, Chinese Academy of Medical Sciences	2, IM	China	Phase 2	NCT044470609
2.	RNA					
	LNP encapsulated mRNA	Moderna/NIAID	2,IM	United state	Phase 3	NCT04470427
		BioNTech/ Fosun Pharma/Pfizer	2,IM	Germany	Phase 3	NCT04368728

		Imperial college London	2,IM	UK	Phase1	ISRCTN17072692
		Curevac	2,IM	Germany	Phase 1	NCT04449276
		People Liberation Army(PLA)Academy of Military sciences/ walvax Biotech.	2,IM	China	Phase 1	ChiCTR2000034112
3.	Protein Subunit					
		Anhui Zhifei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy Sciences	2 or 3, IM	China	Phase 2	NCT04466085
	Nanoparticle	Novavax	2,IM	USA	Phase 2	NCT04368988
		Clover Biopharmaceuticals Inc./ GSK/ Dynavax	2,IM	China	Phase 1	NCT04405908
		Vaxine Pty Ltd/ Medytox	1,IM	Australia	Phase 1	NCT04453852
		Queensland/CSL/ Seqirus	2,IM	Australia	Phase 1	ACTRN12620000674932
4.	Non Replicating viral vector	University of Oxford/AstraZeneca	1,IM	UK	Phase 3	ISRCTN89951424
		CanSino Biological Inc./Beijing Institute of Biotechnology	1,IM	China	Phase 2	ChiCTR2000031781
		Gamaleya Research Institute	1,IM	Russia	Phase 1	NCT04436471
5.	DNA Plasmid					
		Inovio Pharmaceuticals/ International Vaccine Institute	2,ID	USA	Phase2	NCT04447781
		Osaka University/	2,IM	Japan	Phase 2	NCT04463472

		AnGes/Takara Bio				
		Cadila Healthcare Limited	3,ID	India	Phase 2	CTRI/2020/07/026352
		Genexine Consortium	2,IM	Korea	Phase 2	NCT04445389
6.	Plant derived virus like protein					
		Medicago Inc	2,IM	Canada	Phase 1	NCT04450004

CONCLUSION

The Impulsive outbreak of SARSCOV-2 in Wuhan now grew into global pandemic. This is third coronavirus after SARSCOV and MERS, have burden on healthcare and economy system. This virus is highly contagious and spread at very high rate through droplets or close contacts. Scientist believed that SARSCOV-2 originated from bats and intermediate host and transmission from animal to human is still unclear. SARSCOV-2 is RNA virus and can mutate very rapidly. SARSCOV-2 can make a copy of genome in very few hours. Like SARSCOV, it uses same protein or receptors (ACE-2) of host cell to enter in the cell membrane. These receptors are widely distributed throughout the body especially in lung epithelium cells. With SARSCOV-2, it takes about two weeks for symptom to appear. The symptoms may vary from mild to moderate and recover fully but in severe case need ICU mechanical ventilation. Therefore, SARSCOV-2 is a great threat to healthcare system. Rigorous efforts are made by Researcher in this duration of pandemic to develop safe and effective therapy, currently there is no vaccine or antiviral approved by FDA yet. Drug repurposing of many already approved drugs are good options for treatment of this infection. Normally, a vaccine would take decades to develop, investigator now hope that vaccine comes within few months. Different countries public or private sector have race to design a potential vaccine. However, some vaccines are in phase 3 and safety studies starts in large human population, still many are in preclinical trials. Therefore, Researcher has much work to do. Safety of trial, generation of immune response, production on large scale should keep in mind for effective immunization of world population. Now it is hoped that an efficient vaccine will come which reduce the psychosocial effect of this pandemic.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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