JURNAL TEKNOLOGI LABORATORIUM

JTL

POLTEKKES KEMENKES YOGYAKARTA

SPECIAL EDITION

COVID-19

CONTENT
JURNAL TEKNOLOGI LABORATORIUM
Vol 9 No 1 (2020): Special Edition "COVID-19"

In this section, we published a special issue about COVID-19. All papers in this number have been reviewed with all our reviewers. We hope these papers will be useful for cope with COVID-19.

Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 01 – 12
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.214
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Review Article

Modulation of severe acute respiratory syndrome coronavirus (SARS-CoV-2) in the receptor, innate immunity and drug anti-viral candidate

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HIGHLIGHTS

- SARS-CoV-2 has a high affinity than SARS-CoV and MERS-CoV due to the changing of some S protein nucleotide.
- High ACE2 expression in respiratory cells that potential high risk for COVID-19 infection by ACE2 receptor.
- There is a screening of drug potential as an anti-viral against SARS-CoV-2, chemical synthesis, and natural product.

ARTICLE INFO

Article history

Received Date: April 02nd, 2020
Revised Date: May 27th, 2020
Accepted Date: June 06th, 2020

Keywords:

Drug candidate
Novel coronavirus
COVID-19
Modulation

ABSTRACT

The Coronavirus disease-19 (COVID-19) is a contagious acute respiratory infectious disease caused by SARS-CoV-2 as a global pandemic in 2020. This disease most spreads and causes some severe cases, even death in the world. The primary purpose of this review discusses the recent article that was published regarding COVID-19 genomic modulation, the mechanism of innate immunity and the screening of anti-viral drug candidates, for treating COVID-19 patients. This review used the latest paper regarding COVID-19 with 63 journals with high impact factors such as Nature, The Lancet, Cells, International Journal of Biological Sciences, Mol Biol Methods. Journal of Microbiology, Immunology, and Infection, Nat. Rev. Microbiol, and other international journals indexed by Scopus, Elsevier, and Springer through in vivo and in vitro studies. The genomic of SARS-CoV-2 consist high similarly to coronaviruses family, albeit possessing a different pathway even has higher affinity, due to changing some nitrogen bases are supposed to have a significant effect on its pneumonia. Herein, we report review article an update on the recent literature of the COVID-19 modulation genome, mechanism of innate immunity, and medical literature. Moreover, we report anti-viral drugs that have been developed from synthetic drugs and medicinal compounds from plants. Several studies have been re-analyzed using in vitro, in vivo, and modelling using bioinformatics tools.

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1. INTRODUCTION

A novel human coronavirus which is a new evolution of SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) was recognized in Wuhan, China, in December 2019.[1] The virus officially named SARS-COV-2 by ICTV (International Committee on Taxonomy of Viruses), and pneumonia caused by SARS-CoV-2 has been known as COVID-19 (coronavirus disease 2019).[2] World Health Organization(WHO) was announced on 31 January 2020 that COVID-19 was list as the Public Health Emergency of International Concern (PHEIC), it's a might risk to multiple countries and requires a coordinated international response.[3,4] The coronavirus have been known belong to a virus's family that may cause various symptoms such as fever, lung infection, breathing difficulty, and pneumonia.[2,3] Recently, SARS-CoV-2 become a serious epidemic worldwide due to highly contagious disease. Previously, at the beginning the coronavirus family was detected in Saudi Arabia in June 2012, then attributed to Middle East Respiratory Syndrome Coronavirus (MERS-CoV).[4,5] SARS-CoV belongs to the family Coronaviridae with an enveloped, positive-stranded RNA virus with ~30,000 nucleotides.[6] Coronavirus (CoV) is a single strand RNA virus with a diameter of 80-120nm. It is divided into four types; α-Coronavirus (α-CoV), β-Coronavirus (β-CoV), δ-Coronavirus (δ-CoV), γ-Coronavirus (γ-CoV). Based on BLASTP using NCBI, it is known that SARS-CoV-2 like SARS-CoV and MERS-CoV belongs to β-coronavirus, a large class of viruses prevalent in nature. If we compared with SARS and MERS, this virus has highly transmissibility and infectivity despite a low mortality rate.[6,7,8] Somehow, phylogenetic trees show that SARS-CoV-2 in Indonesia and similar to the others countries, which derived from the same ancestor of Severe Acute Respiratory Syndrome Coronavirus genome.[3,6]

Albeit, SARS-CoV and MERS-CoV found that has similar case with SARS-CoV-19, it is supposed that the bat is still a probable species of origin for SARS-CoV-2, with share 96% whole genome identity with a bat CoV, BatCoV, RaTG12, from *Rhinolophus affinis* from Province of Yunnan.[8] However, SARS-CoV and MERS-CoV usually pass into intermediate host, such as civets or camels, before leaping to human.[9,10] This fact indicates that the Virus was probably transmitted to human by other animals. Considering that the earliest coronavirus disease 2019 (Covid-19) patient reported no exposure at the seafood market.[10] It has been analyze from genomic database, that Pangolin-CoV is 91.02% and 90.55% identical to SARS-CoV-2 and BatCoV RaTG13, respectively, at the whole genome level.[11,12,13] The S1 protein of Pangolin-CoV is much more closely related to SARS-CoV-2 than to RaTG13.[11,14] Spike protein can interaction with human ACE2 receptor via five key amino acid residues, which is completely consistent between SARS-CoV-2 and Panglion-CoV, but there is four amino acid mutation in RaTG13.[6,11] Both Panglion-CoV and RaTG13 are lost the putative furin recognition sequences motif at S1/S2 cleavage site that can be observed in the SARS-CoV-2. Somehow, researcher suggest that Panglion species are high probably as natural reservoir of SARS-CoV-2 like other Coronavirus.[11,15]

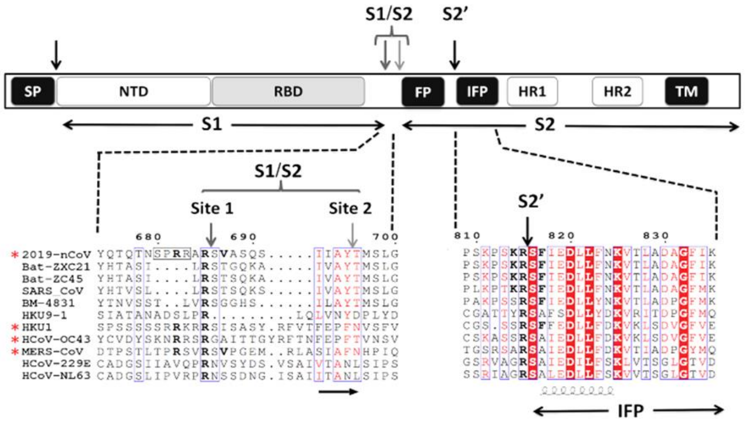

Figure 1. Schematic representation of the COVID-19 contain Spike protein with a focus on the putative maturation sites. The domains were previously characterized in SARS-CoV and MERS-CoV: Signal peptide (SP), N-terminal domain (N-TD), receptor binding domain (RBD), fusion peptide (FP), internal fusion peptide (IFP), heptad repeat ½ (HR ½), and the transmembrane domain (TM).[13] The SP, S1, S2,

and S2’ cleavage sites are indicated by arrows. Insertion of furinlike cleavage site is surrounded by a black frame, while red asterisk indicate the presence of canonical furin-like cleavage motif at the S/S2 site.[13]

If we compare, SARS-CoV (2003) infect 8098 individuals which 9% mortality rate, across around 26 countries in the world, but somehow novel coronavirus (2019) infect more than 935.750 individuals people with mortality rate 2.9~4% till date of this paper writing. It is shows that the transmission rate of SARS-CoV-2 is higher that SARS-CoV due to genetic recombination even at Spike protein in the receptor binding domain (RBD) region of SARS-CoV-2 which is have enhanced its transmission ability.[11,12] Scientists and clinicians have learned much of coronavirus disease 2019, COVID-19, and its pathogenesis: not all people exposed to SARS-CoV-2 are infected and not all infected patients develop severe respiratory illness. Accordingly, SARS-CoV-2 infection can be roughly divided into three stages: stage I, an asymptomatic incubation period with or without detectable virus; stage II, non-severe symptomatic period with the presence of virus; stage III, severe respiratory symptomatic stage with high viral load.[12]

Pathogenity of SARS-CoV-2, uses the SARS-CoV receptor, the angiotensin-converting enzyme 2 (ACE-2) for entry and the serine protease TMPRSS2 for S protein priming. Binding of the ACE-2 receptors in the type II pneumocytes in the lungs triggers a cascade of inflammation in the lower respiratory track. It has been demonstrated that when the SARS spike protein binds to the ACE-2 receptor, the complex is proteolytically processed by type 2 transmembrane protease TMPRSS2 leading to cleavage of ACE-2 and activation of the spike protein, similar to the mechanism employed by influenza and human metapneumovirus, thus facilitating viral entry into the target cell. It has been suggested that cells in which ACE-2 and TMPRSS2 are simultaneously present are most susceptible to entry by SARS-CoV. Early indications are that SARS-CoV-2 virus also requires ACE-2 and TMPRSS2 to enter cells.[13,14] Patients infected with COVID-19 showed higher leukocyte numbers, abnormal respiratory findings, and increased levels of plasma pro-inflammatory cytokines.[15]

Moreover, in this article we report antiviral drugs that have been developed from synthetic drugs and medicinal compounds from plants. Several studies have been re-analyzed using in vitro, in vivo, and modeling using bioinformatics tools.

2. REVIEW METHOD

In this article, we reviewed 63 articles related to SARS-CoV-2, ranging from the structure of viruses, schematic to the proteins that make up viruses, the mechanism of interaction with receptors, the effect of viruses on the immune system, and several drug compounds that have the potential to be used as an anti-viral candidate. We accessed these articles from various primary sources, mainly from the journal website, such PMC (PubMed Central) system, the National Library of Medicine (NIH), and several other journal sites, especially at this time all of the journals related to COVID-19 can be accessed free of charge. Most of the journals that we use as references in this review, published in 2019 and 2020, are the latest and most updated journals related to COVID-19. Besides, these journals have high impact factors such as Nature (Nature communication, Nature review, Nature medicine), The Lancet, Cells, International Journal of Biological Sciences. Journal of Microbiology, Immunology, and Infection, Nat. Rev. Microbiol, and other international journals indexed by Scopus, DOAJ, Springer, and Elsevier. Besides, we also review the database of the National Center for Biotechnology Information (NCBI) regarding schematic constituent proteins and RNA sequences to compare with other virus families. We highlight some of the findings from these journals both in vivo and in vitro studies.[16] In vivo studies are carried out using either human cells or mice, while in vitro is carried out using bioinformatics tools such as molecular docking, CLC sequences viewers, phylogenetic analysis, computational, Q-UEL systems, and several other tools.[17]

3. RESULTS AND DISCUSSION

Virion genetic structure

Coronavirus virions are spherical with diameters of approximately 125 nm as depicted in recent studies by cryo-electron tomography and cyro-electron microscopy.[17] Coronavirus has helically symmetrical nucleocapsids, which is uncommon among positive-sense RNA viruses, but far more common for negative-sense RNA viruses. Coronavirus has gene domain, which is domain possessing four protein, such as spike (S), membrane (M), envelope (E), dan nucleocapsid (N) protein, all of which are encoded within 3' end of the viral genome. Protein S (~150 kDa), utilizes an N-terminal domain signal to gain access to the ER and is heavily N-linked glycosylated. Homotrimers of the virus encoded S protein make up the distinctive spike structure on the surface of the virus.[18]

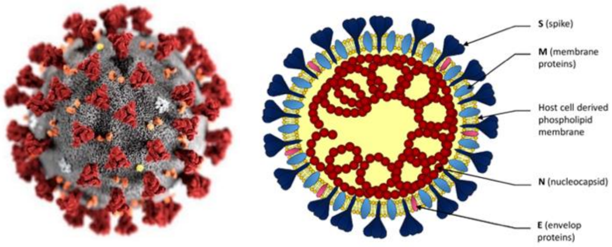

Figure 2. Structure of SARS-CoV-2 (a) Illustration of the SARS-CoV-2 virion Structure, (b) genomic organization of SARS-CoV-2, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins.[4]

M protein is the most abundant structural protein in the virion, has small size (~25-30 kDa) protein with 3 transmembrane domain dan is thought give the virion its shape. This protein has a small N-terminal domain, can experience glycosylated ectodomains and a relatively long C-terminal domain endo-domain that extends 6-8 nm into the viral particle.[18] The main difference in the 2019 coronavirus novel (SARS-CoV-2) compared to the coronavirus incorporated in the beta-coronavirus group is the size and sequence of the S (spike) protein. ORF1a, 1b, proteins E, M, and N-terminal domains do not differ much, but in the genetic sequence the S proteins are relatively different.[19] Like SARS-CoV and MERS-CoV, the SARS-CoV-2 also requires angiotensin-converting enzyme 2 (ACE2) as a receptor to enter the cell.[20]

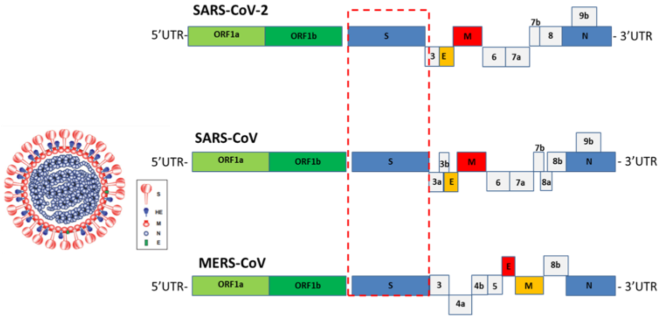

Figure 3. The Scematic of Coronavirus genome organization, including origin replicase complex (ORF), spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins[15](NCBI database)

The original replicase complex (*orf*) *ab* is the most significant gene in the coronavirus family, which encodes the pp 1ab protein and 15 non-structural protein (*nsp*). The *orf1a* gene encodes for pp1a protein which also contains 10 nsp. All of these nsps are known as viral RNA-dependent RNA-polymerase, via N-terminal extension domain (NTE), together with possessing high polymerase activity.[21]

SARS-CoV-2 interaction with receptor binding domain

Coronavirus has Spike protein that significantly determinant of virus entry into host cells.[22] The virus's envelope spike which is glycoprotein will bind to its receptor, ACE2 for SARS-CoV and SARS-CoV-2, CD209L (a C-type lectin, also known as L-SIGN) for SARS-CoV, DPP4 for MERS-CoV.[13,22] When SARS-CoV enter into host cell, it might direct membrane fusion between virus and plasma membrane, then associate with receptor binding, ACE2. The S protein of SARS-CoV-2 consisting of 1255 amino acids (aa) which devided into two subunit, subunit S1 (aa 17-680) and subunit 2 (aa 681-1195). Subunit 1 has function as surface subunit, while subunit 2 as transmembran unit.[23,24]

Receptor ACE2 expression and distribution in human body may indicate the potential infection ways of Coronavirus through the developed single cell RNA sequencing (scRNA-seq) technique dan single-cell transcriptomes based on the public database, the data from researchers show that high ACE2 expression was found in type II alveolar cells of lung, esophagus upper, and strafied epithelial cells, absorptive enterocytes from ileum and colon, cholangiocytes, myocardial cells, kidney proximal tubule cells, and bladder urothelial cells.[24] The epidemic of novel coronavirus 2019 is their ability to associate with respiratory disease and few extrapulmonary sign.[25] That data was confirm, that those organs with high ACE-expressing cells should be considered as potential high risk for COVID-19 infection.[26]

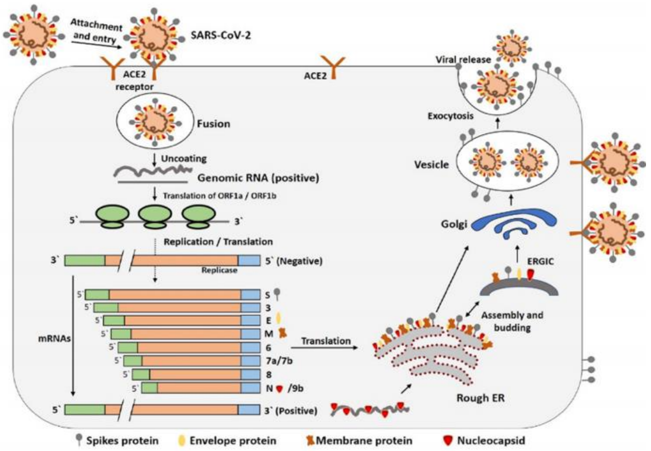

Figure 4. The SARS-CoV life cycle in host cells and its spike (S) protein structure

Figure 4. The SARS-CoV life cycle in host cells and its spike (S) protein structure. The life cylcle will begins when its S protein bind to the receptor ACE2, then changing of S protein conformation facilitates viral envelope fusion with the cell membrane through the endosomal pathway. After that, SARS-CoV releases RNA into host cell. Genome RNA is translated into viral replicase polyproteins pp1a and pp1a, then cleaved into small product by viral proteinases. At the same time, polymerase translated into relevant viral proteins. Both viral protein and genome RNA are subsequently assembled into virion in the ER and Golgi, which are budding into the lumen of the ERGIC and then transported via vesicles and released out of the cell. (ACE2: Angiotensin-converting enzyme; ER, endoplasmic reticulum; ERGIC, ER-Golgi intermediate compartment.[27]

There, by N domain viral genome bud into membranes of the ERGIC containing viral structural proteins, forming mature virions. Albeit, M protein has protein-protein interaction function for assembly of coronavirus, M and E protein function together to produce coronavirus envelopes.[27] ACE2 is receptor used by SARS-CoV-2 that has a higher affinity (~10 fold) due to different conformational of Spike protein than other coronaviruses as like SARS-CoV and MERS-CoV. Specifically, the SARS-CoV-2 mechanism can infect humans through S protein, how strong the interaction is for risk human transmission, and how SARS-CoV-2 causes pathological mechanisms of organ damage that remains unknown yet.[28]

Clinical characteristics of COVID-19

SARS-CoV-2 infection in a person's lungs results in large numbers of lesions. However, it causes viraemia in the body and causes major clinical manifestations such as fever, pharyngalgia, fatigue, diarrhea and other non-specific symptoms. This process includes the incubation phase and the initial phase of the disease. This incubation takes 1–14 days (3-7 regular days). Leukocytes and peripheral blood lymphocytes are not significantly reduced (normal or slightly lower) in this phase. Then, the virus spreads through the bloodstream and especially in the lungs, digestive tract, and heart, possibly concentrated in tissues expressing ACE2 receptors, forming and reducing microthrombus, thereby reducing the risk of damage to major organs.[27,29]

According to medical laboratories in some patients find results, some involve the number of leukocytes, lymphocytes, and eosinophils; percentage of lymphocytes and eosinophils; D-dimer concentration, C-reactive protein (CRP), procalcitonin (PCT), serum amyloid A (SAA), and serum creatine kinase. In several studies, the majority of patients had normal or decreased white blood cell counts, and lymphocytopenia. But in severe patients, the number of neutrophils, D-dimers, blood urea and creatinine levels is significantly higher, and the number of lymphocytes continues to decrease. In addition, inflammatory factors (interleukin (IL) -6, IL-10, tumor necrosis factor-α (TNF-α) increase, indicating the patient's immune status. A number of research data indicate that in plasma patients with severity have increased IL-2 levels , IL-7, IL-10, granulocyte colony-stimulating factor (GCSF), 10 kD interferon gamma-induced protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), inflammatory protein macrophage 1-α (MIP- 1α), and TNF-α.[30]

Clinical diagnosis of COVID-19 is mainly based on epidemiological history, clinical manifestations and some auxiliary examinations, such as nucleic acid detection (RT-PCR), CT scan, immune identification technology (Point-of-care Testing (POCT) of IgM/IgG, enzyme-linked immunosorbent assay (ELISA)) and blood culture. However, the clinical symptoms and signs of patients infected with SARS-CoV-2 are highly atypical, including respiratory symptoms, cough, fever, dyspnea, and viral pneumonia. Therefore, auxiliary examinations are necessary for the diagnosis of COVID-19, just as the epidemiological history.[31,32]

Mechanism innate immunity of COVID-19

The site of initial infection with SARS-CoV-2 is unknown, and the pathogenesis of COVID-19 is still under investigation. Clinically, the immune response caused by SARS-CoV-2 infection occurs in two phases. During the incubation and non-several stages, specific adaptive immune responses are needed to eliminate the virus and to prevent disease progression to a severe stage. The innate immune response that plays a role in SARS-CoV-2 in the lungs involving pro-inflammatory cells such as macrophages and granulocytes because of a binding between the virus with the host cell receptor ACE2 (lung epithelium, macrophages, granulocytes and lymphocytes).[28,33]

In innate immune response occurs since SARSCoV-2 viral particles and viral genome have been detected in monocytes and lymphocytes. SARS-CoV-2 uses the entry receptor by binding ACE2 receptor. The early onset of rapid viral replication may cause massive epithelial and endothelial cell apoptosis and vascular leakage, triggering the release of exuberant pro-inflammatory cytokines and chemokines.[33,34,35] In addition, SARS-CoV-2 infection may also cause pyroptosis in macrophages and lymphocytes.[32,33] In SARS-CoV infection, viroporin 3a has also been shown to trigger the activation of NLRP3 (NOD-like receptor protein 3) inflammasome and the secretion of IL-1b in bone marrow-derived macrophages, suggesting the induction of cell pyroptosis, which can cause the release of large amounts of pro-inflammatory factors.[32]

Innate immune responses that play a role in SARS-CoV-19 infection in the lung involve pro-inflammatory cells such as macrophages and granulocytes. These pro-inflammatory cells can recognize the SARS-CoV-2 viral through pathogen associated molecular patterns (PAMPs).[36] The PAMPs of SARS-CoV-2 viral can be recognized by macrophage cells in the form of RNA at the time of invasion of cells or in the form of dsRNA during replication in cells.[37] SARS-CoV-2 virus RNA and dsRNA are identified by macrophages through their endosomal receptors TLR3 and TLR7 and their cytosolic receptors RIG-1 and MDA-5. Once recognized, there will then be a cascade signaling in the cell which then activates transcription factors in the nucleus such as NF-kB and IRF-3 which mediate the expression of IFN-gamma and other pro-inflammatory cytokines as a form of initial defense due to viral entry.[38,39] IFN-gamma may be used to kill viruses. In contrast, generally the SARS-CoV virals are able to inhibit the formation of IFN-gamma as a strategy to defend itself to stay alive in cells.[30,40] They employ multiple strategies to interfere with the signaling leading to type I IFN production and/or the signaling downstream of IFNAR.[41] This dampening strategy is closely associated with the disease severity. Once type I IFN is secreted, these viruses are equipped with mechanism that inhibit IFN signaling such as decreasing STAT1 phosphorylation. The viral proteins involved in the modulation of this host type I IFN response are both structural proteins (such as M, N) and non-structural proteins (ORF).[42]

However, in COVID-19 patients with severity being able to produce other pro-inflammatory cytokines with very high amounts in periperal blood namely GM-CSF, IL-1ß, IL-2, IL-6, IL-7, IL-10, IP-10, MCP-1 and IL-4, which can potentially cause cytokine storms.[40,43] The researchers found that in the lungs of Covid-19 patients with severity developed a cytokine storm which made the patient's condition very weak, severe and even death. In several studies, similar getting significant results in COVID-19 patients with severity tended to increase the most visible pro-inflammatory cytokines namely IL-6, IL1, GM-CSF, TNF-α while still the cytokines were known and developed as treatment targets.[26,39]

The complement system is an important part of the host's immune response to both bacterial and viral infections. The complement system is one way the innate immune system detects and responds to foreign antigens. Because of its potential to damage the host tissue, the complement system is also tightly regulated through several inhibiting proteins that are constitutionally present in the serum. Host factors that encourage complement response related to protective or pathogens in viral infections are not well understood. Of particular concern, C3a, C4a, and C5a anaphylatoxins are produced during activation of the complement signaling cascade; they have strong pro-inflammatory properties and can trigger inflammatory cell recruitment and neutrophil activation.[41] There is still no evidence of complement in SARS-CoV-2 infection and can be used as reference material in suppressing the occurrence of excessive inflammation in severe COVID-19 patients.[13,29,30,39]

Potential drug to fight SARS-COV-2

The SARS-CoV-2 genome showing drug potential to treat their patient, even by the mechanism, analyze how the SARS-CoV-2 pattern genome, viral mechanism of infecting its host, the researchers have developed the potential of a drug to fight SARS-CoV-2. Especially, tracing from viruses that have high similarly, SARS-CoV and MERS-CoV. The drug compounds developed by the researchers came from various sources, both natural and synthetic. The researcher also has described the effect of RNA interference on replication of the SARS virus, which by interferons could block colds caused by more weakened members of the coronavirus family. Interferon and α-ketoamide can form a complex with SARS-CoV-2 that might effective for antiviral candidates by blocking viral replication. Meanwhile, ribavirin, the nucleoside analog was used as antiviral to be shown to have SARS inhibitory effect in vitro.[30] Interferon and cyclosporine able to reduce the virus replication by increase the immune-suppressive effect of CsA, immunosuppressant agents, while IFN-α is immuno-stimulating protein favoring cell conversion into antiviral agent.[44]

The screening of the drug candidate mostly carried out by in silico to found several antiviral agents that can be used as drugs to inhibit the development of SARS-CoV-19.[45] Some of them are namely wellferon, alferon, and betaferon (through interferon mechanism), then ribavirin (a nucleoside analogous). In another study, candidate drugs from natural ingredients found by Wang et al (2016) were Scutellarin can reduce the expression and activity of ACE in brain tissue. Hesperidin is a bioflavonoid that can inhibit cleavage activity of the 3-like protease (3CL-Pro) of SARS-CoV based on assays[46] for some countries have tried to use chloroquine and hydroxychloroquine as a weapon to fight Coronavirus, this is based on previous research shown that the possibility, partly identical involving alkalinization by chloroquine of the phagolysosome, seems effective against coronavirus among which is the severe acute respiratory syndrome (SARS)-associate coronavirus. Colson finding shows that this drug is also useful as an antimalarial, and anti-inflammatory due to infection.[47] There are also some convention treatment of patients with SARS-CoV-2 infection using several type of treatment which is Lopinavir/Ritonavir has anti-HIV inflammatory activity able to inhibit viral RNA replication of SARS, even MERS[31,48] like favipiravir (T-750)[49] by inhibition of virus replication, remdesivir also known has good anti-viral activity as an alternative of treatment SARS-CoV-2[42,44,50] *in vitro* effective for controlling infections due to the SARS-CoV-2 (Xiao) virus, although further clinical testing is needed.[51] The mechanism by remdisivir is that it inhibits the synthesis of nucleic acid, but has not yet obtained permission for marketing in any country,[44] like Oseltamivir[44] and Chloroquine.[46,50]

Some of the natural product has been reported might potential as a drug candidate, as a compound Glycyrrhizin, was an extract from liquorice effects *in vitro* assays on clinical isolates of coronavirus from patients with SARS.[44] Quercetin and TSL-1 from *Toona sinensis* Roem by Inhibit the cellular entry of SARS-CoV[48] Emodin derived from genus *Rheum* and *Polygonum* it can Inhibit interaction of SARS-CoV spike protein and ACE2,[44,51] Baicalin from *Scutellaria baicalensis,* Inhibit Angiotensin-converting enzyme (ACE), also have a role vital function broad therapeutic effect, including anti-apoptosis, anti-inflammation, anti-oxidative stress,[37,48] Tetrandrine, fangchinoline, and cepharanthine with mode of action Inhibit the expression of HCoV- OC43 spike and nucleocapsid protein Immunomodulation,[52,53] nitazoxamide has been demonstrated by in vitro assay for anti-MERS-CoV activity, that could be reached with two daily oral does,[53] Alisporivir uses as a drug additive anti-MERS-CoV activity when used in combination with ribavirin.[51] Moreover, silvestrol is a molecule of the flavaglines family from plants, know can bind to EIF4a and enhances the affinity of EIF4A for mRNA inhibit protein translation by blocking helicase activity,[55] mycophenolate mofetil (MMF) sems synergistic effect with IFN-β1b/MMF was administered to MERS-CoV patients, even had lower APACHE II score,[38] corticosteroids by delayed virus clearance (this may be an extra treatment for reducing viral distribution).[45] Robson were conducted an analysis using bioinformatics tools use of steroids derivative compounds, emodin can be an inhibitor of SARS-CoV entry, with some of the binding features, such as ketone group.[56] Recent studies have found that mercaptopurine can also act as an inhibitor of both SARS-CoV and MERS-CoV via targeting papain-like proteases.[57] Melatonin also reported having the potential for antiviral infection through anti-inflammatory activity and antioxidant effects.[58] Melatonin indirectly plays a role in regulating ACE2 expression, which is a key entry receptor involved in viral infections of HCoVs.[59] Specifically, melatonin inhibits calmodulin, which interacts with ACE2 by inhibiting the shedding of its ectodomain, a key infectious process of SARS-CoV.[54,60,61] Furthermore, we need to learn much more, carried out some experiment to verify all of the drug candidates can work effectively as soon as possible to stop COVID pandemic.

4. CONCLUSION

Novel-Coronavirus 2019 (n-CoV-19) possessed high similarity to coronavirus family, especially with SARS and MERS, but somehow has a high affinity than SARS-CoV and MERS-CoV due to changing of some S protein nucleotide are supposed to have a significant effect on its pneumonia. SARS-CoV-2 requires angiotensin-converting enzyme 2 (ACE2) as a receptor to enter the cell, which is high ACE2 expression in respiratory cells that potentially high risk for COVID-19 infection. When SARS-CoV-2 enter to host cell, the innate immune response that plays a role in SARS-CoV-2 in the lungs involving pro-inflammatory cells such as macrophages and granulocytes because of a binding between the virus with the host cell receptor ACE2. However in COVID-19 patients with severity being able to produce other pro-inflammatory cytokines with very high amounts in such as GM-CSF, IL-1ß, IL-2, IL-6, IL-7, IL-10, IP-10, MCP-1 and IL-4, which can potentially cause cytokine storms. Several anti-viral drugs candidates have been developed from synthetic drugs and natural products, drug compounds from plants. Albeit, there are have to verify by several scientific studies, to re-analyzed using in vitro and in vivo studies, even by modelling using bioinformatics tools.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ACKNOWLEDGEMENT

Thank you very much for a people who are helping me to write this paper, especially to my university.

FUNDING INFORMATION

This work was unfunded.

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SHORT BIOGRAPHY

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 13 – 20
ISSN 2580-0191 (Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.221
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Original Research

Immunobioinformatics analysis and phylogenetic tree construction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Indonesia: spike glycoprotein gene

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HIGHLIGHTS

- The basis of designing an epitope-based vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

ARTICLE INFO

Article history:

Received Date: April 28th, 2020
Revised Date: May 27th, 2020
Accepted Date: June 07th, 2020

Keywords:

COVID-19
Epitope-based vaccine
Immunobioinformatics
Phylogenetic tree
SARS-CoV-2

ABSTRACT

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), has spread worldwide and as a result, the World Health Organization (WHO) declared it a pandemic. At present, there are no approved vaccines against SARS-CoV-2. Therefore, the aim of this study was to predict epitope-based vaccines using bioinformatics approaches and phylogenetic tree construction of SARS-CoV-2 against the backdrop of the COVID-19 pandemic. In this study, we employed 27 isolates of SARS-CoV-2 spike glycoprotein genes retrieved from GenBank® (National Center for Biotechnology Information, USA) and the GISAID EpiCoV™ Database (Germany). We analyzed the candidate epitopes using the Immune Epitope Database and Analysis Resource. Furthermore, we performed protective antigen prediction with VaxiJen 2.0. Data for B-cell epitope prediction, protective antigen prediction, and the underlying phylogenetic tree of SARS-CoV-2 were obtained in this research. Therefore, these data could be used to design an epitope-based vaccine against SARS-CoV-2. However, advanced study is recommended for confirmation (*in vitro* and *in vivo*).

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1. INTRODUCTION

On 30 December 2019, the Chinese government reported an outbreak of pneumonia disease in Wuhan.[1] Furthermore, the causative agent identified by the International Committee on Taxonomy of Viruses (ICTV) was named SARS-CoV-2.[2] This new virus has rapidly spread across China and other countries.[3,4,5] In addition, the WHO announced a new name for the disease caused by SARS-CoV-2 coronavirus disease 2019 (COVID-19).[6] At present, there are three coronaviruses that cause disease in humans: severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2.[7]

Coronaviruses belong to the *Coronaviridae* family of the order *Nidovirales*. The coronavirus family consists of alpha, beta, gamma, and delta coronaviruses. The term “corona” reflects the crown-like spikes on the surface of the virus. They are roughly 65–125 nm in diameter with a single-stranded RNA of approximately 26–32 kbs in length. The structural proteins are encoded by four structural genes, including the envelope (E), membrane (M), nucleocapsid (N), and spike (S) genes; orf1ab is the largest gene in SARS-CoV-2.[8] Interestingly, the spike glycoprotein plays an important role in binding to the receptors of the host cell and is the major target for neutralizing antibodies.[7,9]

Vaccines have been demonstrated to decrease the morbidity and mortality levels of many infectious diseases.[10] Therefore, the development of an effective vaccine against SARS-CoV-2 infection is urgently required. Progress in molecular biology and biotechnology is driving the construction of novel concepts in vaccinology. Synthetic recombinant proteins containing epitopes could be produced efficiently with modern biotechnology methods.[11] Hence, an epitope-based vaccine is suggested as a novel possibility for effective vaccines against SARS-CoV-2. In this study, we applied bioinformatics analysis to obtain data for B-cell epitope prediction, protective antigen prediction, and molecular phylogenetic tree construction of SARS-CoV-2 isolates.

2. MATERIALS AND METHOD

2.1 SARS-CoV-2 isolates

The isolates of SARS-CoV-2 were retrieved from GenBank® (National Center of Biotechnology Information, USA) and the GISAID EpiCoV™ Database (Table 1).

Table 1. SARS-CoV-2 isolates retrieved from GenBank®.

Accession ID	Origin	Host	Isolation Source	Database
MT240479.1	Pakistan: Gilgit	*Homo sapiens*	Throat swab	GenBank®
MT188341.1	USA: Minnesota (MN)	*Homo sapiens*	Nasopharyngeal or oropharyngeal swab	GenBank®
MN908947.3	China: Wuhan	*Homo sapiens*	Unknown	GenBank®
LC529905.1	Japan	*Homo sapiens*	Unknown	GenBank®
MT039890.1	South Korea	*Homo sapiens*	Unknown	GenBank®
MT066156.1	Italy	*Homo sapiens*	Sputum	GenBank®
MT072688.1	Nepal	*Homo sapiens*	Oropharyngeal swab	GenBank®
MN985325.1	USA: Washington (WA)	*Homo sapiens*	Oropharyngeal swab	GenBank®
MN988713.1	USA: Illinois (IL)	*Homo sapiens*	Sputum	GenBank®
MT253701.1	China: Zhejiang, Hangzhou	*Homo sapiens*	Sputum	GenBank®
MT007544.1	Australia: Victoria	*Homo sapiens*	Unknown	GenBank®
MT192759.1	Taiwan	*Homo sapiens*	Sputum	GenBank®
MT039888.1	USA: Massachusetts (MA)	*Homo sapiens*	Oropharyngeal swab	GenBank®
MT106054.1	USA: Texas (TX)	*Homo sapiens*	Sputum	GenBank®

Accession ID	Origin	Host	Isolation Source	Database
MT233519.1	Spain: Valencia	*Homo sapiens*	Nasopharyngeal exudate	GenBank®
MT126808.1	Brazil	*Homo sapiens*	Nasopharyngeal swab	GenBank®
MN938384.1	China: Shenzhen	*Homo sapiens*	Nasopharyngeal swab	GenBank®
MT012098.1	India: Kerala State	*Homo sapiens*	Throat swab	GenBank®
MT093571.1	Sweden	*Homo sapiens*	Unknown	GenBank®
MT135041.1	China: Beijing	*Homo sapiens*	Unknown	GenBank®
MT121215.1	China: Shanghai	*Homo sapiens*	Throat swab	GenBank®
EPI_ISL_435281	Indonesia: Jakarta	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	GISAID EpiCoV™
EPI_ISL_435282	Indonesia: Jakarta	*Homo sapiens*	Nasopharyngeal and Oro-pharyngeal swab	GISAID EpiCoV™
EPI_ISL_435283	Indonesia: Jakarta	*Homo sapiens*	Nasopharyngeal swab	GISAID EpiCoV™

2.2 Nucleotide sequence preparation

SARS-CoV-2 nucleotide sequences (spike glycoprotein gene) from all isolates were retrieved from GenBank® and the GISAID EpiCoV™ Database. Multiple sequence alignment of nucleotide sequences was performed using Molecular Evolutionary Genetics Analysis X (MEGA X).[10]

2.3 Prediction of B-cell epitopes and protective antigens

A B-cell epitope, which is known as a B-cell antigenic determinant, is a specific antigen region with high affinity for B-cell lymphocytes. This interaction induces B-cells to produce an antigen-specific antibody and memory cells.[12] To predict the B-cell epitope, analysis was conducted using the IEDB online webserver with default thresholds (0.350) and VaxiJen v2.0.[10]

2.4 Molecular phylogenetic analysis

Phylogenetic modeling and tree visualization were achieved by applying MEGA X with the Maximum Likelihood method.[10] The phylogenetic tree was validated by running the analysis on 1000 bootstrapped input datasets[13] and cross-referencing it against the Tamura-Nei substitution model.[14]

3. RESULTS AND DISCUSSION

A novel coronavirus, SARS-CoV-2, spread across the world very rapidly. Human-to-human transmission has been confirmed, and the number of global cases has been increasing at a fast pace.[9] To date, there are more than 2 million people infected with SARS-CoV-2 worldwide based on the online interactive dashboard hosted by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. This interactive web-based dashboard visualizes and tracks reported cases of COVID-19 in real time.[15]

In addition, the spike glycoprotein has recently been regarded as a highly expectant antigen formulation for the construction of a SARS-CoV-2 vaccine. There are two reasons for this: (1) it is involved in surface exposure, directly recognized by the host immune system, and (2) it mediates the interaction with the host cells by binding to the receptor, ACE2.[16] Muthumani *et al*. reported that a DNA vaccine encoding MERS-CoV spike glycoprotein was immunogenic in rhesus macaques, camels, and mice.[17] Moreover, Pallesen *et al*. demonstrated a higher titer of neutralizing antibodies in mice immunized by the recombinant prefusion, MERS-CoV spike glycoprotein.[18] In addition, Zhang *et al*. described various types of SARS-CoV-2 vaccines based on a number of different developmental processes, such as whole-cell killed and live-attenuated vaccines, subunit vaccines, mRNA vaccines, DNA vaccines, live vector vaccines, and synthetic peptides.[16]

Epitopes from all samples of SARS-CoV-2 isolates were predicted using the IEDB online webserver to determine the potential for B-cell recognition with an accuracy of approximately

75%. This prediction was based on the combination of Hidden Markov Model (HMM) statistical methods and trend scale.[10] Furthermore, the peptides were predicted using VaxiJen v2.0 to determine the characteristics of immunogenicity or protective antigens. Therefore, they can be distinguished as either non-antigens or antigens. The prediction has an accuracy of approximately 70-89%. The performance of this server was developed based on the physicochemical properties of the target protein without alignment.[19]

Table 2. Prediction of B-cell epitopes and protective antigens of SARS-CoV-2

Predicted Peptides	Position	Length	Protective Antigens
RTQLPPAYTNS	21-31	11	0.8710 (Probable ANTIGEN)
SGTNGTKRFDN	71-81	11	0.5906 (Probable ANTIGEN)
LTPGDSSSGWTAG	249-261	13	0.4950 (Probable ANTIGEN)
VRQIAPGQTGKIAD	407-420	14	1.2606 (Probable ANTIGEN)
YQAGSTPCNGV	473-483	11	0.0881 (Probable NON-ANTIGEN)
YGFQPTNGVGYQ	495-506	12	0.7136 (Probable ANTIGEN)
RDIADTTDAVRDPQ	567-580	14	0.4400 (Probable ANTIGEN)
QTQTNSPRRARSV	675-687	13	0.1763 (Probable NON-ANTIGEN)
ILPDPSKPSKRS	805-816	12	0.5322 (Probable ANTIGEN)
VYDPLQPELDSF	1137-1148	12	0.0903 (Probable NON-ANTIGEN)
KNHTSPDVDLG	1157-1167	11	1.4039 (Probable ANTIGEN)
FDEDDSEPVL	1256-1265	10	0.3154 (Probable NON-ANTIGEN)

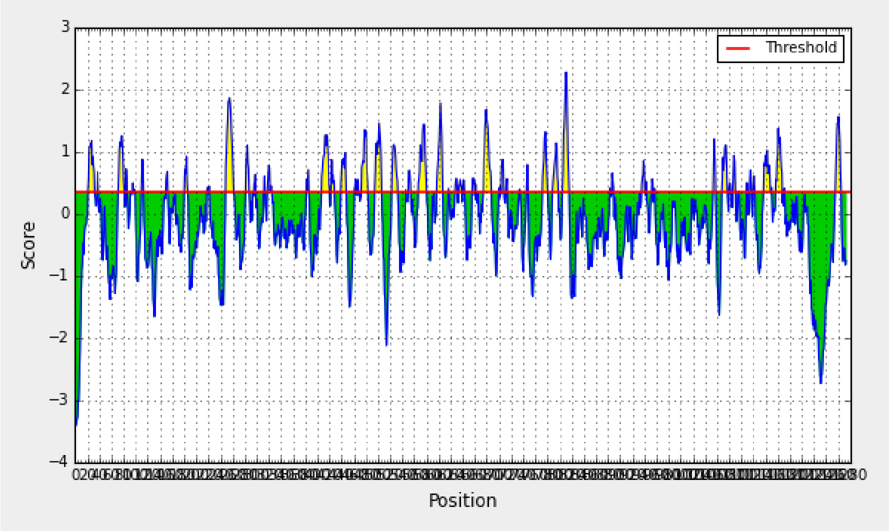

Figure 1. Prediction of B-cell epitopes from the amino acids of spike glycoprotein of SARS-CoV-2. B-cell epitope prediction was performed using the IEDB online webserver. The yellow region was positive, whereas the green region was a negative prediction of B-cell epitopes.

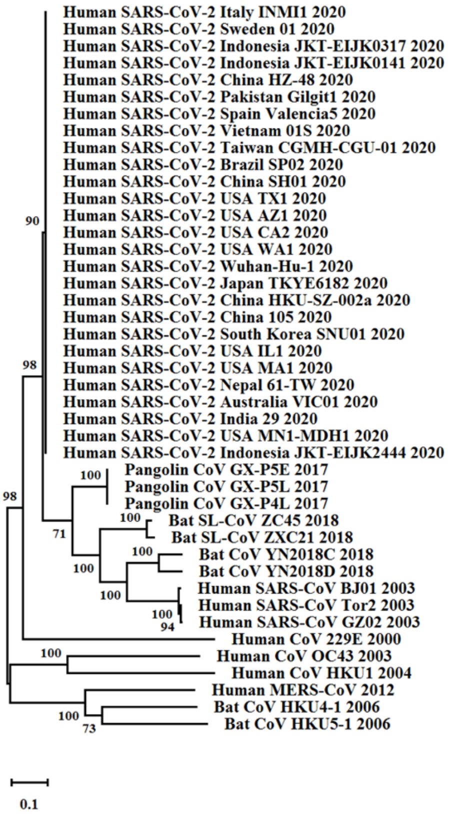

Figure 2. Phylogenetic tree of the first three SARS-CoV-2 in Indonesia and other coronaviruses.

In this study, we obtained 12 predicted peptides and 8 other peptides considered as protective antigens (Table 2 and Figure 1). B-cell epitope prediction is a methodology to predict protein regions that could be recognized as an epitope response to B-cells. An epitope is part of an antigen molecule that binds to the antibodies. This area is essential for designing certain types of vaccines or specific antibodies. The design of a seed vaccine requires an epitope that can determine the active side of the antigen that binds to the antibodies.[12]

Recently, there have been no epitope-based vaccines seen on the market.[20] However, over the past decade, research and development to reveal epitope-based vaccines has garnered much interest within the vaccine industry. Progress has been made utilizing various methods, such as cell culture techniques, recombinant DNA technology, and immunoinformatics.[11] Epitope-based vaccines play an essential part in the present volume of research with several advantages over conventional vaccines, including robust safety and stability, high specificity, and better manufacturing and retention. As a result, epitope-based vaccines have become an increasingly popular field of vaccinology. Several studies have identified the potency of epitope-based antigens that efficiently generate high immunity and protection against various pathogens.[10,12]

Molecular phylogenetic analysis is used to address both applied and fundamental issues of virus research, including phylogeography, diagnostics, evolution, origin, epidemiology, taxonomy, and forensics. It may provide an evolutionary view of the variety of any character that can be measured for a cluster of viruses.[21] The molecular phylogenetic analysis in this study exhibited the genetic relationship between SARS-CoV-2 isolates from various countries and other coronaviruses originating from humans, bats, and pangolins (Figure 2). Interestingly, we identified the newly submitted three Indonesian SARS-CoV-2 isolates (provided by Eijkman Institute for Molecular Biology, Ministry of Research and Technology/National Agency for Research and Innovation of the Republic of Indonesia) from the GISAID EpiCoV™ Database. We revealed that there were not many differences in the spike glycoprotein gene between viruses isolated from various countries and those from Indonesia. On the other hand, Andersen *et al*. stated that SARS-CoV-2 is clearly not a laboratory construct or manipulated virus.[22]

In addition, Lam *et al*. demonstrated that coronaviruses are present in many wild mammals in Asia.[1] Currently, it is crucial to investigate the likelihood of intermediate hosts of SARS-CoV-2 to contain COVID-19 spread. Pangolin CoV is 91.02% identical to SARS-CoV-2 at the whole-genome level.[23] Previously, Zhou *et al*. stated that SARS-CoV-2 shares a 96% whole genome with a bat CoV from *Rhinolophus affinis* (BatCoV RaTG13) collected from Yunnan Province, China.[24] Based on this study, we recommend that further surveillance studies be carried out on bats and pangolins in the natural environment, especially in China and Southeast Asia, in order to understand the risk of zoonotic transmission in the near future.

Overall, vaccination is an effective method for controlling diseases and regulating human and animal health. Specifically, vaccines are agents that enhance the adaptive immune response and can reduce the effects of infections and diseases. This is accomplished through the immune system recognizing the vaccine as a foreign object, then destroying it, and placing it in memory. Moreover, the novel concept of reverse vaccinology has revolutionized the study of vaccine development. The ability to obtain a whole-genome sequence from a virulent organism has led to the *in silico* analysis of the most protective antigens before conducting confirmation experiments (*in vitro* and *in vivo*). Several advantages, such as low cost, speed, along with the success of the bioinformatics approach, depend on the accuracy of predictions, which are supported by many tools.

4. CONCLUSION

This study supplied data for B-cell epitope prediction, protective antigen prediction, and the phylogenetic tree of SARS-CoV-2. In summary, this study could serve as the basis to design an epitope-based vaccine against SARS-CoV-2. However, an advanced study is suggested for confirmation (*in vitro* and *in vivo*).

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ACKNOWLEDGEMENT

We thank Editage for editing the manuscript.

FUNDING INFORMATION

PMDSU Scholarship Batch III from the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia.

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 21 – 28
ISSN 2580-0191 (Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.219
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Review Article

Herd immunity and COVID-19 in Indonesia

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HIGHLIGHTS

- Herd immunity in the case of Indonesia is still controversial to be applied in Indonesia because until now no vaccine has been found as a substitute for the formation of partial immunity due to the formation of natural antibodies in patients.

ARTICLE INFO

Article history:

Received Date: April 26, 2020
Revised Date: June 8, 2020
Accepted Date: June 14, 2020

Keywords:

COVID-19
Herd Immunity
SARS-CoV-2
Indonesia

ABSTRACT

Herd immunity or herd effect is a phenomenon that occurs in groups of people who are resistant to disease. The purpose of conducting this research is to predict the number of cumulative cases of COVID-19 in Indonesia. Covid-19 cases in Indonesia on April 6, 2020, were 2235 cases spread in 34 Provinces. As many as 2491 cases in Indonesia, there were 192 patients recovered (including those treated, so they have natural antibodies in the end) while the total who died was 209 people. It is assumed that around 13% of the total cases have natural antibodies. This is also the case with SARS-CoV-2 and may explain why some individuals (perhaps those most recently able to recover from seasonal coronavirus infections) have asymptomatic infections. Finally, the theoretical concept of increasing herd immunity in pandemic and epidemic cases in Indonesia which aims to control COVID-19 still needs to be reviewed because it is seen from the mortality data that CFR COVID-19 is predicted to be around 8.39% of the population in Indonesia where the risk of death still available. The best alternative is to do a healthy lifestyle, social distancing, and waiting for the vaccine to be found.

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1. INTRODUCTION

The main principle of the immune system against microbes is divided into two types, namely innate systems and adaptive systems. Innate immune response to microbes is rapidly and non-specific, whereas adaptive immunity is specific to microbes and has memory cells used when microbes re-infect.[1] Herd immunity or herd effect is a phenomenon that happens of the group people resistance of disease such people without a fully working immune system, including those without a working spleen, people on chemotherapy treatment whose immune system is weakened, people with HIV, newborn babies who are too young to be vaccinated, elderly people and many of those who are very ill in hospital. Herd immunity, also known as not everyone in a population needs to be immunized to eliminate the disease.[2,3] Vaccines are one of the most cost-effective measures in health care, but this benefit is eroded as the cost per dose rises. Another advantage of an effective vaccination program is the ‘herd immunity’ that it confers on the general population. By lowering the number of susceptible members of a population, vaccination decreases the natural reservoir of infected individuals in that population and so reduces the probability of transmission of infection. Thus, even unvaccinated members will be protected because their chance of encountering the pathogen is decreased.[4]

Herd immunity is an important concept of the epidemic that concerns immunity to prevent transmission of pathogens through vaccination programs. Herd immunity can be obtained in a limited way such as the pathogens are fairly species-specific, the pathogens are spread contagiously by fairly direct means and host exposure or vaccination confers fairly strong immunity.[5] Herd immunity also is known as the totality of naturally acquired and vaccine-based immunity to a given infectious agent like a virus, bacteria, fungi as the proportion of the whole population so can reduce the risk of infection for the individual. Herd immunity also obviously dynamic because will disappear over time through the reduced immune response system so the memory of infectious agents like bacteria, fungi, and the virus will be decreased or can be the death of individual and do vaccination for the mechanism of this herd immunity.[6] The indirect effect of vaccination can be increased the level of herd immunity is important in the disease elimination program.[7] Herd immunity is the indirect protection from infection conferred to susceptible individuals when a sufficiently large proportion of immune individuals exist in a population. Herd immunity threshold: the point at which the proportion of susceptible individuals in a population falls below the threshold needed for transmission.[8]

Mechanism of herd immunity from before and after vaccination is when infectious agents like viruses, bacteria, and fungi into the body, many infected cells because of lack of immunity was to fight the disease yourself. When an infectious agent into a vaccinated body, the spread of the disease becomes limited. The indirect effect protects individuals who not immunized, including those who cannot be vaccinated and those who have vaccinated but not successful, which is the principle of herd immunity. Succesful herd immunity will increase if the body is vaccinated around 40% but depends on the disease.[3,7]

The mechanism of action of herd immunity in people before and after being vaccinated through the picture below is taken from various sources[2,5] :

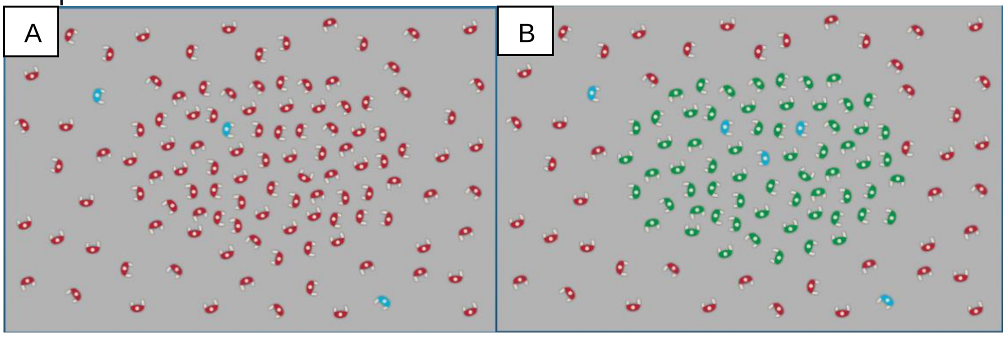
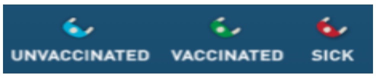


Figure 1. Mechanism of herd immunity

Picture A, infectious agents such as bacterial and fungal viruses enter the body; many infected cells due to lack of immunity will fight the disease themselves. Picture B, when an infectious agent comes a vaccinated body, the spread of the disease becomes limited. The indirect effect will protect individuals who are not immunized, including those who cannot be vaccinated and those who have vaccinated but are not successful, which is the principle of herd immunity. Successful herd immunity will increase if the body is vaccinated around 40% but also depends on the disease.

Note:



There are seven COVs known to cause humans which are divided low pathogenic and highly pathogenic COVs. Four corona-viruses (HCOVs, namely HCOVs 229E, NL63, OC43, and HKU1) are known as non-severe acute respiratory syndrome (SARS) like COVs. The disease caused by SARS-CoV-2 is named Corona-virus Diseases-2019 (COVID-19). COVID-19 arises through transmission from humans to a human pathogen which causes a broad spectrum of clinical patients with COVID-19. Clinical symptoms that appear in the form of no symptoms at all (being asymptomatic) to having fever, cough, sore throat, general weakness, fatigue, and muscular pain, for case severe such as severe pneumonia, acute respiratory distress syndrome, sepsis, and septic shock, additional some countries such as South Korea, China, and Italy that patients with confirmed SARS-COV-2 infection have developed anosmia/hyposmia/loss of smell.[11,12]

In general, innate and adaptive immune systems play a direct role in eliminating viruses. Besides, an immune response will fight HLA class-I and class-II-restricted viral epitopes mediated by CD8+ and CD4+ T lymphocytes. Clinically T lymphocyte responsible has an opportunity to be antiviral in the body. When analogous with another COVs. SARS-COV-2 may induce lymphocyte-mediated by an immune response, and this is evidenced by the presence of patients treated at the hospital often showing signs of lymphopenia so cellular immune can be suppressed by the presence of SARS-COV-2 infection will quickly clear SARS-COV-2 without a mild clinical sign of infection or virus causing excessive immunosuppression and the defence of the human body.[10,12]

Innate herd immunity is a type of herd immunity that genetically determined physiological changes with respect to antibody production or other defence mechanisms in a herd. It does not depend on the previous exposure of herd with infection, or it may arise in a herd through prolonged exposure to an infection or natural selection. Acquired herd immunity is a type of herd immunity where a sufficient number of its members have actually been exposed naturally or artificially to infectious agents during their lifespan.[13,14]

There are many opinions about the relationship of herd immunity and COVID-19 that are still being debated by scientists. Scientists said that herd immunity would appear in people who have the flu and people from abroad have gotten flu vaccines that can protect individuals who are not immunized. The problem with flu strains is still unknown from the flu about variations in strains in certain races; that's why the flu vaccine isn't always 100% effective. But the biggest problem now with coronavirus, which is a novel virus that has never spread before, which means everyone is at risk of infection. Herd immunity can only be achieved if vaccinating but until now there has been no vaccine, and it still takes a long time to make an effective vaccine for coronavirus or individuals who fall ill and then recover it will develop natural immunity against viruses such as influenza viruses. Herd immunity against Covid-19 will be achieved if it is infected with Covid-19 first, but currently, there is no vaccine, so it is quite dangerous if it has to be infected first and then recovered. Social distancing is currently ridden by the government to manage and build herd immunity so that it is more effective against Covid-19. Besides, herd immunity will make it more difficult to spread to the person to person because it has been vaccinated.[3,6,7]

Numerous clinical trials to evaluate novel vaccine candidates and drug repurposing strategies for the prevention and treatment of SARS-CoV-2 infection are currently ongoing. However, it is unknown whether these trials will produce effective interventions, and it is unclear how long these studies will take to establish efficacy and safety, although an optimistic estimate for any vaccine trial is at least 12–18 months.[15,16], Particularly in the context of attaining herd immunity to SARS-CoV-2, regard for finite healthcare resources, cannot be overstated, as this policy inherently relies on allowing a large fraction of the population to become infected. The ability to establish herd immunity against SARS-CoV-2 hinges on the assumption that infection with the virus generates sufficient, protective immunity. At present, the extent to which humans are able to generate sterilizing immunity to SARS-CoV-2 is unclear.[17] In a cohort of 175 recovered COVID-19 patients, SARS-CoV-2-specific serum neutralizing antibodies (NAbs) were detected at considerable, albeit variable, titers in most (n = 165) individuals. Even if reinfection can occur after sterilizing immunity wanes, enduring memory cells of the adaptive immune system would likely facilitate immune control of the virus and limit disease pathology, which would hopefully decrease the clinical severity of subsequent infections.[18, 19]

2. REVIEW METHOD

The purpose of conducting this research is to predict the number of cumulative cases of COVID-19 in Indonesia. The research data was taken from secondary data sourced from the Indonesian Ministry of Health's website portal and data from the government portal that monitors the COVID-19 case data. Using journal calculation method from a journal reference used by the Chinese government in reviewing COVID-19 cases. In this case, what we are analyzing is that the situation occurs COVID-19 so that it can be used as a reference for Indonesian data. And then we specify data and processing data.[20,21] Data processing procedures have several stages, including:

a. Secondary data is obtained from data information from valid national and regional sources of each province, including the number of cases, the number of healing, and the number of dead.
b. Data tabulation is secondary data that has been obtained is processed using Microsoft Excel.
c. Before conducting a strategy to measure distribution modelling, it is necessary to estimate the reproductive number (R0) and simulate the real effective data (Rt) of a population. R0 is the number of secondary cases produced by the presence of one infected person in a full population that has the potential or susceptibility to infection and mingling. Whereas Rt is a simulated version of life in the field using secondary data of cases found to estimate the number of epidemics that are taking place so that this research simulation uses the exponential growth method using data on the latest COVID-19 case numbers in Indonesia as of March 6, 2020. While the serial intervals include the mean (π = 4.7 days), the standard deviation (dv = 2.9 days) and the significant level (R = 0.05). Using Rt values, we can calculate the (critical) minimized (Pkris) level of a population of immunity obtained through medical treatment or natural induction after recovery from COVID-19. So to stop the spread of infection in the population used the formula: (Pkris) = 1-(1/Rt). After being calculated the data obtained are analyzed .[21]

3. RESULTS AND DISCUSSION

Covid-19 cases in Indonesia on April 6, 2020, were 2235 cases spread in 34 Provinces. The three provinces with the highest number of infections are DKI Jakarta (n = 1151), West Java (n = 252), East Java (n = 187). We know the provinces above are densely populated cities and large cities in Indonesia with many activities so that the spread of infection virus through aerosol.

As of March 13 2020, there were 32 countries outside China with over 100 COVID-19 cases. The seven countries with the highest number of infections were: the United States (n = 2294), France (n = 3671), Germany (n = 3675), Spain (n = 5232), Korea (n = 8086), Iran (n = 11,364) and Italy (n = 17,660).[2] Herd Immunity and SARS-CoV-2. The ongoing SARS-CoV-2 pandemic has caused over 3.5 million clinically confirmed cases of COVID-19 and has claimed more than 250,000 lives worldwide (as of May 4, 2020). Numerous clinical trials to evaluate novel vaccine candidates and drug repurposing strategies for the prevention and treatment of SARS-CoV-2 infection are currently ongoing. However, it is unknown whether these trials will produce effective interventions, and it is unclear how long these studies will take to establish efficacy and safety, although an optimistic estimate for any vaccine trial is at least 12–18 months. In the absence of a vaccine, building up SARS-CoV-2 herd immunity through natural infection is theoretically possible. However, there is no straightforward, ethical path to reach this goal, as the societal consequences of achieving it are devastating.[2,9] The number of confirmed cases in the other 25 countries were less than 1200 in 2003, the Chinese population was infected with a virus that caused Severe Acute Respiratory Syndrome (SARS) in Guangdong province. This virus that infects patients shows symptoms of pneumonia with an alveolar spreading injury which causes acute respiratory distress syndrome. Whereas in

Saudi Arabia, it was also detected as confirmed as a member of the coronavirus and named as the Middle East Respiratory Coronavirus (MERS-CoV). MERS-CoV infection starts with mild upper respiratory injury while its development leads to severe respiratory illness. Similar to SARS coronavirus, patients infected with MERS-coronavirus suffer from pneumonia, followed by symptoms of acute respiratory distress syndrome and kidney failure. Latest at the end of 2019, WHO was notified by the Chinese government of several cases of pneumonia with the known etiology COVID-19 spread from human to human spread of the virus occurred because of close contact with infected people, coughing, sneezing, respiratory droplets or aerosols. This aerosol can penetrate the human body (lungs) through inhalation through the nose or mouth.[20]

The lowest case is Papua (n = 25), around 1.1% of the total cases in Indonesia. This does not mean there are no cases because the facilities in Papua have not been fulfilled, and data access is difficult. Most COVID cases have no symptoms, especially cases of teenagers and the elderly or elderly. The risk exposure ratio is based on the sex of the patient even the male sex in the COVID-19 study with the proportion of men ranging from 51.4% to 73.2% (Lai et al., 2020). Meanwhile, according to research, the ratio of exposure to 0-9 years of age is 0.00%. Age 10-19 years as much as 0. 04%. Age 20-29 years 1.04%. Age 30-39 years as much as 3.43%. Age 40-49 years as much as 4.25%. Age 50-59 years as much as 8.16%. Ages 60-69 years as much as 11.8%. Age 70-79 years as much as 16.6% and the highest ratio at the age of ≥80 years as much as 18.4% so that from the data above the highest death ratio is based on cases of age exposed at age ≥80 years.[21] At age> 65 years, the clinical manifestations of COVID-19 pneumonia were confirmed, and 1,399 (32.6%) with very severe cases. As noted above, the overall mortality rate for COVID-19 pneumonia is 4%. Whereas age <65 years, all mortality ratio (0.3%). This data shows that the majority of patients with COVID-19 pneumonia will recover from the disease, especially younger people. Our current data shows that patients in the deceased group are susceptible to multiple organ failure, especially heart failure and respiratory failure. One of the best laboratory parameters is cardiac injury inflection to predict COVID-19 pneumonia death is cardiac troponin I, and these parameters remain valid in the sex, age, and disease underlying the analysis of suitable controls.[22]

If secondary data parameters are observed, and the implication is further cases that the difference in Ro and Rt is related to the proportion of individuals who already have natural immunity (antibodies) and who are treated who are infected with pathogens in a population. Besides, the way to count for pathogens in a particular population is by transferring Ro to the proportion of the population that does not yet have natural immunity (antibodies) in the sense that they are susceptible to this pathogen infection. Therefore, Ro is the same as Rt when no individual with natural immunity (antibody) is found in a population (all susceptible to infection). It is said that partial immunity has already appeared before the transmission agent so that it has an impact on reducing the number of secondary expected to emerge. The obstacle SARS-CoV-2 is a new coronavirus, and pandemic cases first appeared. However, a possible source of partial immunity is because the mechanism of cross-reactivity antibodies results in partial immunity from previously common seasonal coronavirus infections that have infected in the human population for decades as noted for SARS-CoV. As many as 2491 cases in Indonesia, there were 192 patients recovered (including those treated so they have natural antibodies in the end) while the total who died was 209 people. It is assumed that around 13% of the total cases have natural antibodies. This is also the case with SARS-CoV-2 and may explain why some individuals (perhaps those most recently able to recover from seasonal coronavirus infections) have asymptomatic infections. Finally, the theoretical concept of increasing herd immunity in pandemic and epidemic cases in Indonesia which aims to control COVID-19 still needs to be reviewed because it is seen from the mortality data that CFR COVID-19 is predicted to be around 8.39% of the population in Indonesia where the risk of death still available. The best alternative is to do a healthy lifestyle, social distancing, and waiting for the vaccine to be found. When there are no immune individuals in the population (i.e. when all are susceptible), this means that any partial, pre-existing immunity to the infecting agent can reduce the number of expected secondary cases arising because of some possible antibody cross-reactivity and partial immunity from previous infections with the common seasonal coronaviruses.[28] Social distancing is the practice of reducing the spread of viruses and limiting contact between individuals. When these conditions apply, methods to achieve herd immunity serve an important role in preventing disease epidemics and are an important component of programs for disease elimination or eradication. But Understanding herd immunity requires consideration of infection dynamics, modes of transmission, as well as the acquisition of immunity by individuals in the population.[23] Several countries have implemented, namely the USA, Italy, China now also applied in several pandemic countries. Besides, the benefits of Social Distancing also reduce the burden of the medical team to treat cases exposed to COVID-19 due to the lack of health facilities so that many patients will be treated. In conclusion, social distancing is a realistic solution to dealing with Covid-19 pandemic.[23]

Besides candidates for immunotherapy for COVID-19 including monoclonal antibody testing, plasma therapy, immunoglobulins for T-cell respondents, and angiotensin-converting enzyme 2 (ACE2) therapy for vaccine candidates still need to be tested at the in vivo stage. But a history of SARS is very effective using a monoclonal antibody test, so it recommends immunotherapy for 2019-nCoV on the grounds and evidence of previous research on two other coronaviruses SARS-CoV and MERS-CoV.[24.]

4. CONCLUSION

Herd immunity in the case of Indonesia is still controversial to be applied in Indonesia because until now no vaccine has been found as a substitute for the formation of partial immunity due to the formation of natural antibodies in patients. On the other hand, the risk of death in patients is very inappropriate if the concept of herd immunity is applied in Indonesia. The best solution at this time is still social distancing, eating a nutritious life, always maintaining immunity, and always adhering to a healthy lifestyle such as always washing hands and wearing a mask when an emergency when outside activities.

DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

ACKNOWLEDGEMENT

Thank you very much to information institutions that have provided data for Ministry Health of Indonesia, government and regional institutions.

FUNDING INFORMATION

This work was unfunded.

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SHORT BIOGRAPHY

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 29 – 40
ISSN 2580-0191 (Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.223
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Article Review

Human immune response to SARS-CoV-2 infection

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HIGHLIGHTS

- The SARS-CoV-2 infection causes innate and adaptive immune responses
- SARS-CoV-2 RNA mutations result in impaired immune system work

ARTICLE INFO

Article history:

Received Date: May 07, 2020
Revised Date: June 04, 2020
Accepted Date: June 18, 2020

Keywords:

COVID-19
SARS-CoV-2
Innate Immune
Adaptive Immune

ABSTRACT

COVID-19 is an acute respiratory infection caused by a new type of Coronavirus, SARS-CoV-2, which first appeared in Wuhan, China in December 2019. COVID-19 then became a pandemic in various countries in early 2020. In this article it contains review that discusses the immune response in humans due to SARS-CoV-2 infection, using the narrative literature review method, a total of 36 articles (6 from Elsevier, 24 from PMC, and six from Springer). It is known that the pathogenesis of COVID-19 and the manufacture of drugs and vaccines are still under investigation, but in infected patients, innate immune responses in the form of alveolar macrophages, dendritic cells, airway epithelial cells, congenital lymphocytes, and neutrophils work together in the fight against infection. Next comes the adaptive immune response in the form of antibodies (immunoglobulins) which help in fighting infections due to SARS-CoV-2. These immune responses include increasing levels of cytokines, coagulation parameters, C-reactive protein, neutrophils, and decreasing total lymphocytes. It is also known that COVID-19 patients with severe disease often experience higher total antibody, IgM responses, and IgG responses than COVID-19 patients without the congenital disease. IgG antibodies are present in the serum, so the serum in COVID-19 patients who have recovered can be used for therapy in COVID-19 patients who have not healed, as long as the drug and vaccine are under investigation.

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1. INTRODUCTION

The disease called coronavirus disease 2019 (COVID-19) has become an epidemic in Wuhan City, Hubei Province, China, in December 2019. COVID-19 is caused by severe acute respiratory Coronavirus two syndromes (SARS-CoV-2), previously known as 2019-nCoV.[1]

SARS-CoV-2 became a pandemic in various countries until early 2020.[2] The virus was named SARS-CoV-2 because it has genetic similarities with SARS-CoV of the genus Betacoronavirus which was an epidemic in 2002-2003.[3,4]

On April 30, 2020, WHO stated globally 3,090,445 positive cases were confirmed to be COVID-19 with 217,769 deaths.[5] While in Indonesia, as of May 08, 2020, there were 13,112 confirmed positive cases of COVID-19, with 2,494 victims recovered, and 943 victims died.[6] In Indonesia, it's also known that COVID-19 fatality rate is quite high in five provinces, namely Banten (11.1%), East Java (10.7%), DKI Jakarta (8.6%), Central Java (7%), and West Java (6.6%). In addition to these provinces, there are five provinces that report the highest cure rates, namely Riau Islands, Bali, Aceh, Gorontalo, and DI Yogyakarta.[7]

SARS-CoV-2 infects humans thought to have originated from bats sold in the largest seafood market in Wuhan.[1] Infected humans can transmit the SARS-CoV-2 virus through sparks from the nose or mouth when coughing, sneezing, and or when breathing.[8] These splashes can fall on nearby objects or surfaces, and people who touch the object or surface and then touch their eyes, nose or mouth can also be infected with SARS-CoV-2 because the virus from the hands moves and enters the lungs.[8,9]

SARS-CoV-2 infection can cause mild, moderate or severe symptoms.[10] The main clinical symptoms are fever >38 ° C, dry cough, and difficulty breathing.[11] In some cases, even to experience heavy congestion, severe fatigue, muscle aches, and diarrhoea.[12] Some patients experience shortness of breath for one week with severe cases such as acute respiratory distress syndrome (ARDS), shock, septic, metabolic acidosis, and bleeding.[10,12]

SARS-CoV-2 is an RNA virus that has a spike glycoprotein (protein S) that can bind to the angiotensin-converting enzyme 2 (ACE2) receptor.[13] The S protein enters the host cell by attaching and binding to the ACE2 receptor, so the receptor-binding domain (RBD) of the S protein automatically recognizes that the receptor belongs to the host.[14] This can eventually combine the virus with the host membrane, and then the virus antigen will be exposed to the antigen presentation cell (APC).[15] ACE2 in the lungs is found in type 2 alveolar cells,[16] this is what causes in the case of COVID 19 symptoms such as peneumonia.[7]

The pathogenesis of COVID-19 and the manufacture of drugs and vaccines are still under investigation. For most patients, COVID-19 can only affect the lungs because most are respiratory diseases, with the main mode of infection being human-to-human transmission through direct contact from infected individuals through coughing or sneezing. COVID-19 has a possible asymptomatic incubation period of 2-14 days during which the virus can be transmitted.[12] When the virus infects, the antigen will be recognized by the immune system, so that an immune response is formed. To find out the immune response formed, the researchers conducted a review of various journals related to SARS-CoV-2 and the immune response in the human body.

2. REVIEW METHOD

The review method used in this article is a narrative review, which is a way to review existing literature and lean to the qualitative interpretation of prior knowledge, by summarizing or synthesizing what has been written on a particular topic but not looking for generalizations or cumulative knowledge from what is reviewed.[17] Articles were searched for keywords COVID-19 and SARS-CoV-2. 19.940 articles were obtained (849 from Elsevier, 9,547 from PMC, and 830 from Springer). The inclusion criteria we used were articles that discussed human immune responses to SARS-CoV-2 infection (based on previous SARS-CoV infections), which were fully accessible. From these criteria, 36 articles were obtained (6 from Elsevier, 24 from PMC, and 6 from Springer). The articles used are articles published in 2002-2020. This article discusses how SARS-CoV-2 infects host cells, and the human immune response infected with SARS-CoV-2. The data obtained is then described and supported by relevant references obtained from credible sources.

3. RESULTS AND DISCUSSION

How to SARS-COV-2 infect a human (Based on SARS-COV)

SARS-CoV-2 is a new Coronavirus subfamily that belongs to the β-coronavirus family which has 79.5% genetic similarity with SARS-CoV, the causative agent of the epidemic in 2002-2003.[18] SARS-CoV-2 has a genome structure like Coronavirus in general, which includes RNA viruses with particle sizes of 120-160 nm.[15] SARS-CoV-2 is thought to originate from bats which then mutate and infect humans. SARS-CoV-2 is closely related to Coronavirus in bats (Bat-SL-CoV ZC45 2018) and also Coronavirus in pangolins (Pangolin-CoV GX-P5E 2017).[7] There are no significant differences in the SARS-CoV-2 glycoprotein spike gene sequence found in Indonesia and the Wuhan-Hu-1 isolate from China.[19] SARS-CoV-2 has 96.2% genetic similarity with bat Coronavirus,[1] and have a 91% genome similarity to the anteater Coronavirus.[15] The structure of SASR-CoV-2 also has similarities with other SARS viruses.[20] The body structure of SARS-CoV-2 is presented in figure 1.

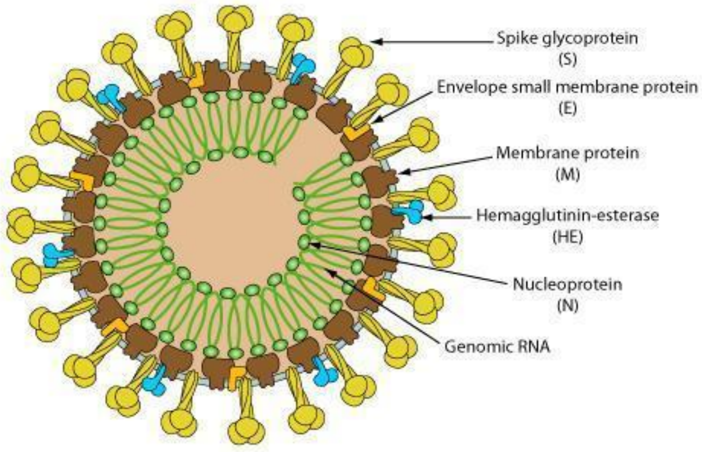


Figure 1. SARS-CoV-2 Body Structure[3]

The structure of SARS-CoV-2 consists of spike glycoprotein (protein S), protein envelope (E), membrane protein (M), hemagglutinin-esterase (HE), nucleocapsid (N), and genomic RNA. S protein is the only viral protein responsible for the entry of the virus into the host cell,[21] S protein protrudes from the virion envelope and plays an important role in attaching the body of the virus to the host receptor.[22,23] Protein E is the smallest protein that is abundantly expressed in infected cells, where it participates in the assembly and development of CoV.[24] M protein is the most abundant protein and forms E protein, M protein is directly related to nucleocapsid and together encourages the formation of protein E in viruses.[24] Hemagglutinin-esterase (HE) is a part of protein E which acts as a lectin and is a receptor-destroying enzyme.[25] The nucleocapsid (N) is seen as a ribonucleoparticle (RNP) because its components correspond to the RNA genome, which is coated by a nucleocapsid protein molecule. Nucleocapsid proteins interact with the RNA genome and coat the genome extensively.[26] RNA genome is the genetic material in the form of a ribonucleic acid single strand (ssRNA) or double-strand (dsRNA) that functions as a store of genetic information and can replicate.[27] In SARS-CoV-2 RNA that is owned is single-stranded RNA (ssRNA).[28]

SARS-CoV-2 has similarities with SARS-CoV which requires specific cellular receptors to infect host cells, namely, angiotensin-converting enzyme 2 (ACE2).[29] ACE2 is a receptor in the form of a central enzyme in the renin-angiotensin system, which negatively regulates the renin-angiotensin system by deactivating Angiotensin II.[30] ACE2 attaches to the outer surface (membrane) of cells in several organs such as the lungs, arteries, heart, kidneys, and intestines.[31]

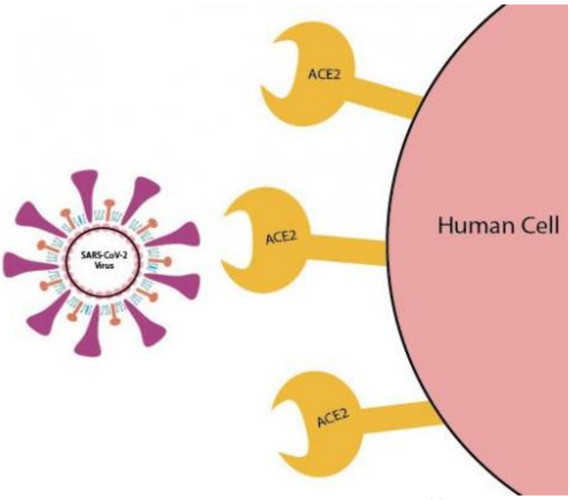


Figure 2. ACE2 Receptors[32]

The SARS-CoV-2 infection process begins with the attachment of Protein S to the ACE2 receptor, then SARS-CoV-2 enters the host cell and releases RNA.[8,32] In the host cell, the RNA genome is translated into polyprotein (pp1a/pp1ab), then replicated (split) into small products by viral proteinases. The polymerase enzyme produces a series of subgenomic mRNAs through RNA replication which ultimately translates into relevant viral proteins. The new viral proteins and genomic RNA are then assembled into virions in the endoplasmic reticulum, and the Golgi is then transported through vesicles,[8] eight released out of the cell and can infect other cells.[8,32] The process can be observed in figure 2.

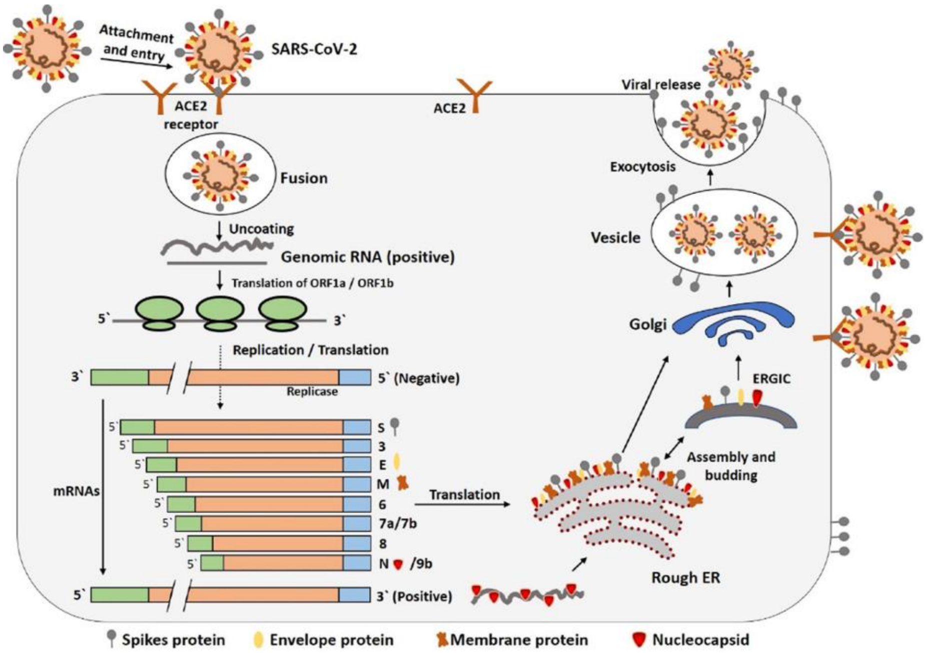


Figure 3. How SARS-CoV-2 Infects Humans[8]

Immune response to human

Humans have the body's defence against various microorganisms, especially those that are pathogenic. This defence system has a complex and complex defence mechanism.[33] The defence system is called the immune system.[34] A person's immune response to an antigen depends on the ability to do the right reaction to eliminate the antigen.[35] This ability is possessed by the components of the immune system that are found in the lymphoreticular tissue which is located throughout the body, for example in the lymph glands; respiratory tract; digestive tract; and other organs.[36]

One of the body's efforts to defend itself against the entry of antigens, such as viruses, is to destroy the virus non-specific (innate) with the process of phagocytosis, in this case, leukocytes and macrophages, including phagocytic cells that play an important role.[37,38] Besides being non-specific, the process of destroying antigens is also done specifically (adaptive). These specific defence mechanisms produce very small groups of proteins called antibodies.[39] These antibodies bind to specific antigens which then facilitate the destruction of antigens, the synthesis of these antibodies is encoded by DNA arranged together with the preparation of a new genome.[34,40]

In the human body, there is a defence system called the immune system. The body's immune system functions to help repair DNA; prevent infections caused by fungi, bacteria, viruses, and other organisms; and produce antibodies to combat antigen attacks.[34] Its job is to find and damage foreign organisms and their toxin products that can harm the human body.[37] When antigens enter the human body, the body will automatically give a response called the immune response. Immune responses that are generally formed consist of 2 types, namely innate immune responses and adaptive immune responses.[41]

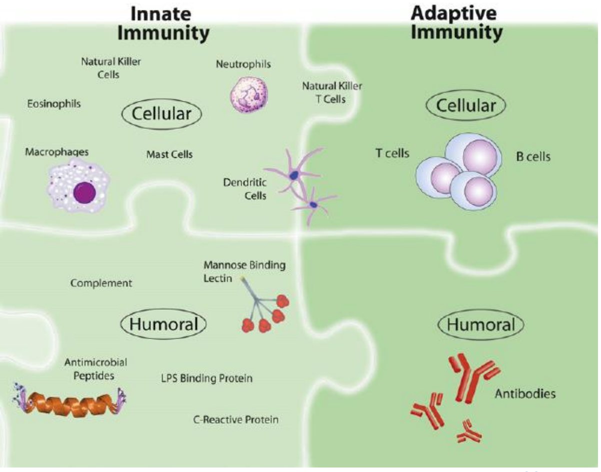
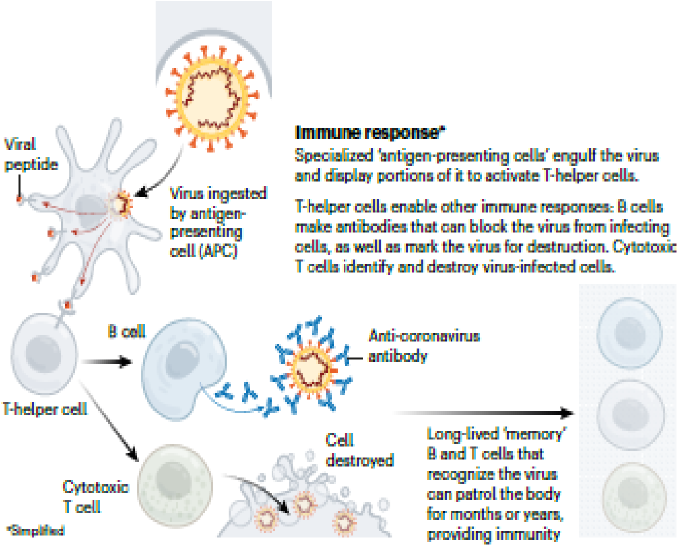


Figure 4. Innate and Adaptive Immunity[38]

The innate immune response becomes the first line of defence against antigens that enter the body, and then this response will trigger another immune response called the adaptive immune response.[42] This adaptive immune response then helps the innate immune work and is tasked with remembering the antigens that infect the body to prevent future infections.[39]



Immune response*

Specialized 'antigen-presenting cells' engulf the virus and display portions of it to activate T-helper cells.

T-helper cells enable other immune responses: B cells make antibodies that can block the virus from infecting cells, as well as mark the virus for destruction. Cytotoxic T cells identify and destroy virus-infected cells.

Long-lived 'memory' B and T cells that recognize the virus can patrol the body for months or years, providing immunity

Figure 5. Immune Response to SARS-CoV-2[32]

The immune system can work when antigens are captured by antigen-presenting (APC) cells, and they will swallow viruses and display viral peptides on active T-helper cells. T-helper cells then send signals to other immune cells, such as cytotoxic T cells and B cells. Cytotoxic T cells will then identify and destroy cells infected with SARS-CoV-2, whereas B cells will recognize SARS-CoV-2 and form antibodies against SARS-CoV-2 antigens. Long-term memory of T cells and B cells can be in the body for a long time and can provide immunity when SARS-CoV-2 re-enters the host body.

Innate immune response

The innate immune response is the response carried out by the innate immune system that first appears when antigens begin to infect.[39] The innate immune response has cellular and humoral systems, including cellular immune systems mediated by natural killer cells (NK), eosinophils, monocytes, basophils, mast cells, and phagocytes (dendritic cells; macrophages; neutrophils.[38] NK cells are part of lymphocytes that can kill target cells directly without causing allergies.[40] Eosinophils are part of leukocytes which make up 1-6% of total leukocytes and many appear in allergies.[43] Monocytes are parts of leukocytes which account for 3-8% of total leukocytes and are classified as mononuclear phagocytes that have receptor sites on the membrane surface.[43] Basophils are the smallest part of leukocytes (less than 2%) and play a role in hypersensitivity reactions that are associated mainly with IgE.[43] Mast cells are cells that contain lots of granules that contain histamine and heparin.[42]

Phagocytes consisting of dendritic cells, macrophages, and neutrophils.[43] Dendritic cells are cells that process antigenic material and present it on the cell surface to be recognized by other immune cells, called antigen-presenting cells (APC), these cells serve as a connecting bridge between innate and adaptive immunity.[44] Macrophages are the main mononuclear phagocyte cells in the tissue in the process of phagocytosis against foreign molecular complexes, monocytes that leave the blood circulation can become macrophages.[45] Neutrophils, which are part of leukocytes that make up 50-70% of the total leukocytes and function as a defensive line that is phagocytic and can enter the infected tissue.[46]

In addition to the cellular immune system, there is also a humoral immune system that plays a role in the innate immune response, namely complement, mannose-binding lectin, antimicrobial peptides, LPS binding protein, and C-reactive protein.[39] Complement is a serum protein that is activated by an antigen that functions as an enzyme in helping protect the body from infection.[47] Mannose-binding lectin is a lectin that acts as a receptor connecting the virus with phagocytes.[42] Antimicrobial peptides are peptide components in the form of amino acids located on the surface of epithelial cells, which help increase immunity by functioning as an immunomodulator.[48] LPS binding protein is a serum lipid-binding glycoprotein that activates monocytes and macrophages to release inflammatory cytokines.[49] C-reactive protein is an inflammatory protein found in plasma and appears in acute phase infections.[50]

The innate immune response, plays an important role in response to SARS-CoV-2, the first response to an incoming virus is regulated mainly by alveolar macrophages, dendritic cells, airway epithelial cells, innate lymphocytes, and neutrophils.[51] Macrophages then present CoV antigens to T cells.[52] This process leads to activation and differentiation of T cells, including the production of cytokinins associated with T cell parts, followed by massive cytokinin release for the amplification of the immune response.[52,53] Cytokines are polypeptides that are produced in response to antigens that mediate and regulate immunological actions and inflammatory reactions.[53] The component of the innate immune response that arises due to SARS-CoV-2 infection in COVID-19 patients is presented in table 1.

Table 1. Default Immune Response to SARS-CoV-2 infection[12,53,54,55]

No	Type of innate immune	The amount
1	Cytokines (IL-1β, IL-2, IL-6, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α / CCL3 and TNF)	increased
2	Coagulation parameter (D-dimer)	increased
3	Neutrophil	increased
4	C-reactive protein	increased
5	Total lymphocytes	decreased

Table 1 shows that in COVID-19 patients the total lymphocytes (T lymphocytes and B lymphocytes) in the blood are significantly decreased, inflammatory cytokinins such as IL-6 will also increase significantly, coagulation parameters such as D-Dimer increase abnormally, so CT shows expansion of lung lesions, other immune systems that are also affected by SARS-CoV-2 namely neutrophils and significantly increased C-reactive protein.[53] Adults infected with SARS-CoV-2 can experience a decrease in lymphocyte counts until lymphocytopenia occurs, especially those who suffer from severe illness. In children with SARS-CoV-2, peripheral blood lymphocytes remain largely in the normal range and show more immune dysfunction.[53]

In a report on 99 cases in Wuhan, the immune response formed by SARS-CoV-2 infection increased total neutrophils (38%), reduced total lymphocytes (35%), increased serum IL-6 (52%) and increased protein c -reactive (84%).[12] Most of the severe COVID-19 patients experienced markedly elevated serum pro-inflammatory cytokinin levels including IL-6 and IL-1β, as well as IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1 , MIP1α (CCL3) and TNF, which are characterized by cytokine storm syndrome. C-reactive protein and D-dimer are also found to be very high.[54] Research in Wuhan, China, shows that COVID-19 damages T lymphocyte cells by reducing the number of T lymphocytes to lymphocytopenia in 44% of 452 patients with congenital diseases (hypertension, diabetes, chronic obstructive pulmonary disease). In this severe group, there was also an increase in the number of pro-inflammatory neutrophils and cytokines (TNF-α, IL-1, IL-6, and IL-8).[55] High levels of pro-inflammatory cytokines can cause shock and damage to some tissues, such as the heart; heart; and kidney, and respiratory failure due to mediating extensive pulmonary pathology, which leads to infiltration of neutrophils and macrophages, diffuse alveolar damage with hyaline membrane formation, and diffuse thickening of the alveolar wall.[56] In addition, splenic atrophy and lymph node necrosis in patients who die also results from excessive immunity.[56,57]

Adaptive immune response

The adaptive immune system is a development of the innate immune system. The adaptive immune system has the advantage of immunological memory, but it is entirely dependent on the innate immune system as the originator of the response. Interactions of the innate and adaptive immune systems that lead to the efficient introduction of antigens, but mal-adaptive interactions between the two can cause dangerous immunology such as allergies and autoimmune.[43]

The adaptive immune response also has a cellular and humoral system. Cellular systems in adaptive immune responses include T cells[38] namely cellular immunity that plays a role in the specific immune system consisting of CD4 + cells, CD8 + cells, naive T cells, NK T cells, and Th3,[58] B cells[38] a collection of cell populations that express various immunoglobulin receptors on the cell surface to recognize various antigens,[59] and dendritic cells that act as a link between the innate immune system and the adaptive immune system.[38]

As for the humoral system in the adaptive immune response that is mediated by antibodies (immunoglobulins secreted by B cells).[38] Immunoglobulin (Ig) is a molecule found in plasma that can specifically respond to antigens that stimulate its production. Immunoglobulin is a heterotetramer molecule that contains four polypeptide chains consisting of two long chains called heavy chains (H), and two short chains called light chains (L).[60] Data on adaptive immune responses that arise due to SARS-CoV-2 infection are presented in table 2.

Table 2. Adaptive Immune Responses to SARS-CoV-2 Infection[56,60]

No	Adaptive immune type	The amount
1	Total antibody titer	increased
2	Immunoglobulin M	increased
3	Immunoglobulin G	increased

In COVID-19 patients, it was reported that patients with severe disease often experienced increased IgG responses and higher total antibody titers, which were associated with poorer outcomes from SARS-CoV-2 infection.[57] Research conducted in China in March 2020, showed that of 173 patients known to have a total antibody titer increased 93.1% on the 11th day, IgM increased 82.7% on the 12th day, and IgG increased 64.7% on the day 14th, with 12 patients were found to have negative antibodies which may be a lack of blood samples at a later stage of the disease.[56] Antibodies can neutralize viruses by inhibiting the attachment of viruses to receptors on host cells, thereby preventing intracellular penetration and multiplication.[61] IgM and IgG antibodies in the human body are present in the serum. Serum COVID-19 patients who have recovered can be used for therapy of COVID-19 patients who have not healed because they contain antibodies.[60,61] Convalescent plasma therapy in COVID-19 patients out of 5 patients showed viral elimination and increased antibody titer in 2 patients, also showed improvement in lung lesions in patients on the third day after plasma transfusion.[60]

Viruses may remain uncontrolled despite an increase in host immunity because the virus has mutated during the transmission process in the host body.[7] RNA virus mutations contribute to the adaptation of viruses in creating a balance between the integrity of genetic information and genomic variability.[20,62] The sequence of mutations is identified singly or double in coding 3C-Like Protease from a virus that is resistant to inhibitors. Inhibited resistant viruses show delays and reduce the production of infectious virus particles.[63] The ability of the virus to defeat the immune response makes the immune response inadequate, causing viral replication and tissue damage. On the other hand, an excessive immune response can also cause autoimmune diseases.[15] Autoimmune occurs when the immune system that is formed incorrectly identifies antigens, where cells, tissues or organs of the human body are actually considered as foreign objects so that they are damaged by antibodies.[64]

4. CONCLUSION

SARS-CoV-2 is known only to be able to infect if it finds an appropriate receptor, in this case, the ACE2 receptor. Viruses that have entered cells can divide RNA and damage cells, new viruses that form will exit the cell and infect other living cells. COVID-19 has an asymptomatic incubation period of 2-14 days, during which time the body will form an immune response. These immune responses include increasing levels of cytokines, coagulation parameters, C-reactive protein, neutrophils, and decreasing total lymphocytes. It is also known that COVID-19 patients with severe disease often experience higher total antibodies, IgM responses, and IgG responses than COVID-19 patients without congenital diseases. This review was carried out while the COVID-19 pandemic was still ongoing, so that not many published research data were available, it is hoped that further studies and reviews related to the human immune response to SARS-CoV-2 infection with more data would be obtained to obtain deeper and larger knowledge.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ACKNOWLEDGEMENT

We would like thank to STIKes Hutama Abdi Husada Tulungagung, Jurnal Teknologi Laboratorium (Journal of Laboratory Technology), editor and reviewer for editing the manuscript.

FUNDING INFORMATION

STIKes Hutama Abdi Husada Tulungagung, Indonesia.

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SHORT BIOGRAPHY

Lia Yosaneri Wina Nurtias in a Medical Laboratory Technology STIKes Hutama Abdi Husada Tulungaguung, Indonesia.

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 41 – 48
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.220
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Original Research

Investigation of revers-transcriptase polymerase chain reaction values of patients with COVID-19 findings in lung computed tomography results

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HIGHLIGHTS

Evaluating chest CT results with RT-PCR can be an appropriate alternative approach in the diagnosis and treatment of disease.

ARTICLE INFO

Article history

Received Date: Apr 27, 2020
Revised Date: May 27, 2020
Accepted Date: Jun 4, 2020

Keywords:

Covid-19
Chest CT
Coronavirus
RT-PCR
Pandemic

ABSTRACT

It turned out that the cause of pneumonia cases that occurred in China was due to SARS-CoV-2. The aim is to compare chest computer tomography and Revers-Transcriptase Polymerase Chain Reaction methods used in the diagnosis of COVID-19 disease with each other and to evaluate this disease with risk factors.

The study was carried out on 66 patients. Epidemiological history, clinical symptoms, chest CT and RT-PCR results of the cases were examined. RT-PCR results of 1, 4, and 7 days were evaluated for each case with positive chest CT results. Thirty-seven of them were found to be positive on day 1, 5 of them were found to be positive on day 4, and 2 of them were found to be positive on day seven from 52 patients whose RT-PCR results were examined. In the remaining 8 cases, no positive findings were found. The most common findings are; cough (78.8%), fever (55.8%), and shortness of breath (28.8%). It was observed that 51.9% of the cases had chronic disease history and 50% of the patients using cigarettes had bilateral lung involvement in their CT results. Seven cases received intensive care support, 3 cases were intubated. Two of the intubated cases were exitus (3,8%). The positive results of RT-PCR were found to be negative in most of the cases which have positive chest CT; suggests that chest CT is more reliable in making a diagnosis. Therefore, evaluating chest CT results with RT-PCR can be an appropriate alternative approach in the diagnosis and treatment of disease. However, in order to be fully diagnosed, the patient's history, chronic diseases, age, symptoms, imaging, blood, and test findings must all be considered as a whole.

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1. INTRODUCTION

Coronaviruses are enveloped, positive-polarized, single-stranded RNA viruses from the Coronoviridia family and their etiological agent is SARS-CoV, which previously appeared in China.[1] It was revealed that the cause of pneumonia cases occurring in Wuhan city of Hubei province of China in December 2019 was caused by SARS-CoV-2, a new species belonging to the SARS-CoV family.[2] The World Health Organization (WHO) has declared this disease called COVID-19 and originating from SARS-CoV-2 as "International Public Health Emergency".[2] Later, WHO declared the COVID-19 outbreak on Mar 11, 2020, as a pandemic due to its alarming spread around the world.[3] COVID-19 still maintains its effect in 202 countries with serious mortality rates.[4]

Coronaviruses (CoV) are a large family of viruses that lead to symptoms that can progress from the common cold to severe illnesses leading up to death.[5] This new coronavirus, called SARS-CoV-2, is a new strain that has not been previously identified in humans and is thought to be due to bat.[6] Common symptoms of COVID-19 disease caused by this zoonotic virus include fever, cough, and shortness of breath.[7] In addition, symptoms such as weakness, muscle aches, sputum, hemoptysis, anorexia, nausea, vomiting, diarrhoea, and abdominal pain are also encountered.[8,9] In recent studies, it is stated that symptoms such as taste, smell loss[10] and conjunctivitis may be due to this virus.[11]

There is no protective and therapeutic vaccine or specific antiviral drug for COVID-19 disease yet. The treatments are symptomatic, and oxygen therapy is applied to patients with severe signs of infection. While mechanical ventilation may be necessary in cases of resistant respiratory insufficiency, hemodynamic support is essential in the cases with septic shock. Although any antiviral therapy has not been approved; Various medications such as lopinavir/ritonavir (400/100 mg in 12 hours), chloroquine (500 mg in 12 hours), and hydroxychloroquine (200 mg in 12 hours) are recommended. Alpha-interferon (for example, 5 million units with aerosol inhalation, twice a day) is also used in treatment.[12] In addition, vitamin C,[13] vitamin D,[14] plasmapheresis, immunotherapy,[15] and even stem cells are considered among the treatment options.[16]

COVID-19 is more severe in chronic patients such as old age, cardiovascular disease, diabetes mellitus, chronic respiratory diseases, hypertension, and cancer.[6] In addition, obesity and smoking affect the course of the disease negatively.[17] Smoking cessation is thought to be important in reducing viral contamination and the severity of the disease.[18]

The SARS-Cov-2 virus, which is a COVID-19 agent, is transmitted from person to person through respiratory droplets, close contact, and touching the infected surfaces. Among the methods of protection from this disease, which the incubation period ranges from 2 days to 14 days, are avoiding non-compulsory travel, early diagnosis, and isolation of patients, attention to social distance, attention to hand, respiratory and environmental hygiene.[19] Viruses can be found in any environment, so it is difficult to keep our environment clean at all times. In recent studies, researchers are working on ultraviolet devices to solve this problem. In this respect, researchers think that these rays will inactivate the SARS-Cov-2 virus, based on the past literature data and the effect of UV rays on similar viruses.[20]

The current method recommended by WHO in the diagnosis of Covid-19 disease is RT-PCR. The detection of SARS-Cov-2 nucleic acid by RT-PCR test from the throat swab sample taken constitutes the standard diagnostic test. In addition, chest CT is used to diagnose COVID-19 disease in the early stages as a supporter.[21] Abnormal lung tissue (83%), ground-glass opacity (73%), lung consolidation (27%), and pleural effusion (11%) are among the most common chest CT findings.[9] The aim of this study is to compare the results of chest computer CT and RT-PCR methods used in the diagnosis of COVID-19 disease and to evaluate this disease together with risk factors.

2. MATERIAL AND METHOD

This is a retrospective study performed with 66 cases diagnosed with COVID-19 in Siirt state hospital and whose treatment was completed. Fourteen cases of these cases with negative Computerized Tomography results were excluded from the study. Epidemiological history, clinical symptoms, Computed Tomography (CT), and Revers-Transcriptase Polymerase Chain Reaction (RT-PCR) results were evaluated.

All Chest CT images were examined by the radiologist, and the decision was made based on positive or negative CT findings. Positive CT results were grouped as unilateral lung involvement (mild involvement), bilateral lung involvement (moderate involvement), and bilateral severe lung involvement (severe involvement). RT-PCR results of the patients, who have COVID-19 findings at their Chest CT results, were evaluated at regular intervals, and the cases were grouped (Figure 1). The data obtained in the study were analyzed using SPSS (Statistical Package for Social Sciences for Windows 22.0) program. Number, percentage, average, and standard deviation were used as descriptive statistical methods in the evaluation of the data.

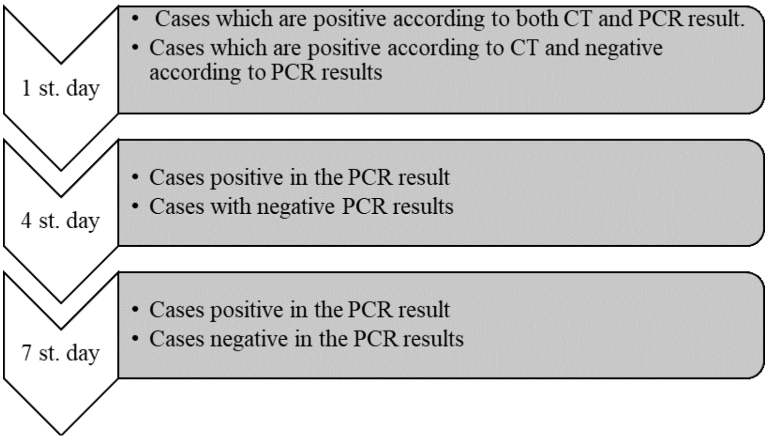

Figure 1. CT and RT-PCR results of cases

3. RESULTS AND DISCUSSION

In the study, a total of 52 cases, 27 males and 25 females diagnosed with COVID-19, were examined. The cases were divided by gender and age group (Table 1). There were Cough in 41 cases (78.8%), fever in 29 cases (55.8%), shortness of breath in 15 cases (28.8%), headache in 9 cases (17.3%), muscle pain in 9 cases (17.3 %), there were symptoms of nausea and vomiting in 1 female case.

Table 1. Distribution of cases by age and gender

Age	Male	Female	Total (%)
20-29	4	8	12 (23.1)
30-39	4	3	7 (13.5)
40-49	8	6	14 (28.8)
50-59	5	2	7 (13.5)
60-69	3	5	8 (15.4)
70-79	3	1	4 (5.7)
Total	27 (51.9%)	25 (48.1%)	52 (100)

It was found that all cases with diabetes mellitus (DM), hypertension (HT), pulmonary disease (PD) were over 40 years old, and all cases with cardiovascular disease (CVD) were over 50 years old. In addition, two females had both DM and HT, and one female had DM, HT, and PH (Table 2). It was determined that 20 cases (38.4%) had no chronic illness and did not smoke. Considering the CT results of these cases, 3 cases had bilateral lung involvement (moderate involvement), and 17 cases had unilateral lung involvement (mild involvement). Two of the cases with moderate involvement were male (21 and 41 years old), and 1 was female (43 years old). It was found that these patients generally applied to the hospital late. It was observed that 18 male cases (34.6%) were smokers (7 cases using for 1-9 years, 7 cases using for 10-19 years, 4 cases using for more than 20 years). Considering the CT results of these cases, 6 cases (33.3%) were unilateral lung involvement (mild involvement), 9 cases (50%) were bilateral lung involvement (moderate involvement), and 3 cases (16.7%) were bilateral severe lung involvement (severe involvement).

Table 2. Chronic cases in cases

Gender	Diabetes mellitus	Hypertension	Pulmonary disease	Cardiovascular disease	Total
Male	5	3	2	2	12
Female	3	6	5	1	15
Total	8 (15.4%)	9 (17.3%)	7 (13.5%)	3 (5.8%)	27(51.9%)

Unilateral lung involvement (mild involvement) in the CT of 25 cases (11 males 14 females), bilateral lung involvement (moderate involvement), in CT of 20 cases (11 males nine females), bilateral severe lung involvement in 7 cases (five males two females) was present. It was observed that cases with bilateral severe lung involvement in their CT had either smoking or chronic disease, and all were over the age of 40.

It was determined that RT-PCR results in 52 cases were positive in 37 cases and negative in 15 cases on Day 1, positive in 5 cases and negative in 10 cases on day 4, positive in 2 cases, and the remaining 8 cases were negative on day 7. It has seemed that 6 cases with negative results were mild involvement, and 2 cases with moderate involvement in the result of CT of the cases which are determined as negative (Table 3). 7 cases of 52 cases (13.4%) received intensive care support, and 3 cases (5.8%) were intubated. Two of the intubated cases were exitus (3.8%), and one was extubated. The other 4 cases were referred to the service after the intensive care treatments were finished. The first exitus case is a 49-year-old male with pulmonary disease and smoking. The second exitus 42-year-old male did not have a chronic disease, was a smoker.

Table 3. RT-PCR results

RT-PCR	CT			Total (%)
	Mild (Patient)	Moderate (Patient)	Severe (Patient)	
Positive on first day	16	16	5	37 (71.2)
Positive on fourth day	3	2	0	5 (9.6)
Positive on seventh day	0	1	1	2 (3.8)
Negative	6	2	0	8 (15.4)

Since December 2019, a new outbreak of coronavirus (COVID-19; previously known as 2019-nCoV) has appeared in the world.[1,6] The rapidly increasing number of cases suggests that the virus is transmitted from person to person and is more contagious than SARS-CoV and MERS-CoV. It was reported that this disease, which caused a pandemic, caused advanced alveolar damage and respiratory insufficiency.[1,22]

It was stated in the studies conducted that the majority of the cases were male in the 30-69 age group,[23,24,25] and in a different study, they were male again between the ages of 39-55.[21,26] We found that the majority of cases were in the 20-29, 40-49 age groups, and men. Although it is stated in the literature studies that the average age is high, it was seen that the average age was lower in our study. It is known that asymptomatic children and adolescents make it difficult to control the outbreak.[27] For this reason, we think that children and adolescents should be evaluated among risk factors.

The most common symptom of the disease is indicated as fever; the most important symptoms after fever are cough, shortness of breath, sputum removal, myalgia, headache, hemoptysis, diarrhoea, and nausea.[1,24,25,28] Although similar symptoms were observed in our study, as different, the most common symptom was cough.

It is stated in the cases where the virus is severe and has a high mortality rate, that the average age is older, and most of them are chronic patients.[23,28,29] DM, HT, CVD, and PD are the most common chronic diseases[2,24,25,26,30,31] (Table 4). In our study, it was seen that most patients were with HT that support the literature. We think that this virus, which is pandemic nowadays, where chronic diseases are common, will increase the morbidity and mortality rates. In addition, it was observed that 20 cases (38.4%) had no chronic illnesses and did not smoke, and their CT results were positive. It is known that these patients were late for admission to the hospital. Early diagnosis and treatment, as with any disease, will reduce possible complications in the COVID-19 outbreak.

Table 4. Chronic disease distribution

	Diabetes mellitus (%)	Hypertension (%)	Pulmonary disease (%)	Cardiovascular disease (%)	Smoking (%)
Yang et all	22	17	8	5	2
Guan et all	16	23.7	-	8	15
Zhang et all	12	30	-	3	27
Zhou et all	18	30	-	8	-
Yen et all	14	14	-	-	-
This Research*	15.4	17.3	13.5	5.8	34.6

Cigarette exposure, inflammatory processes in the lung, increased mucosal inflammation, expression of inflammatory cytokines and tumour necrosis factor α (TNF-α), increased permeability of epithelial cells, excessive mucous release and impaired mucociliary clearance.[32] Besides, it is known that cigarette exposure is a major risk factor for lung diseases,[32] and bacterial and viral infections are more common in smokers.[33] Studies have reported that chest CT findings and prognosis in COVID-19 patients who smoke are more severe than non-smoking patients.[34] In a similar study, the rate of smoking was found to be significantly higher in the group with the progressive disease compared to the stable group ($p <0.05$).[4] In our study, it was observed that smoking was higher than the literature[2,24,31] (Table 4), and 33.3% of them were mild involvement, 50% were moderate involvement, and 16.7% were heavy involvement when the CT findings of the cases were analyzed. In addition, two of the ex-patients were smoking. Consequently, the prognosis of the disease and chest CT findings are affected negatively in COVID-19 patients who smoke.

Since there is no vaccine and a specific drug developed for COVID-19 disease yet, early diagnosis and rapid isolation of patients from the community is very important. The main methods to diagnose the disease are RT-PCR, and chest CT could be to be an alternative. Several studies have also indicated that chest CT is more effective than RT-PCR test in the diagnosis of COVID-19.[21,35] In a study that is made on 1014 patients who admitted to the hospital with suspicion of COVID-19, the diagnosis rates were found to be 59% reliable for RT-PCR and 88% reliable for CT.[35] In our study, the first RT-PCR tests performed in 71.2% of patients whose chest CT findings were compatible with COVID-19 were positive. This result suggests that CT is more reliable than RT-PCR in the diagnosis of COVID-19.

In this study, as of the 7th day, the RT-PCR negativity rate of patients admitted to the hospital with suspicion of COVID-19 was 15.4%. 75% of these cases were evaluated as a mild involvement of CT. Negative results of RT-PCR may be caused by low sensitivity, improper sample intake from throat swabs, disruptions during transport, and poor performance of kits. This situation leads to delay in diagnosis and treatment, infection of a large number of people, deterioration of the prognosis of the disease, an increase in mortality, and morbidity. Therefore, evaluating chest CT results with RT-PCR can be an appropriate alternative approach in the diagnosis and treatment of disease.

Another important issue in the effective fight against epidemics such as COVID-19 is the protection of health workers from the illness at the highest level. As a matter of fact, in the COVID-19 outbreak, which we are in, health workers and other personnel working in the health sector face the risk of disease.[36] This situation creates life-threatening risks for healthcare professionals and their families, as well as other healthy individuals who apply to hospitals. Therefore, the contamination risks that may arise during the application and transportation process of RT-PCR tests should also be taken into consideration. The problems that have been experienced recently during the Ebola epidemic 'sampling and transfer' process can be shown as an example of this.[37] To prevent these problems; it may be useful to use the CT method in diagnosis.

4. CONCLUSION

In the diagnosis of COVID-19, it is difficult to say that a single method is sufficient. For this reason, we think that it is more accurate to evaluate the patient's history, chronic diseases, age, symptoms, CT images, laboratory and RT-PCR test results as a whole.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

Thank you very much for people who are helping to write this paper, especially to university.

FUNDING INFORMATION

The authors declared that this case has received no financial support.

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SHORT BIOGRAPHY

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 49 – 57
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.222
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Article Review

Diagnosis and epidemiology of Coronavirus (COVID-19) outbreak in Indonesia

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HIGHLIGHTS

- Patients diagnosed were aged 30-49 years
- Highest comorbidity are hypertension, diabetes mellitus and cardiac disease
- Large-scale Social Restrictions or “Pembatasan Sosial Berskala Besar (PSBB)” policy

ARTICLE INFO

Article history

Received Date: May 04, 2020
Revised Date: June 04, 2020
Accepted Date: July 02, 2020

Keywords:

Covid-19
PSBB
Coronavirus
Epidemiology
Outbreak

ABSTRACT

COVID-19 has experienced an increase in 2,995,758 positive cases and 204,987 deaths, in distribution areas of more than 213 countries. This research using a systematic literature review, policy brief and internet-based questionnaire with the aim of finding out the diagnosis and epidemiology of Coronavirus (COVID-19) outbreak in Indonesia. The cases in Indonesia have reached 9,771 and killed 784 people, possibly as many as of 19 cases are asymptomatic but can be carriers of the virus. The diagnosis is carried out by rapid testing and using a polymerase chain reaction (PCR). Most of the patients diagnosed were aged 30-49 years (38.91%), the highest accompanying diseases were hypertension (34.85%), diabetes mellitus (25.76%) and cardiac disease (17.05%). The most signs and symptoms are that the patient has a cough, shortness of breath and has a history of internal medicine and fever. Based on sex mostly experienced by men (58.94%) compared to women (41.06%). The Government of Indonesia is making efforts to reduce the COVID-19 outbreak by implementing a health protocol and a Large-scale Social Restrictions or “Pembatasan Sosial Berskala Besar (PSBB)” policy.

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1. INTRODUCTION

Coronavirus outbreak occurred in the world Since mid-December 2019. This disease was first discovered in Wuhan City, Hubei, China, which is found in Wuhan's Huanan Seafood Wholesale Market or a market of fish and live animals that sell various species of animals.[1] It has been reported that this disease is caused by SARS-CoV-2 (formerly 2019-nCoV or HCoV-19). In general, the incubation period is 1 to 14 days (average: 5-6 days) but can reach 92 for 24 days.[2] The most commonly seen characteristics of COVID-19 are fever, cough and abnormal chest computed tomography (CT).[3] On Feb 11, 2020, The World Health Organization (WHO) declared a 2019 coronavirus disease (Covid-19) as a pandemic. To date Apr 29, 2020, COVID-19 has experienced an increase in cases of 2,995,758 positive and 204,987 died. Spread areas occur in more than 213 countries including Indonesia, this is a global threat.[4] The prevalence of the COVID-19 pandemic in Indonesia, based on a report on Apr 29, 2020, showed an increase in cases, of which 9,771 were positive, 1,391 recovered and 784 died.[5]

This disease appears inseparable from the role of animals or zoonoses in transmission to humans, bats, anteater and dogs that are suspected to be their hosts.[6,7] Human to human transmission from Covid-19 occurs mainly through respiratory droplets, direct contact, asymptomatic transmission, and intrafamilial transmission.[2,8]

COVID-19 can affect any demographics, including old age, children, and pregnant women. Therefore to stop transmission of the virus and save the lives of its citizens, the Indonesian government implements strategies and tactics including protocol directives from the World Health Organization (WHO) such as free testing with rapid tests and PCR, free treatment, the establishment of COVID-19 hospitals, large-scale social restrictions, and volunteering and involving social organizations to handle this pandemic.

To see an overview of the outbreak and management of COVID-19 handling in Indonesia, there needs to be an epidemiological study based on data reports from various sources, especially the Task Force for the Acceleration of Handling COVID-19 from the Ministry of Health of the Republic of Indonesia and a review of articles from various available sources and references. It is hoped that this study will be able to explain the understanding of COVID-19, which causes high mortality rates and suggest developing strategies to overcome future epidemic threats.

2. REVIEW METHOD

A narrative review was conducted using Elsevier, Medline / PubMed, Scopus, and Web of Science. The search terms use keywords: "Novel coronavirus," "Novel coronavirus 2019", "2019 nCoV", "Covid-19", "diagnosis". Observation studies and case reports for calculating prevalence were obtained from the policy brief and the report of the Task Force for the Acceleration of COVID-19 Handling of the Ministry of Health of the Republic of Indonesia. We also presented a survey policy brief to assess compliance with the physical, social distancing of the Research and Development Center for Humanities and Health Management, Litbang RI online (internet, Facebook, Instagram, Twitter, WhatsApp) 19.654 respondents spread in 34 provinces in Indonesia.

3. RESULTS AND DISCUSSION

Pathogenesis

Coronavirus 2019 is an inflammatory lung parenchymal disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clinical symptoms that arise vary from shock without complications (mild) to septic (severe).[9,10] Coronavirus is the only positive RNA virus, which is encapsulated, not segmented. There are four genera, namely alpha coronavirus, betacoronavirus, deltacoronavirus and gamma coronavirus. Severe respiratory failure in COVID-19 is commonly associated with the hyperinflammatory syndrome. Cytokine storms may cause hyper inflammation in severe SARSCoV-2 disease.[11]

Coronaviruses have capsules, round or elliptical particles, often pleomorphic with diameters of around 50-200 nm and coronavirus structures form cube-like structures with protein S located on the surface of the virus.[12,13] The envelope is studded with projecting glycoproteins and surrounds a core consisting of matrix protein enclosed within which is a single strand of positive-sense RNA (Mr 6 × 10^6) associated with nucleoprotein. The envelope glycoproteins are responsible for attachment to the host cell and also carry the main antigenic epitopes, particularly the epitopes recognized by neutralizing antibodies. OC43 also possesses a haemagglutinin.

Coronavirus is sensitive to heat and can effectively be activated by disinfectants containing chlorine, lipid solvents with a temperature of 56 ℃ for 30 minutes, ether, alcohol, peroxyacetic acid, non-ionic detergents, formalin, oxidizing agents and chloroform. Chlorhexidine is not effective in deactivating the virus. Coronavirus infections usually occur frequently in winter and humidity is not too high.[12,13]

This new type of Coronavirus pneumonia can occur in all people, especially immunocompromise. If exposed to large amounts of the virus at one time, can cause disease even though the body's immune system is functioning normally. People with weak immune systems such as the elderly, pregnant women, and other conditions, can cause the disease progressively faster and more severely. Coronavirus infection causes a weakened immune system against this virus so re-infection can occur.[12,13]

The main clinical symptoms of COVID-19 infection are fever (temperature> 38ºC), cough and difficulty breathing. Moreover, it can be accompanied by heavy tightness, fatigue, myalgia, gastrointestinal symptoms such as diarrhoea and other respiratory symptoms. In some patients, mild symptoms are not accompanied by fever.[12,13] Besides, fever and cough are reported to be the most common symptoms associated with COVID-19 patients.[3] However, mild symptoms such as nausea, headaches, respiratory problems, sore throat, myalgia, vomiting and sometimes diarrhoea were also shown in COVID-19 patients.[3,14]

A meta-analysis study from journals concluded that COVID 19 patients would be exacerbated by comorbidities including was fever (91 ± 3, 95% CI 86-97%), followed by cough (67 ± 7, 95% CI 59-76%), fatigue (51 ± 0, 95% CI 34-68%) and dyspnea (30 ± 4, 95% CI 21-40%). The most prevalent comorbidities were hypertension (17 ± 7, 95% CI 14-22%) and diabetes (8 ± 6, 95% CI 6-11%), followed by cardiovascular diseases (5 ± 4, 95% CI 4-7 %) and respiratory system disease (2 ± 0, 95% CI 1-3%). Compared with the Non-severe patient, the pooled odds ratio of hypertension, respiratory system disease, cardiovascular disease in severe patients were (OR 2.36, 95% CI: 1.46-3.83), (OR 2.46, 95% CI: 1.76-3.44) and (OR 3.42, 95% CI: 1.88-6.22) respectively.[15]

The spread of the virus can be through close contact, environment or objects contaminated with the virus, airway droplets, and airborne particles. A droplet is particle-containing water with a diameter of> 5um. Droplets can pass up to a certain distance (usually 1 meter) to the vulnerable mucosal surface. Droplet particles are large enough so that they will not last or settle in the air for a long time. Droplet production from the airways includes coughing, sneezing or talking as well as invasive respiratory procedures such as sputum aspiration or bronchoscopy, tracheal tube insertion. Airborne particles are particles with a diameter of less than 5um, which can spread over long distances and are still infectious. Airborne pathogens can spread by contact. Direct contact is the transmission of pathogens directly with the skin or mucous membranes, blood or blood fluid that enters the body through mucous membranes or damaged skin.[12,13]

Diagnosis

Laboratory markers of disease progression and clinical outcomes, such as D-dimer, C-Reactive Protein (CRP), procalcitonin, neutrophil count, lymphocyte count and inflammatory cytokines were monitored.[2,11] Regarding laboratory findings, decreased albumin (75.8%, 95%CI 30.5–100.0%), high C-reactive protein (58.3%, 95%CI 21.8–94.7%), and high lactate dehydrogenase (LDH) (57.0%, 95%CI 38.0–76.0), lymphopenia (43.1%, 95%CI 18.9–67.3), and high erythrocyte sedimentation rate (ESR) (41.8%, 95%CI 0.0–92.8), were the most prevalent laboratory results (Morales, 2020). Diagnostic tests for COVID-19 have been developed such as reverse transcription-polymerase chain (RT-PCR), real-time PCR, real-time quantitative RT-PCR (rRTqPCR), COVID-19-RdRp/Hel real-time RT-PCR assay, POCT/bedside testing, loop-mediated isothermal amplification (RT-LAMP), full genome analysis by next-generation sequencing (NGS), fluorescence-based quantitative PCR assay, enzyme-linked immunosorbent assay (ELISA), computed tomography technique (CT) imaging and X-Ray.[9,16,17]

Histologically, tissue biopsy of the lungs, liver and heart tissue reveals pneumococcal desquamation, hyaline membrane formation, diffuse bilateral alveolar damage in conjunction with cellular fibromyxoid exudates. Multiple nucleated syncytial cells, atypical enlarged pneumocytes, interstitial mononuclear inflammatory infiltrates along with the presence of the majority of lymphocytes in the affected lung configuring significant cytopathic effects.[11] Nearly all COVID-19 patients are reported to have varying degrees of disability in lung pneumonia with other viruses on CT imaging. In addition, other findings include bilateral multilobular sub-lateral consolidation of the lungs at an early stage followed by multiple mottling and ground-glass opacity.[9,11]

Severe pulmonary lesions are seen around the 10th day after initial symptoms in most patients recovering from COVID-19 disease.[18] The blood profile of COVID-19 patients showed lymphopenia, leukopenia, thrombocytopenia and anaemia, along with higher aspartate aminotransferase levels and hypersensitive troponin.[9,11] Initially reported to be normal, but a slight increase in level in the later stages was noted, indicating the possibility of secondary infection.[11] Cytokine storm associated with the rampant inflammation resulted into the release of proinflammatory cytokines and chemokines like IFN-γ, IL-1β, IP-10, MCP-1, TNF-α, G-CSF, MCP-1, IP-10, and MIP-1A which severely damages pulmonary tissues leading to death in severe COVID-19 patients. Although, lymphopenia, leucopenia, thrombocytopenia, and RNAaemia occurs with a decrease in helper T cells, regulatory T cells, and memory T cells in severe COVID-19 cases notably the levels of Th1 and Th2 cytokines are found elevated. However, elevated levels of ALT, AST, LDH, CPK, creatinine, γ-GT and α-HBDH in a severe form of the disease suggests multiorgan involvement.[16]

COVID-19 Epidemiology in Indonesia

On April 28, 2020, Data from World Health Organization (WHO), there were 2,954,222 positive COVID-19 cases and 202,597 dead, spreading over 213 countries. Cases in several countries show European Region 1,386,693 confirmed (27,313) 126,429 deaths (1,904), Region of the Americas 1,179,607 confirmed (39,087) 60,211 deaths (1,722), Eastern Mediterranean Region, 171,238 confirmed (5,305), 7,148 deaths (157), Western Pacific Region 145,385 confirmed (1,264) 5,998 deaths (40), South-East Asia Region 48,348 confirmed (2,288) 1,917 deaths (93) African Region, 22,239 confirmed (769) 881 deaths.[19]

The official statistics of the epidemic in Indonesia were reported by the Ministry of Health through the Task Force for the Acceleration of Handling COVID-19, 2020, on Apr 29, 2020, the cumulative number of positive cases was 9,771 recovered 1,391 and 784 people died (cluster/web). Figure 1 shows the distribution of confirmed cases[5] in April 2020.

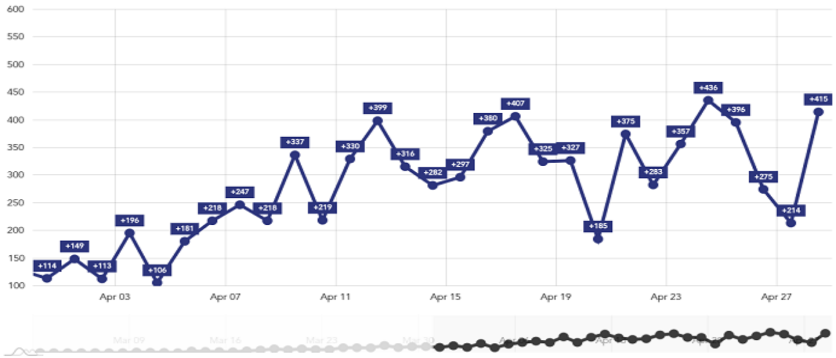

Figure.1 Distribution of Confirmed Cases during April 2020 in Indonesia[5]

At the beginning of April, there were 114 confirmed cases, further increasing until the second week, there were 407 cases. Fluctuations occurred from the third week to the fourth week and even dropped by only 214 cases, but the next day there was an increase again. Starting at the end of the month, the fluctuation case has begun to stabilize or decrease, and this is due to the Indonesian government making policies with large-scale social restrictions. The number of COVID-19 cases above is substantially possible still below proportion because the data are only based on rapid tests or by polymerase chain reaction (PCR) while many people have not been confirmed even though they have the potential to be carriers of asymptomatic viruses. It has been speculated that the total number of COVID-19 infected people is about five times higher than the official statistics. This bias must be taken into account when interpreting any COVID-19 statistics.[20]

The distribution of the COVID-19 case area almost occurred in all provinces in Indonesia, the highest being in DKI Jakarta, West Java, East Java, Central Java, followed by other provinces. The age of COVID-19 patients in Indonesia who had been diagnosed was mostly 30-49 years (38.91%), seen in figure 2.

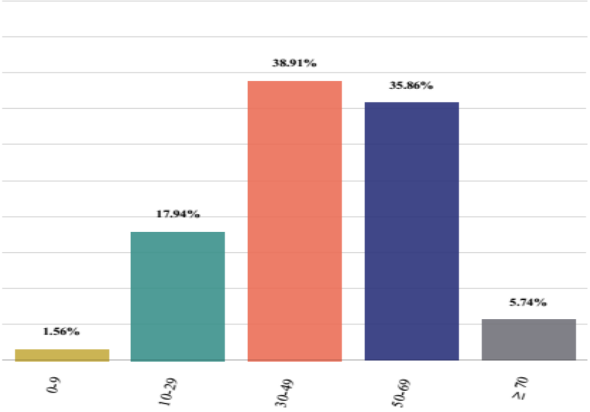

Figure 2. Distribution of COVID-19 Cases By Age[5]

Figure 2 shows that many cases of COVID-19 death are experienced in older people; this is associated with a weak immune system that allows faster development of viral infections and comorbidities.[21,22] The results of previous studies, most patients heal themselves, while the mortality rate is around 10-14%, especially patients over the age of 40 years with comorbidities such as heart disease, asthma, chronic lung disease and diabetes.[21] The highest incidence of COVID-19 with comorbidities in Indonesia was hypertension (34.85%), diabetes mellitus (25.76%) and cardiac disease (17.05%).

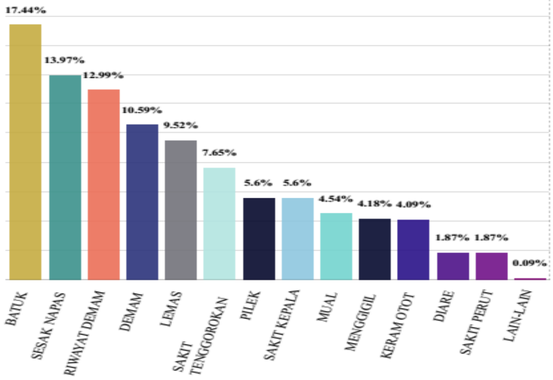

Figure 3. Signs and Symptoms of COVID-19 patients[5]

Based on the signs and symptoms of COVID-19 in figure 3, many patients experience coughing, shortness of breath and a history of internal medicine and fever. Research that has been done shows signs and symptoms of fever (96%) and cough (51.47%) were the most common symptoms.[23] Coronavirus infection in Indonesia by sex is mostly experienced by men (58.94%) while women (41.06%).[10]

Physical and Social Distancing Compliance

The results of an online survey with Google form via the internet, Facebook, and WhatsApp related to community compliance with physical and social distancing calls, based on the results of the RI Ministry of Health Research and Development briefing policy of 19,654 samples, 99.96% knew about the government's recommendations for physical distancing. However, only 54.29% were obeyed keep distance and 8.82% stay at home, 32.09% stated that leaving the house to shop for basic needs, 43.99% stated leaving the house for urgent needs. from 19,654 respondents who carried out the health protocol, 93.40% used masks, 17.20% wore gloves, 47.37% said they always brought a hand sanitizer, and 31.79% said they gave greetings without contact.[24]

Activities that are still a concern in the results of the compliance survey are outdoor activities. This cannot be denied because the community needs to fulfil their daily needs, besides the policy taken by the government by implementing Large Scale Social Restrictions (PSBB) which it is still considered loose not applying total lockdown so that people are free to leave the house.

Prevention and Control of COVID-19 Spread

Public health and control measures are needed to reduce the transmission of COVID-19 people to people by limiting the global spread of the virus.[22] Experience from the early phase of SARS-CoV-2 Pneumonia strongly highlighted that travel history, rather than chest radiography, is of paramount importance for early detection and isolation of SARS-CoV-2 pneumonia cases.[24] Public services and facilities must provide antiseptic reagents for regular hand washing WHO recommends that it is crucial to limit transmission of infection by Physical distancing to avoid close contact, especially with health care workers and to prevent international outbreaks of avoiding countries or regions of the red zone. In addition, people with symptoms of respiratory tract infections should practice cough etiquette, which is to keep a distance, cover the cough and sneeze with disposable tissues or clothing, and wash hands, and in health care facilities improve infection prevention standards and control practices. especially in the emergency department.[4]

The Ministry of Health of the Republic of Indonesia issued Regulation No. 9 of 2020 concerning Large-Scale Social Restriction Guidelines. Some activities are limited as long as the regions carry out the Large-Scale Social Restrictionincluding restrictions on schools, work at the office, religious activities, public facilities, social culture, public transportation and defence and security. Efforts that are no less important are guarding public panic, social media one-sidedly increases knowledge and understanding, but also has the potential to spread false information or false news. Therefore the government needs to provide accurate knowledge and prevent hoax information. It is also necessary to make efforts by all components of the community by exploring the potential of the community to be empowered and able to play a role in preventing the transmission of Covid-19.

4. CONCLUSION

The COVID-19 outbreak has become a health threat worldwide, Indonesia is one of the countries affected by more than 9,000 cases, and more than 784 people have died. Epidemiologically the COVID-19 case in Indonesia occurred in the 34 highest provinces in Jakarta, most patients were aged 30-49 years, male sex and many were accompanied by comorbid hypertension, DM and heart disease. Efforts to reduce Covid-19 cases by implementing health, social and physical distancing protocols through the Large Scale Social Restrictions policy.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

All the authors acknowledge and thank their respective Institutes, Task Force for the Acceleration of Handling COVID-19 Ministry of Health of the Indonesian government, which has provided COVID-19 update data.

FUNDING INFORMATION

The authors declared that this case had received no financial support.

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SHORT BIOGRAPHY

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 58 – 66
ISSN 2580-0191 (Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.218
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Article Review

Perspective of molecular immune response of SARS-COV-2 infection

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HIGHLIGHTS

SARS-CoV-2 increases the number of neutrophils as APC, suppresses IFN, increases the activity of Th1 / Th17, B cells, CD8 + and CD4 +, and causes cytokine storms especially pro-inflammatory cytokines which can increase respiration disorders and multi-organ damage.

ARTICLE INFO

Article history:

Received Date: May 07, 2020
Revised Date: June 04, 2020
Accepted Date: June 18, 2020

Keywords:

COVID-19
SARS-CoV-2
Immune Response

ABSTRACT

COVID-19 is a type of Pneumonia that caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). When COVID-19 arise in Wuhan China and rapidly spread throughout to the World, we need to learn how pathogenesis and immune responses occur in the bodies in more detail. COVID-19 is third Severe Respiratory Disease outbreak caused by the Coronavirus in the past two decades after Severe Acute Respiratory Syndrome (SARS) in the 2002 and Middle East Respiratory Syndrome (MERS) in the 2012. The Articles from PUBMED and Research Gate were searched for studies on the immune response of COVID-19 infection by SARS-CoV-2. SARS-CoV-2 increases the number of neutrophils, suppresses IFN, increases the activity of Th1/Th17, B cells, CD8+ and CD4+, and causes cytokine storms especially pro-inflammatory cytokines which can increase respiration disorders and multi-organ damage. This review tries to explain about pathogenesis and immune responses of COVID-19 to provide a reference in designing the appropriate immune intervention for treatment and therapeutic such as drug or vaccine based on the recent research progress SARS-CoV-2 and previous studies about SARS CoV and MERS CoV.

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1. INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a pathogenic viral infection caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that initially arises in Wuhan, Hubei Province, China and rapidly spread to the world. Initially, the cases arise in Wuhan's Hunan Seafood Wholesale Market which trade in fish and variety of live animals such as bats, marmots, and snake. The causative agent for the disease was identified from throat swab samples conducted by the Chines Centre for Disease Control and Prevention (CCDC), and was subsequently named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The sequences-based analysis suggested that bat is a key reservoir of SARS-CoV-2 and can be transmitted to humans.[1] Furthermore, the transmission will occur from human to human as it is today.

On 30th January 2020, the World Health Organization (WHO) declared that COVID-19 from Wuhan was to be a Public Health Emergency of the International Concern posing a high risk to countries. WHO announces this disease outbreak a pandemic diseases. It is due to the rapid increase in the number of cases outside China over the past 2 weeks that has affected a growing number of countries in the world.[2] There were 7734 cases have been confirmed in China on that day. In a number of counties also confirmed 90 cases.[3] On 30th March 2020, WHO confirmed that there were 638.146 cases with 30105 death in 203 countries in the World.[4] In Indonesia, Ministry of Health Indonesia confirmed that there are 1285 positive cases with 114 deaths and 64 recovering on 30th March 2020.[5]

COVID-19 symptoms appear after an incubation period of approximately 5.2 days. The period from the onset of the symptoms to death ranges is from 6 to 41 days with a median of 14 days.[6,7] This period is dependent on the age of the patient and the status of the immune system. The most clinical symptoms at the onset of COVID-19 are fever, dry cough, and fatigue. The other symptoms include headache, sputum production, hemoptysis, diarrhea, dyspnea, lymphopenia, and bilateral lung infiltrates on imaging.[7]

The main problems of COVID-19 are high transmission and the absence of specific drugs and vaccines. For this problem, the immune system is an important aspect to support the host's self-defense and the patient's recovery. This review may help in designing the appropriate immune intervention for treatment and therapeutic such as drug or vaccine in preventing more wide transmission and in the development of the COVID-19 drugs based on the recent research progress SARS-CoV-2 and previous studies about SARS -CoV, and MERS-CoV.

2. REVIEW METHOD

We performed this review of the Pubmed and Research Gate databases from inception to 30 March 2020 to find articles providing information on the molecular immunopathogenesis of COVID-19 infection. The initial search identified 87 sources. Based on the inclusion criteria, we get 13 articles that discuss the Immunopathogenesis of Coronavirus including SARS-CoV, MERS-CoV, and SARS-CoV-2. Initially, this review explains the immune response in SARS-CoV and MERS-CoV, and subsequently, explains the pathogenesis and immune response in SARS-CoV-2. As a coronavirus family, we assume that the immune response of SARS-CoV-2 is similar to SARS-CoV and MERS-CoV. The search was expanded using a snowballing method applied to the references of retrieved papers.

3. RESULTS AND DISCUSSION

As the coronavirus family, the immune response of SARS-CoV-2 is similar to SARS-CoV and MERS-CoV, although it has little difference in clinical manifestation. The summary of the immune response is described in table 1**.**

Table 1. Immune Response Summary of SARS-CoV, MERS-CoV, and SARS-CoV-2

Coronaviruses	Immune Responses	Source
SARS-CoV	1. Cytotoxic T Lymphocyte (CTL)	[8]
	2. MHC class I and II (HLA)	[8]
	3. HLA-B * 4601, HLA-B * 0703, HLA-DR B1 * 1202	[9]
	4. HLA-Cw * 0801	[10]
	5. HLA-DR0301, HLA-Cw 1502 and HLA-A * 0201	[11]
	6. CD8 + T cells	[12]
	7. Cytokine (IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGF-β) and Chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10)	[38, 39, 40]

Coronaviruses	Immune Responses	Source
MERS-CoV	1. MHC class II molecules: HLA-DRB1 * 11: 01 and HLA-DQB1 * 02: 0	[13]
	2. Mannose Binding Lectine (MBL) gene polymorphism associated with APC	[14]
	3. CD8+ T cells	[15]
SARS-CoV-2	1. Neutrofil as APC	[15]
	2. High Proinflammatory cytokine: IL6, IP-10, MCP-1, MIP-1A, and TNFα.2	[15]
	3. Th1/Th17	[15]
	4. Low Antiinflammatory cytokine: Type I IFN	[15]
	5. B cell, IgM, and IgG	[16]
	6. CD4+ CD8+ T cells	[17]

Immune response to SARS-CoV-2 infection based on clinical manifestation approach, clinical laboratory test results, and studies of immune response on SARS-CoV and MERS-CoV can be shown in figure 1.

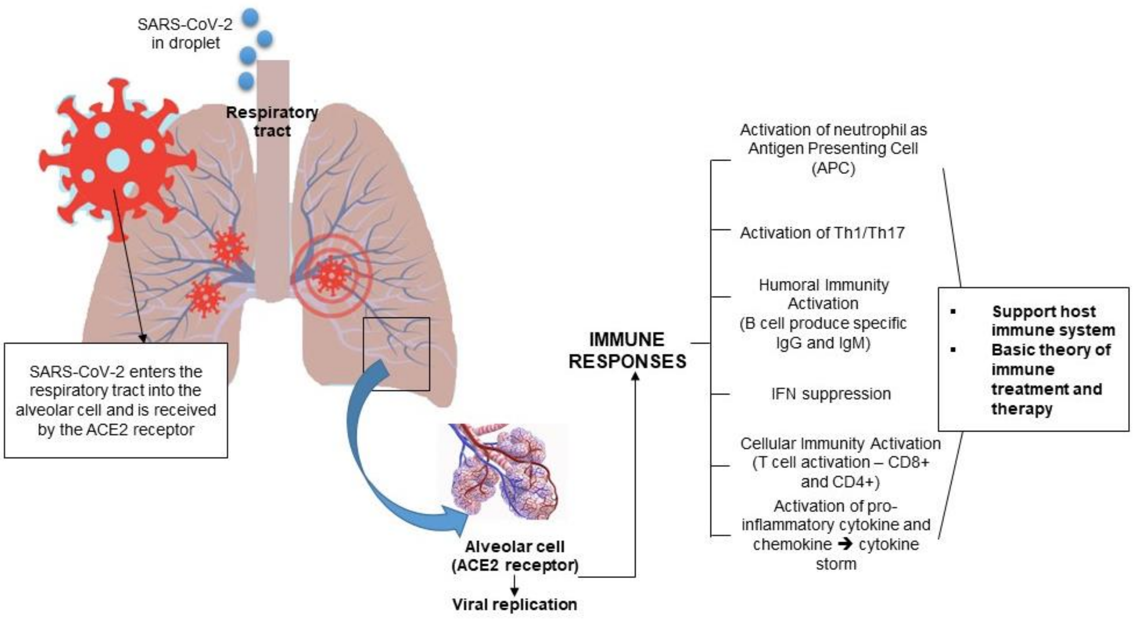

Figure 1. Host immune responses prediction during SARS-CoV-2 infection

According to genotypically and serologically, coronavirus is divided into 4 genera such as α, β, ɣ, and δ Coronaviruses. The subfamily α and β CoV usually causes Human CoV infections. SARS CoV and MERS CoV is included in β CoV. The SARS-CoV-2 is novel β CoV was named by the International Virus Classification Commission.[18,16]

Coronaviruses (CoV) are enveloped viruses, non-segmented, and positive-sense single-stranded RNA (ssRNA) virus genomes in the size 26 to 32 kb, the largest known viral RNA genome. SARS-CoV-2 had a length of 29.9 kb. SARS-CoV-2 has four main structural proteins including Spike Glycoprotein (S), small envelope glycoprotein (E), membrane glycoprotein (M), and nucleocapsid protein (N), and several accessory protein. The structure of CoV virion is shown in figure 1.[16]

The Spike glycoprotein (S protein) is a transmembrane protein with a molecular weight of about 150 kDa, forms homotrimers stand out in the viral surface. S protein facilitates the binding of envelope viruses to Angiotensin-Converting Enzyme 2 (ACE-2) receptors in lower respiratory tract cells. S protein consist of 2 part, S1 and S2. S1 is responsible for the determination of the host-virus range and cellular tropism with the receptor-binding domain make-up while S2 functions to mediate virus fusion in transmitting host cells[18,16]

SARS-CoV-2 rapidly is transmitted from human to human pathogen and causes a wide spectrum of clinical manifestations in COVID-19 patients. The major clinical manifestation of

COVID-19 is similar to other human CoV infection. In general, SARS-CoV-2 is associated with high viral loads in upper respiratory tract secretion. COVID-19 is developed into Severe Acute Respiratory Infections and Acute Distress Syndrome especially in elderly, comorbidities, and immunosuppression patient.[19]

Human to human transmission of COVID-19 mainly occurs via respiratory droplets when patients with coughs, sneezes, or talks can infect another person via direct contact with the mucous membranes. The infection can also occur if a person touches an infected surface and then touches their eye, nose, or mouth. The droplet does not travel that 2 meters and not linger than 3 hours in the air.[20]

Based on the presence of clinical features, COVID-19 is divided into 2 groups such as asymptomatic and symptomatic infection. Asymptomatic COVID-19 infection shows positive SARS-CoV-2 nucleic acid testing of the pharyngeal swab samples but does not show the clinical feature until the first day of continuous negative tests (1-21 days). Only 20.8% asymptomatic cases develop into symptoms cases during hospitalization such as fever, cough, fatigue and etc, 50% cases showed typical CT images of the ground-glass chest, and five (20.8%) presented stripe shadowing in the lungs. The remaining 29.2% cases showed a normal CT image and had no symptoms during hospitalization (young patient estimated 14 years). However, no one cases develop into severe COVID-19 pneumonia or died. Asymptomatic carrier patient has the potential to transmit the disease to others. This study also shows that Asymptomatic COVID-19 carrier can transmit the virus into his family that develops to Severe COVID-19 pneumonia.[21]

According to some studies, an incubation period of COVID-19 is 5 – 14 days following exposure and cause the respiratory and systemic disorder.[13] The spectrum of symptomatic COVID-19 infection ranges from mild to critical. The mild stage is signed with high fever, dry cough, headache, fatigue, sputum production, lymphopenia, and diarrhea. COVID-19 infection cause cough (days 1 – 14), chills (days 1 – 9), high fever (days 7 – 9), fatigue (days 9 – 14), and shortness of breath (days 9-14). The severe stage is signed with dyspnea and hypoxia. The pathological finding of COVID-19 associated with acute respiratory disruption syndrome. The critical disease is signed with respiratory failure, shock, or multi-organ dysfunction. COVID-19 infection causes acute respiratory distress syndrome (ARDS) until death.[6]

The infection show laboratory features such as lymphopenia and elevated some molecules (liver enzymes, lactate dehydrogenase, elevated inflammatory markers, D-dimer, prothrombin time, troponin, creatine phosphokinase, and acute kidney injury. The laboratory testing shows positive in Viral RNA PCR testing and Immunoglobulin M (IgM), and there is ground-glass chest, stripe shadowing in the lungs in CT Scan, and pneumonia in x-ray test.[22,23]

COVID-19 is a positive-sense single-stranded RNA (ssRNA) that entry to the patient via respiratory droplets when patients with coughs, sneezes, or talks can infect another person via direct contact with the mucous membranes and touching the infected surface the body (eye, nose or mouth).

COVID-19 S protein (envelope spike glycoprotein) will bind to ACE2 for SARS-CoV,[19] ACE2 SARS-CoV-2,[24] CD209L (a C lectin or called L-SIGN) for SARS-CoV,[25] DPP4 for MERS-CoV[26] then occur fusion between the viral cell membrane and the target cell.[27] Proteolytic cleavage occurs in S SARS-CoV protein in position (S2') mediated by membrane fusion and virus infectivity.[28] MERS-CoV has also evolved through the activation of two abnormal steps for furin membrane fusion.[29] In addition to membrane fusion, clathrin-dependent and clathrin-independent endocytosis also mediate the process of SARS-CoV virus entry into cells.[30,31]

After that viral RNA enters the cytoplasm of cells in the form of polyproteins and structural proteins which then replicates into new viral RNA. The cell will then be controlled by the structural protein SARS-CoV to form an envelope protein that will be inserted into the endoplasmic reticulum (ER) and Golgi apparatus while the nucleocapsid is formed by a combination of viral RNA genomes and nucleocapsid proteins. In the end, the virus particles will be brought to endoplasmic reticulum-Golgi intermediate compartment (ERGIC) for maturation and will then be removed by ERGIC with vesicles containing infectious viruses through vesicle vesicles which will docking with membranes so that the virus will be released again and ready to infect other healthy cells.[19]

Viral antigen entry stimulates an immune response. COVID-19 viral antigen will be presented to the antigen-presenting cell (APC). The antigenic peptide will be presented by MHC (human leukocyte antigen or HLA in humans) to the specific cytotoxic T lymphocyte virus. MHC Class I played the most role in this Ag COVID-19 presentation, although MHC class II also played a role.[8] The COVID-19 presentation antigen was not fully understood but can be associated with antigen presentation on SARS-CoV or MERS-CoV. Certain HLA polymorphisms have a risk of being susceptible to SARS-CoV infection, namely HLA-B * 4601, HLA-B * 0703, HLA-DR B1 * 1202[9] and HLA-Cw * 0801,[10] while HLA-DR0301, HLA-Cw 1502 and HLA-A * 0201 has protection against SARS infection.[11] MHC class II molecules cause susceptibility to MERS-CoV infection,[13] such as HLA-DRB1 * 11: 01 and HLA-DQB1 * 02: 0. In addition, the Mannose Binding Lectine (MBL) gene polymorphism associated with antigen presentation is associated with susceptibility to SARS-CoV infection.[14]

SARS-CoV-2 stimulate innate and adaptive immune responses. SARS-CoV-2 leads to infection of ACE2 expressing target cells such as alveolar type 2 cells or other cell in lung. SARS-CoV-2 causes an increase in total neutrophils, reduces total lymphocytes, increases serum IL-6, and increases C-reactive protein. Increased neutrophils and decreased lymphocytes also correlate with disease severity and death. COVID-19 has higher plasma levels of many pro inflammatory cytokines, IP-10, MCP-1, MIP-1A, and TNFα.2 that show highly pro-inflammatory conditions in the disease progression and severity. Activation of neutrophil as Antigen Presenting Cell (APC) increase Th1/Th17 expression. Th1/Th17 is induced adaptive response by increase specific antibodies production. Viral antigen may decrease antiviral IFN responses resulting in uncontrolled viral replication whereas IFN is effective innate immune response against viral infection and induce effective adaptive immune response. Type I IFN suppression and hyper pro-inflammatory cytokine production be the basis of viral infection treatment including SARS-CoV infection.[15]

In adaptive immune response, COVID-19 viral antigen will stimulate humoral and cell mediated immunity by B cells and T cells. B cells will form IgM and IgG anti SARS-CoV. IgM will disappear on the 12th day while IgG will last a long time in the body which will function as a protector against infection with the same virus strain.[16] IgG anti SARS-CoV is S-specific and N-specific.[19] In general, Th1 plays a dominant role in an adaptive immunity against to viral infections. Specific Th1/Th17 may be activated and contributes to exacerbate inflammatory responses. B cells/plasma cells produce SARS-CoV-2 specific antibodies that may help neutralize viruses. In previous study showed the peak of specific IgM at day 9 after disease onset and the switching to IgG by week 2. Whether the titer of specific antibody correlates with disease severity remains to be investigated.[15]

In cellular immune response, the number of $CD4^+$ and $CD8^+$ cellular immunity in peripheral blood will drop significantly in SARS-CoV-2 infection.[17] Patients recovering from SARS-CoV will have $CD4^+$ and $CD8^+$ memory T cells that last for 4 years so that when there is repeated viral exposure the body will be able to produce IFN-4.[32] In some patients the specific T cell memory of SARS-CoV can still be identified.[12] CD8 + T cells specific to MERS CoV are also found in mice that are infected with the virus.[12] In general, CD8+ T cell responses are more frequent with greater magnitude than CD4+ T cell responses in SARS Coronavirus. Strong T cell responses correlated significantly with higher neutralizing antibody while more serum Th2 cytokines (IL-4, IL-5, IL-10). In MERS-CoV infection, early rise of CD8+ T cells correlates with disease severity and at the convalescent phase, dominant Th1 type helper T cells are observed. In an animal model, airway memory CD4+ T cells specific for conserved epitope are protective against lethal challenge and can cross react with SARS-CoV and MERS-CoV. Th1 type and CD8+ T cell are keys for successful control of SARS-CoV and MERS-CoV and probably true for SARS-CoV-2 as well to suppress lung pathogenesis.[15]

The leading cause of death in COVID-19 infection is Acute Respiratory Distress Syndrome (ARDS) and multi-organ dysfunction. ARDS is the leading cause of death. This ARDS is an immuno-pathological event from SARS-Cov-2, SARS-CoV and MERS-CoV infections.[16] More patients also developed lymphopenia and pneumonia with characteristic pulmonary ground glass opacity changes on chest CT. COVID-19 infection also show cytokine storm. Cytokine storm is inflammation that cannot be controlled and is very deadly due to the body's response due to the large production of inflammatory cytokines such as (IFN-α, IFN-γ, IL-1β, IL-6, IL-12 , IL-18, IL-33, TNF-α, TGF-β etc.), as well as some chemokines such as (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10 etc) produced by several immune effector cells produced by SARS-CoV infection.[33,34,35] For example in patients with severe MERS-CoV infection, an increase in the production of cytokines IL-6, IFN-α, CCL5, CCL8 and CXCL-10 in their serum compared with patients who have mild to moderate symptoms.[36] The production of pro-inflammatory cytokines mentioned above causes the patient's body to experience ARDS which can cause multiple organ failure (MOF) and ARDS until death. The presence of lymphopenia and cytokine storm may important role in COVID-19 pathogenesis. The Cytokine storm can initiate viral sepsis and inflammatory-induced lung injury which lead to other complications including pneumonitis, ARDS, respiratory failure, shock, multi-organ failure and potentially death.[17]

SARS-CoV and MERS-CoV use various strategies in order to survive against host immunity. Microbes have pathogen-associated molecular patterns (PAMPs) that can be recognized by pattern recognition receptors (PRRs). SARS-CoV and MERS-CoV can induce the production of double membrane vesicles containing little PRRs and can replicate in these vesicles, so that SARS-CoV dsRNA can avoid detection of the host immune system.[37] Hosts can produce IFN-α and IFN-β which can protect the effects of SARS-CoV infection.[38,39] Another corona virus, MERS-CoV, has a protein accessory molecule 4a that can inhibit MDA5 activation to induce IFN production by interacting directly with dsRNA.[40] MERS-CoV also has ORF4a, ORF4b, ORF5 and viral protein membranes that can inhibit the transport of IFN regulatory factor 3 (IRF3) and inhibit the activation of IFN-β promoters.[41] MERS CoV can also hold genes that play a role in the presentation of Ag viruses.[42]

4. CONCLUSION

Specific studies on the immune response to SARS-CoV-2 are still developing. Various study approaches can be carried out by looking at the clinical manifestations that emerge, the results of examinations and literature review from their predecessors, namely SARS-CoV and MERS-CoV. So far, it can be concluded that molecular studies related to SARS-CoV-2 immunopathology states that SARS-CoV-2 increases pro-inflammatory cytokine production, suppresses IFN, increases neutrophil count, increases Th1/Th17 expression, B cell response, and CD8+ and CD4+ responses. Cytokine storms are known to be a trigger for serious respiratory problems and multi-organ damage that can cause death.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

ACKNOWLEDGEMENT

We would like thank to STIKES Rumah Sakit Anwar Medika, Jurnal Teknologi Laboratorium (Journal of Laboratory Technology), editor and reviewer for editing the manuscript.

FUNDING INFORMATION

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SHORT BIOGRAPHY

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Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 67 – 77
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.227
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Article Review

Indirect exposure to novel coronavirus (SARS-CoV-2): an overview of current knowledge

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HIGHLIGHTS

- People may get infected from contact with surfaces, wastewater and air
- The virus can be transported in the air up to 6 m from the source depending on wind speed

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ARTICLE INFO

Article history
Received Date: April 02nd, 2020
Revised Date: May 27th, 2020
Accepted Date: June 06th, 2020

Keywords
Air pollution
COVID-19
Transmission
Wastewater

ABSTRACT

This review chronicles the indirect transmission method which seems to be overlooked by most people and makes attempts to document the various transmission ways with a hope that such information may strengthen the knowledge base of researchers towards methods of eradicating the pandemic. Current knowledge of transmission and exposure of SARS-CoV-2 has been explained. Various researchers have put forward different ways of exposure and transmission. Literature does not reveal whether the indirect transmission route is the dominant one. However, total lockdown could be a veritable means to reduce both direct and indirect transmission routes. In many countries where the indirect transmission has been reduced, the scourge of the virus is less. The work creates awareness on the need to watch out for those routes of transmissions that may not be popular and suggested vital knowledge gaps that need to fill.

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1. INTRODUCTION

The global pandemic of coronavirus disease 2019 (COVID-19) that is ongoing is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is currently a public health emergency of international concern (WHO, 2020). As of April 21st, 2020, data showed that there are two million, four hundred and ninety thousand, five hundred and sixteen (2,490,516) cases of SARS-CoV-2 infection in 215 countries with the death of one hundred and seventy thousand, five hundred and ninety (170,590) (death rate 6.8 %).[1] This trend is on the continuous increase daily as more cases are being recorded, despite the global efforts to stop the outbreak. This, therefore, is responsible for the global lockdown or movement restriction and thus the global economy is being affected.[2] Other efforts to stop the spread involve encouraging proper hygiene which includes, hand washing, social distancing and other personal hygiene directives such as avoid face touching especially the eyes, nose and mouth.[3]

Generally, three possible transmission routes of COVID-19 by the patient to people have been identified including symptomatic, pre-symptomatic and asymptomatic transmission. By way of definition, a symptomatic COVID-19 case is a case that has developed signs and symptoms compatible with COVID-19 virus infection. The pre-symptomatic transmission occurs during the incubation period (5-14 days) for COVID-19, i.e. between the infected time to time when symptoms begin to manifest while the asymptomatic transmission is an infected person without showing any symptoms but transmits the virus to another person. However, to date, there are no reports of asymptomatic transmission, but few documents of laboratory-confirmed cases exist (WHO, 2020). The reported symptoms of COVID-19 patients include cough, fever, difficulty in breathing and diarrhoea. SARS-CoV-2 ribonucleic acid (RNA) has been detected in faeces of not only symptomatic but also asymptomatic patients and on surfaces, in aerosols and wastewater.[4,5,6,7,8,9,10] In other to document clearly the various ways of transmission and exposure for this new virus, we as a result of this present an overview on the current knowledge on the sources of the exposure of human to COVID-19. This will provide an overall quick, concise yet precise reference material with both scholars and public office holders for public enlightenment and could assist policymakers in formulating policies that would help reduce the spread of this virus.

2. REVIEW METHOD

Materials such as preprint servers, statistical bulletins, blogs, journals articles and conference/workshop/seminar papers that were published online were used for sourcing information. In other to maintain our idea of current information, we focused on publications within March and April 2020. All searches were restricted to articles from articles written in English and focused mainly on SARS-CoV-2, and few other articles searched with the following keyword "coronaviruses", "microplastics" "nano plastics" and "airborne particulates". Information not backed by empirical data was avoided, and interviews were completely left out. Overall, 5 preprint (not peer-reviewed) papers included were Zhang et al.,(2020)[5]; Wu et al., (2020)[11]; Santarpia et al., (2020)[12]; Enyoh et al., (2020)[3] and Dipak et al., (2020)[13].

3. RESULTS AND DISCUSSION

Indirect exposure routes to SARS-CoV-2

Preventing the transmission in the family settings, public and healthcare systems is of importance to prevent any further spread of the virus. Two possible routes of exposure include the direct or indirect transmission of the virus from an infected person to an uninfected person or a new host.

Direct exposure: Direct exposure is one of the major routes of transmission of coronavirus from person-to-person. This has been described both in hospitals where medical personnel contact the diseases from patient or healthy person contacting the disease from an infected medical personnel. In this case, it could be said to be a nosocomial infection. Another typical direct exposure route involves family settings.[14] Here, a member of the family transmits the virus to one or other members of the same family.

Indirect exposure: Indirect viral transmission involves an uninfected person coming in contact either by hand or with a contaminated surface. Transmission of coronaviruses from contaminated dry has been demonstrated and reviewed.[15] Another indirect route could be from inhaling contaminated air or ingesting, swallowing, and/or breathing in untreated wastewater spray or mist. The schematic below emphasizes the indirect exposure routes to SARS-CoV-2 based on current information are presented in Figure 1.

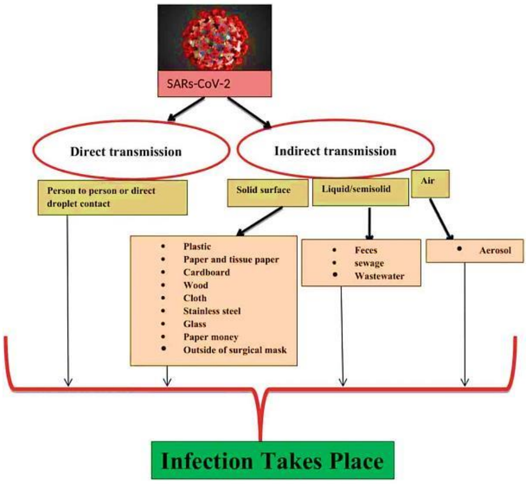

Figure 1. Overview of plausible exposure pathway to SARS-CoV-2 based on current information

Solid surfaces/fomites

This route is based on contact transmission when viral particles emitted from the respiratory tract of an infected individual are deposited on a solid surface such as plastic, paper and tissue paper, cardboard, wood, cloth, stainless steel, glass, paper money and outside of surgical masks. Then the virus can infect another person when the person comes in contact with the contaminated surface by hand and then touches their nose, mouth or eyes, then sneaks into the body via the mucous membranes. The survival and stability of SARS-CoV-2 on surfaces has been shown in some reports, in both laboratory and natural settings. These studies are summarized in Table 1. Viral particles found on surfaces such as toilets, and frequently touched surfaces at the University of Nebraska Medical Center.[12] Similarly, on March 26th, the CDC (Center for Disease Control) published a report on the coronavirus-stricken Diamond Princess Cruise ship in which they found traces of RNA from SARS-CoV-2 on surfaces throughout the cruise ship, in the cabins of both symptomatic and asymptomatic infected passengers, up to 17 days later.[16]

In hospital settings, there will be high risks of infection by contact with surfaces especially in low and middle-income countries (LMICs) with inadequate health care systems[13], where patient's documentation is mostly done using paper-based records. Most of the hospitals in LMICs are using paper to keep patient records, owing to poor funding and lack of proper databases. Each record file is exchanged many times between health care workers, patient caretakers as well as various administrative people in the hospital. These paper files and records also it travels to different wards or rooms for various purposes along with patients during medical check-up and treatment. The virus could be transmitted during this process. At present, very little is known regarding the surface distribution of SARS-CoV-2 in hospitals of LMICs which rely on paper documentation of patients. However, this pathway of infection may also occur in public places. In other studies, it was indicated that surfaces in general hospital wards and intensive care units are infectious, of which the highest rates were for computer mice, followed by trash cans, sickbed handrails and doorknobs.[17,18] The further study revealed that sleeve cuffs and gloves of medical staff presented significant positive results for the virus.[19] Due to these findings, encouraged proper hygiene such as proper handwashing or sanitizing be practised by medical staff immediately after patient contact.[17]

Table 1. Persistence of novel coronavirus on surfaces at different conditions

Surface	**Strain/ isolate**	**Inoculum (Viral Titer in $TCID_{50}$ per mL)**	**Temperature (ºC)**	**RH (%)**	**Persistence**	**Reference**
Plastics	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1)	$10^{5.25}$	21 to 23	40	≤ 3 days	Neeltje et al., 2020
	N/A	$10^{7.8}$	22	65	4 days	Chin et. al., 2020
Paper and tissue paper	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1)	$10^{5.25}$	21 to 23	40	3 hours	Neeltje et. al., 2020
	N/A	$10^{7.8}$	22	65	< 1 hour	Chin et al., 2020
Cardboard	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1)	$10^{5.25}$	21 to 23	40	24 hours	Neeltje et al., 2020
Copper	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1)	$10^{5.25}$	21 to 23	40	≤ 3 days	Neeltje et al., 2020
Stainless steel	N/A	$10^{7.8}$	22	65	4 days	Chin et al., 2020
	SARS-CoV-2 nCoV-WA1-2020 (MN985325.1)	$10^{5.25}$	21 to 23	40	4 hours	Neeltje et al., 2020
Wood	N/A	N/A	N/A	N/A	4 days	WebMD Medical Reference (2020)[a]
	N/A	$10^{7.8}$	22	65	< 2 days	Chin et al., 2020
Ceramics	N/A	N/A	N/A	N/A	≤ 5 days	WebMD Medical Reference (2020)[a]
Aluminium					2 ≤ 8 hours	
Glass	N/A	N/A	N/A	N/A	≤ 5 days	WebMD Medical Reference (2020)[a]
	N/A	$10^{7.8}$	22	65	2 days	Chin et al., 2020

Metal	N/A	N/A	N/A	N/A	≤ 5 days	WebMD Medical Reference (2020)[a]
Cloth	N/A	$10^{7.8}$	22	65	< 2 days	Chin et al., 2020
Banknote	N/A	$10^{7.8}$	22	65	2 days	Chin et al., 2020
Masks	N/A	$10^{7.8}$	22	65	4 days (inner layer) and 7 days (outside layer)	Chin et al., 2020

RH: Relative Humidity; N/A: Not available. A peer-reviewed medical web, but did not make the used references visible.

Liquid and/or semisolid

Feces/stool

According to the World Health Organization[20], a significant proportion (16 – 73%) of patients with SARS tend to have diarrhoea in addition to respiratory symptoms. As one of the symptoms of COVID-19 is diarrhoea, the patient tends to excrete faeces/stool often. Recent reports show that SARS-CoV-2 has been detected in stool samples of COVID-19 patients[21,22,14,8,23,24] and for more than nine days after the patients recovered.[4]

The shedding of SARS-CoV-2 was studied in a cluster of 9 cases and was 10000000 RNA copies/g faeces one week after symptom onset and decreased to 1000 RNA copies/g three weeks after symptom onset.[10] In another study, reported up to 1000000 viral particles per mL in a single faecal sample.[5] Although these studies did not evaluate the replication in culture, they, revealed the likelihood of infection through contact with contaminated stool or faeces, even though, there are currently no reports of infection through this process. Further, because the virus is detectable in the faeces just like the previous coronaviruses.[25] Therefore aerosolization of the virus in contaminated faeces during toilet flushing was believed to be the mode of transmission of this outbreak similar to the previous coronaviruses.[26]

Wastewater

By adopting a wastewater - based epidemiology (WBE) approach, the prevalence of viruses in a particular wastewater treatment plant (WWTP) catchment population was understood by researchers because wastewater contains viruses excreted from symptomatic and asymptomatic individuals in a catchment.[27,6] The WBE approach is essential in predicting and providing early warning for a potential outbreak of disease. Therefore, informing the efficacy of public health interventions, as previously demonstrated for enteric viruses, such as norovirus, hepatitis A virus, and poliovirus.[28,29] The viruses mentioned above are, however, not enveloped like SARS-CoV-2 virus and may not behave exactly alike in wastewater. Recent studies have shown that SARS-CoV-2 is present in untreated wastewater suggesting a potential, significant source or route of infection to the people of most community.[22] The presence of SARS-CoV-2 in untreated wastewater is linked to the stool of an infected person passed into the sewer system. In stool samples with high RNA copies, viable SARS-CoV-2 was detected.[21] Although it is unlikely that wastewater will become a critical transmission pathway for SARS-CoV-2, increasing the circulation of the virus in the population will increase the virus load into the sewer systems of our cities.[30]

The N_Sarbeco and NIID_2019-nCOV_N assays were used by Ahmed in their study to detect SARS-CoV-2, and subsequently sequencing the RT-qPCR (quantitative reverse transcription-polymerase chain reaction) products recorded to avoid false results due to wastewater being a complex matrix.[6] Results showed that 22.2 % (2 out of 9) of wastewater samples analyzed were positive for SARS-CoV-2 using the N_Sarbeco assay while NIID_2019-nCOV_N assay failed to detect. Prevalence in the wastewater based on excreted human faeces was estimated according to Eq. (1). Results suggest a median SARS-CoV-2 infection of 0.096 %

in the catchment basin during the study period. By implication, the clinical prevalence would be equivalent to 450 cases in the catchment, but the upper bound of the 95 % confidence interval around the median would suggest up to 764 total cases to 314 undiagnosed cases or roughly seven undiagnosed infections for every ten diagnosed infections.

$$Infected\ person = \frac{\left({}^{RNA\ copies}/_{Wws\ (L)}\right)*\left({}^{Wwt\ (L)}/_{day}\right)}{\left({}^{g\ feces}/_{person-day}\right)*\left({}^{RNA\ copies}/_{\ faeces}\right)} \quad (1)$$

Note: Wws = wastewater sample in liters; Wwt = total wastewater collected per day in liters.

By use of PEG 8000 (polyethylene glycol) concentration method, Wu et al., (2020) were able to recover SARS-CoV-2 successfully from wastewater. Following a 24h flow-dependent composite sample, the authors assayed using CDC N1, N2, and N3 primers with a virus concentration of ~ 10 to 240 Copies 40 mL^{-1}, ~ 40 to 140 Copies 40 mL^{-1} and ~ 10 to 160 Copies 40 mL^{-1}. The prevalence rate was estimated to range from 0.000001 to 0.000035 %. Similarly, Medema (2020) followed a 24h flow-dependent composite sampling technique in collecting tested sewage samples of 7 cities in the Netherland.[30] With the use of RT-PCR against three fragments of the nucleocapsid protein gene (N_{1-3}) and one fragment of the envelope protein gene (E), the samples were analyzed. No SARS-CoV-2 detected in samples with N_2 throughout the study period while others had a detection rate of 58.3, 33.3 and 20.8% respectively. The prevalence rate was estimated to range from 0.000001 to 0.000035% (Table 2).

Overall, the information provided by these studies shows that not only there is a risk to sewage workers, but also the circulating SARS-CoV-2 in the communities can be monitored through sewage surveillance. They were thereby complementing the limited current clinical surveillance done in many world countries (e.g. mostly low and middle-income countries), where tests are mainly done when there are severe symptoms. Furthermore, it could be used as an early warning tool for increased circulation in the rainy period on unaffected populations. However, there is no evidence to date that SARS-CoV-2 has been transmitted via sewerage systems, with or without wastewater treatment. We, therefore, recommend that future wastewater sampling efforts and analysis adhere to already established safety procedures to curb the potential spread.

Air

The transmission of SARS-CoV-2 in the air is aided by aerosols as people emit aerosol particles when talking, and that louder speech volumes correlate to more aerosol particles being emitted. The transportation of aerosols is largely depended on environmental factors such as airflow and direction, humidity, ambient temperature, wind speed and gravity as well as landscape and densities of buildings.[31,32,33,34] Recent studies have demonstrated this scenario of SARS-CoV-2 surviving in aerosols.[35,17] Neeltje (2020) used the SARS-CoV-2 nCoV-WA1-2020 (MN985325.1) strain in their study and compared the stability in aerosols (< 5 µm).[35] Using a three-jet nebulizer, the aerosol was filled with viruses with 50% tissue-culture infectious dose; $TCID_{50}$ mL^{-1} of $10^{5.25}$. The decay rate was determined to measure stability at different environmental conditions such as temperature (21 to 23 °C) and relative humidity of 40 %. Results showed that SARS-CoV-2 was stable and remained throughout the period of study (three hours). In a hospital setting, Zhen-Dong (2020) collected air samples in intensive care units (12 air supplies and 16 air discharges hour $^{-1}$) and general wards (8 air supplies and 12 air discharges $hour^{-1}$).[17] Results from the study showed that 35 % and 12.5 % of the air samples were contaminated respectively, indicating that the virus can travel in the air. Overall, the aerosol mainly concentrated downstream up to 13 feet from the source while smaller quantities were found up to 8 feet upstream with the maximum transmission of ~ 4 meters from the source. However, there are limitations to the study.[17] First, the results of the nucleic acid test do not indicate the amount of viable virus. Second, for the unknown minimal infectious dose, the aerosol transmission distance cannot be strictly determined. Although we have warning signs, there is still a lack of evidence of transmission via aerosols as the minimal viral load is unknown. These studies (Neeltje, 2020 and Zhen-Dong**,** 2020) did not fully mimic real-life outdoor scenario considering many other environmental factors.[35,17]

Following this need for a deeper understanding of the fluid and particles transport emanating from our respiratory tracts from coughing, sneezing, speaking, or breathing, Dbouk and Dimitris (2020) using an advanced three-dimensional model was able to study the phenomenon.[36] The applied model which is based on fully coupled Eulerian-Lagrangian techniques taking the flowing factors into account including droplet phase-change, relative humidity, evaporation, turbulent dispersion forces, and breakup in addition to the interactions of droplet–droplet and droplet–air. Results from their study showed that saliva-disease-carrier droplets from a mild human cough at the temperature of 20°C, relative humidity of 50% and at wind speeds which vary from 4 to 15 km/h could travel up to 6 meters (farther than the current recommended 2 meters distance between people). However, at a wind speed of 0 km/h, the droplets could not travel 2 meters (current recommended distance between people).

Reviewed studies have confirmed that SARS-CoV-2 aerosol exposure poses significant risks. Therefore, there is a need for more studies on indoor and outdoor environments where many environmental factors are in play such as the presence of airborne microplastics and other particulates in the ambient environment that may aid in the transmission of the virus over longer distances (> 4 m). While indoor activities are ongoing, whether in the hospital or household setting, large volume/quantity of small size plastics (microplastics or nano plastics) are often generated and deposited (could be as high as > 11, 000 particles day^{-1} m_{2}^{-1}) on ground surfaces.[33]

Virus emitted from a patient can drop on the surface of these particles and can be accommodated up to 3 days if the room isn't cleaned properly. During the period of accommodation, the particles can be re-suspended in the air with dust particles when there is a disruption of indoor air, thereby putting inhabitant at risks of infection when these plastic particles (with the virus) are inhaled. Following a simulated study, MPs particles as much as 272 particles can be inhaled by inhaling indoor air.[37] In the outdoor environment, the process is similar except that the virus may potentially be transported with particles over long distances depending on the wind events, relative humidity, ambient temperature and anthropogenic activities as well as particle shape, size and length.[3]

4. CONCLUSION

SARS-CoV-2 can be transmitted indirectly through contact with solid surfaces or fomites, liquid or semisolids, including wastewater and faeces as well as through air (aerosol). Although reports have not shown any case transmitted through these pathways, the likelihood is high as suggested by available data. The survival and stability of SARS-CoV-2 on surfaces is largely depended on environmental factor such as relative humidity, temperature and pH as well as the surface morphology. Smooth surfaces will increase the chances of survival by becoming more stable. Wastewater Based Epidemiology (WBE) may be an effective tool in monitoring outbreaks of this virus, and there is a need to treat wastewater before disposing of in the environment. The primer-probe used in wastewater studies included N_Sarbeco, E_Sarbeco, NIID_2019-nCOV_N, and CDC ($\text{N}_{1\text{-}3}$) of which CDC N_2 and NIID_2019-nCOV_N have poor detection rate. The virus can be transported in the air up to 4 m from the source (at current, in the confined environment) and human saliva-disease-carrier droplets may travel up to 6 m, depending on the wind speed. Therefore there is a need to review or re-evaluate the current guideline of generally keeping 2 m distance between people (both in confined and open places) as recommended by WHO.

Further studies should focus on the contribution of airborne plastic particles such as MPs and NPs on the survival and transport of the virus in the air over long distances. Studies should also be conducted on the survival of the virus food produce as previous coronaviruses have been shown to survive on lettuce at low temperature. Studies should also be conducted on the survival of the virus in drinking water as previous coronaviruses have been shown to survive. As people died from COVID-19, they are buried in the soil. So, the potential impact on groundwater should be studied. These studies would inform on the extent of risks of exposure and damage this unwanted virus would cause to the public and ecosystems health.

DISCLOSURE STATEMENT

The authors reported no potential conflict of interest. This paper was published on the preprint server (10.20944/preprints202004.0460.v1).

ACKNOWLEDGEMENT

We acknowledge the efforts of frontline health workers and scientists helping to combat the COVID-19pandemic.

FUNDING INFORMATION

This work was unfunded.

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Table 2. Available data on the detection of SARS-CoV-2 in wastewater samples

Location	**Sampling date**	**Sampling technique**	**Assay used**	**Detection rate (%)**	**Virus concentration (^{a}Copies/100 mL ^{b}Copies/40 mL)**	**Prevalence rate (%)**	**References**
Southeast Queensland, Australia	24/02/20 to 01/04/20	Composite grab sample and autosampler (conventional refrigerated and/or submersible in-situ high-frequency autosampler)	N_Sarbeco	22.2	Approx. 1.90 to 12.0^a	0.096	Ahmed et. al., 2020
			NIID_2019-nCOV_N	0	ND	N/A	
Massachusetts, USA	18/03/20 to 25/03/20	24h flow-dependent composite sample	CDC N_1	N/A	Approx. 10 to 240^b	0.026	Wu et. al., 2020
			CDC N_2	N/A	Approx. 40 to 140^b	0.026	
			CDC N_3	N/A	Approx. 10 to 160^b	0.026	
7 locations in Netherlands	5/02/20 to 5/03/20	24h flow-dependent composite sample	CDC N_1	58.3	N/A	0.000001	Medema et al., 2020
			CDC N_2	0	N/A	N/A	
			CDC N_3	33.3	N/A	0.000035	
			E_Sarbeco	20.8	N/A	0.000035	

*Primer probe were CDC N_1, N_2, N_3 = nucleocapsid protein gene ($N_{1\text{-}3}$) (USA); Sarbeco N = envelope protein gene (N) and E = envelope protein gene (E); NIID_2019-nCOV_N (Japan)
*N/A = Not available; ND = No data

Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 78 – 86
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.232
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Original Research

Comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM and IgG antibody test for the diagnosis of SARS-CoV-2 infection

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HIGHLIGHTS

Instead of using the tests as an alternative to each other, using them as complementary will be indispensable for definitive diagnosis.

ARTICLE INFO

Article history
Received Date: July 29th, 2020
Revised Date: August 01st, 2020
Accepted Date: August 04th, 2020

Keywords
SARS-CoV-2
RT-PCR
IgM antibody test
IgG antibody test

ABSTRACT

In the present study, we compared the real-time reverse-transcription polymerase chain reaction (RT-PCR) and total IgM-IgG antibody tests for diagnosis SARS-CoV-2 patients. SARS-CoV-2 patients were divided into four additional subgroups according to clinical examination, and Computed Tomography (CT) for SARS-CoV-2. Groups were included 60 mild cases, 111 moderate cases, 53 severe cases and 105 normal cases. In a mild group, 52.5% of 60 cases were found to be male, 45.9% female, and the average age was found as 38.4 ±2.011. The positive ratio was found as 80.3% in the RT-PCR test, while 39.3% in total IgM/IgG. In a moderate group, 49.1% of 111 cases were found to be male, 50% female, and the average age was 45.05 ±1.519. The positive ratio was found as 85.7% in the RT-PCR test, while 54.5% in total IgM/IgG. In a severe group, 53.7%of 53 cases were found to be male, 44.4% female, and the average age was 55.5±2.122. The positive ratio was found as 75.9% both in RT-PCR and total IgM/IgG test. In a normal group with no involvement according to Computed Tomography (CT), 49.1%of 105 cases were found to be male, 50% female, and the average age was found as 34.8±1.391. The positive ratio was found as 95.3% in the RT-PCR test, while 5.7% in total IgM/IgG. Chronic diseases were detected more in severe cases, suggesting that persons who have chronic diseases or decreased immunity, such as diabetes mellitus, cardiovascular diseases, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2. According to our results, we can suggest a higher detection sensibility in RT-PCR than in total IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT.

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1. INTRODUCTION

The new coronavirus (SARS-CoV-2) spread rapidly all over the world and caused coronavirus disease (COVID-19) in infected people after its appearance in Wuhan, China, in December 2019. The World Health Organization identified COVID-19 (2019 coronavirus disease) disease. COVID-19 caused an epidemic and led to a major challenge for health systems. For reducing the risk of spread, it should be investigated and developed effective treatment and diagnostic options.[1] The signs and symptoms of SARS-CoV-2 infection are known to be not specific; most are found to be associated with respiratory complications such as cough dyspnea, and viral pneumonia. However, the mortality of critical patients with SARS-CoV-2 pneumonia is also noteworthy.[1,2] For this reason, specific COVID-19 diagnostic tests are necessary in order to confirm suspected cases.[3,4]

In order to diagnose SARS-CoV-2 infection, the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test, which detects the presence of viral RNA, is used. This molecular test is known to be useful in the first three weeks of infection and is currently the WHO recommended reference standard.[5] RT-PCR tests were known to be based on the RNA-dependent RNA polymerase (RdRp) gene of the ORF1ab sequence, E gene, N gene, and S gene of the SARS-CoV-2 genome.[6] Among these tests, RT-PCR analyses targeting the RdRp test has the highest analytical precision.[4]

To determine epidemiological surveillance, the immunological tests can be thought of as supplementary diagnostic aid and important support. These tests are based on the detection of immunoglobulin IgM and IgG seen in the second week of infection against SARS-CoV-2.[5] Rapid serological tests based on the detection of antibodies in venous and capillary blood give results in a few minutes. Nevertheless, the sensitivity of the test depends on the timing when the sample was taken and can be more than 90% since the second week of symptom beginning.[7] The use of these tests may contribute significantly to improve the accuracy of the clinical diagnosis, especially in hospitalized patients with negative molecular test results and in patients who have just undergone RT-PCR.[5,7]

In this study, we aim to evaluate the rapid serological test for detection of IgM and IgG antibodies, by means of comparing its additional diagnostic performance with the one of the RT-PCR, for detecting SARS-CoV-2 infections under field conditions.

2. MATERIAL AND METHOD

In this study, 329 patients diagnosed with SARS-CoV-2 in Siirt training and research hospital (Siirt University, Turkey) from March 15th to July 01st, 2020, were included as the case group. To diagnose patients, pneumonia diagnosis protocol for novel coronavirus infection was followed, subjected to the tests including clinical examination, Computed Tomography (CT) and RT-PCR for SARS-CoV-2. SARS-CoV-2 patients were divided into four additional subgroups according to CT results. Groups were included 60 mild cases with unilateral pulmonary involvement (32 males and 28 females, median age were 34 [17-77]), 111 moderate cases with bilateral pulmonary involvement (55 males and 56 females, median age were 43 [19-99], 53 severe cases with bilateral diffuse involvement (28 males and 24 females, median age was 55 [20-92]) and 105 normal cases with no involvement according to CT (52 males and 53 females, median age was 32 [17-84]).

Data on biochemical parameters were obtained from 329 confirmed SARS-CoV-2 infection patients, validated by a wide range of studies including clinical examination, laboratory tests, and

RT-PCR for SARS-CoV-2, with SARS-CoV-2 RdRpq PCR detection kit (Bioeksen, İstanbul, Turkey), as well as using a SARS-CoV-2 IgM/IgG detection kit (Colloidal Gold, China). Clinical and laboratory information was collected during routine clinical studies, and this study was approved by the Siirt University Non-Interventional Clinical Research Ethics Committee (Decision No: E-5739).

SPSS software version 22.0 was used for statistical analysis. All quantitative data in non-normal or unknown distribution were expressed as median and interquartile range $p<0.05$ was defined as statistically significant in all tests.

3. RESULTS AND DISCUSSION

In the present study, we compared the real-time reverse-transcription polymerase chain reaction (RT-PCR) and total IgM-IgG antibody tests for diagnosis SARS-CoV-2 patients. SARS-CoV-2 patients were divided into four additional subgroups according to clinical examination, and Computed Tomography (CT) for SARS-CoV-2. Groups were included 60 mild cases (32 males and 28 females, median age were 34 [17-77]), 111 moderate cases (55 males and 56 females, median age were 43 [19-99]), 53 severe cases (28 males and 24 females, median age were 55 [20-92]) and 105 normal cases (52 males and 53 females, median age were 32 [17-84]). Liu et al. 2020 reported that there were 44 moderate cases, 52 severe cases, and 37 critical cases with no significant difference in gender and age among three subgroups. According to our results, there is no significant difference of gender, whereas the age of severe (55.5) and moderate (45.05) group's means higher than mild (38.4) and normal (34.8) group's means.

Table 1 shows the comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and IgM-IgG antibody test for the diagnosis of SARS-CoV-2 infection. In mild group, 52.5% (n=32) of 60 cases were found to be male, 45.9% (n=28) female, and the average age was found as 38.4 ±2.011. The positive ratio was found as 80.3% (n=49) in RT-PCR test, while 39.3% (n=24) in total IgM/IgG. In moderate group, 49.1%(n=55) of 111 cases were found to be male, 50%(n=56) male, and the average age was 45.05 ±1.519. The positive ratio was found as 85.7%(n=96) in RT-PCR test, while 54.5% (n=61) in total IgM/IgG antibody test. In severe group,53.7%(n=29) of 53 cases were found to be male, 44.4%(n=24) female and the average age was 55.5±2.122. The positive ratio was found as 75.9%(n=41) both in the RT-PCR test and total IgM/IgG antibody test. In normal group with no involvement according to CT, 49.1%(n=52) of 105 cases were found to be male, 50%(n=53) female, and the average age was found as 34.8±1.391. The positive ratio was found as 95.3%(n=101) in the RT-PCR test, while 5.7%(n=6) in IgM/IgG antibody test. According to the results we obtained from this study, we can suggest a higher detection sensibility in RT-PCR than in total IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT(Figure 1).

In recent, Liu et al.,[1] studied in RT-PCR detection for viral RNA in 133 patients the instead of an infected with SARS-CoV-2. They reported the positive ratio was 65.91% in moderate cases, 71.15% in severe cases and 67.57% in critical cases, respectively. In our study, according to RT-PCR detection results for viral RNA in 329 patients infected, the positive ratio was detected as 80.3% in mild cases, 85.7% (n=96) in moderate cases, 75.9%(n=41) in severe cases and 95.3%(n=101) in normal cases (Table 1). In our study, the positive ratio of RT-PCR was higher than in their study. RT-PCR test is known to be useful in the first three weeks of infection and is currently the WHO recommended reference standard.[5] It is known that RT-PCR analyses targeting the RdRp test have the highest analytical precision.[4]

Liu et al.,[1] reported that the positive ratio of IgM/IgG antibody detection in patients was 79.55%/93.18%, 82.69%/100%, and 72.97%/97.30% in moderate, severe, and critical cases, respectively. In this study, we measured the positive ratio of total IgM/IgG antibody in patients. We found the positive ratio of total IgM/IgG antibody as 39.3% (n=24) in mild cases, 54.5% (n=61) in moderate cases, 75.9%(n=41) in severe cases and 5.7%(n=6) in normal cases. Supplement serum IgM / IgG antibody detection against SARS-CoV-2 internal nucleoprotein (NP) and surface spike protein receptor binding site (RBD) may compensate for RT-PCR deficiencies in some cases.[6,8] After infection with the virus, a humoral immune response produces the antibody. In general, IgM antibodies rise a few days after a viral infection and can be detected after a week of incubation, and IgG antibodies are known to appear in the middle and late stages of infection. There is a continuous increase known in the antibody titter and remains in the bloodstream for a long time. The supplementary antibody test can make up for the missed diagnosis of RT-PCR. However, it still cannot diagnose all infected patients.[6] The IgM and IgG testing, in combination, are known to be important to improve the clinical sensitivity of early COVID-19 diagnosis. Certainly, the detection sensitivity was confirmed to be higher in the IgG-IgM combined antibody test than in the individual IgG or IgM antibody test.[9] In our study, we found that the positive ratio of total IgM/IgG antibody detection in severe cases was 75.9% and in normal cases was 5.7%. Wang et al.,[6] stated that the detection of IgM and IgG antibodies could only achieve a sensitivity of 70% at 4 up to 6 days after admission for COVID-19 patients. Vidal-Anzardo et al.,[5] stated that the rapid serological tests were useful as a complementary test to PCR, especially during the second and third week of illness as well.

Since COVID-19 disease symptoms are not specific enough to diagnose, NAT (nucleic acid tests) to directly detect the targeted pathogen are applied as standard. However, high false negativity rates have been reported.[10,11] In addition, problems are encountered such as RT-PCR molecular tests take a long time, difficulties encountered in the application, the application only in reference laboratories, being expensive, requiring trained personnel, only detecting acute infection. CT was used in patients with COVID-19 suspected clinical signs to confirm RT-PCR tests. When only CT is used in the diagnosis of COVID-19, it can be confused with pneumonia caused by another viral infection agent. Serological tests - ELISA (Enzyme-dependent Immune Assay), in addition to NATs, are essential for monitoring surveillance studies. This is important in identifying both the sick and healed individuals and knowing the immune status of the community. However, serological tests are not suitable for the diagnosis of acute disease. When seropositivity occurs, SARS-CoV-2 antibodies and secreted antibodies for other Coronavirus infections may cross-react. Therefore, it has no value for early diagnosis. In clinical cases, it can only be valuable for determining immunity or useful in answering epidemiological questions.[12] Serological tests are not useful in diagnosing acute cases. Because, the first week of the disease, IgM and IgG antibody response is insufficient and only detected after about 6-15 days from the onset of the disease reaches the acceptable level.[13]

Table 2 shows the clinical features of SARS-CoV-2 patients. The most symptoms observed in mild group were cough (65.6%), respiratory distress (26.2%), malaise (45.9%), sore throat (18%) and headache (8.2%), in moderate group were cough (81.3%), malaise (32.1%), respiratory distress (30.4%), fewer (25.0%), sore throat (14.3%) and headache (8.9%), in severe group were respiratory distress (48.1%), malaise (40.7%), fewer (31.5%), headache and nausea/vomiting (13%),myalgia(25.9%), stomach pain(7.4%) and loss of taste/smell (3.7%) and in normal group were cough (61.3%), malaise(29.2%),respiratory distress (28.3%), fewer (17.9%) and headache and myalgia (10.4%).

It is known that the aged population (age above 65 years), and persons who have decreased immunity or chronic diseases, such as cardiovascular diseases, diabetes mellitus, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2. Therefore, to prevent the infection, they must take special precautions.[14] In our study, cardiovascular diseases were detected 4.9%(n=3) of mild, 5.4%(n=6) moderate, 7.4%(n=4) of severe and 0.9%(n=1) normal cases, pulmonary diseases were detected 3.6%(n=4) of moderate, 11.1%(n=6) of severe and 1.9%(n=2) of normal cases, diabetes mellitus were detected 6.6% (n=4) of mild, 8.9%(n=10) of moderate, 14.8%(n=8) of severe and 4.7%(n=5) of normal cases, cancer were detected 0.9%(n=1) of moderate, 3.7%(n=2) of severe and 0.9% of normal cases (n=1) and hypertension were detected 9.8% (n=6) of mild, 7.1% (n=8) of moderate, 13%(n=7) of severe and 2.8(n=3) of normal cases. In a recent study, Özüdoğru et al.,[15] reported that diabetes mellitus, cardiovascular diseases, pulmonary diseases and hypertension detected 15.4%, 5.8%, 13.5%, 17.3% of 52 SARS-CoV-2 patients, respectively. In another recent study, Yung et al.,[16] found that diabetes mellitus, cardiovascular diseases, pulmonary diseases and hypertension detected 22%, 5%, 8%, 17% of 52 SARS-CoV-2 patients, respectively. In recent studies, Guan et al.,[17] Zhang et al.,[18] Zhou et al.,[19] and Yen et al.,[20] also detected some chronic diseases in COVID-19 patients. In our study, chronic diseases were detected more in severe cases, suggesting that persons who have decreased immunity or chronic diseases, such as cardiovascular diseases, diabetes mellitus, hypertension, and lung disease, are at a higher risk for developing severe COVID-19 if they are infected with SARS-CoV-2.

4. CONCLUSION

In conclusion, according to the results that we obtained from this study, we can suggest a higher detection sensibility in RT-PCR than in IgM/IgG antibody test for mild, moderate and normal group, while the detection sensibility of IgM/IgG antibody increases in a severe group with diffuse bilateral involvement according to CT. The use of IgM/IgG antibody tests may contribute significantly to improve the accuracy of the clinical diagnosis, especially in hospitalized patients with negative molecular test results and in patients who have just undergone RT-PCR. Instead of using the tests as an alternative to each other, using them as complementary will be indispensable for definitive diagnosis. If it is necessary to diagnose with the IgG-IgM combined serological and/or molecular tests specifically for the patient's condition and to evaluate the CT findings together with the biomarkers to be detected from the patient's blood will help us get through this pandemic process best. The immediate priority for the diagnosis of COVID-19 is the development of nucleic acid and protein tests and their integration into multiplex panels over time. To improve surveillance efforts, to support patient-healing and vaccination studies, it is best to use test methods appropriate for the period of the disease and to verify with clinical findings.

DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

FUNDING INFORMATION

The authors declared that this case had received no financial support.

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SHORT BIOGRAPHY

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Table 1. Comparison of real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and total IgM-IgG antibody test for the diagnosis of SARS-CoV-2 infection

	Results				
	Mild (n=60)	**Moderate (n=111)**	**Severe (n=53)**	**Normal (n=105)**	**Total** (n=329)
IgM/IgG (+)	39.3% (n=24)	54.5%(n=61)	75.9%(n=41)	5.7%(n=6)	40%(n=132)
SARS-CoV-2 RNA (RT-PCR) (+)	80.3% (n=49)	85.7%(n=96)	75.9%(n=41)	95.3%(n=101)	87%(n=287)
Age-range	17-77 (SD:15.577)	19-99(SD:16.001)	20-92 (SD:15.452)	17-84(SD:14.251)	17-99 (SD:16.831)
Mean (age)	38.4(SE:2.011)	45.05 (SE:1.519)	55.5(SE:2.122)	34.8(SE:1.391)	42.25 (SE:0.928)
Median (age)	34	43	55	32	40
Male	52.5% (n=32)	49.1%(n=55)	53.7%(n=29)	49.1%(n=52)	50.15%(165)
Female	45.9% (n=28)	50%(n=56)	44.4%(n=24)	50%(n=53)	49.85%(164)

Table 2. Clinical characteristics of patients with COVID-19 pneumonia

Symptoms	**Mild (n=60)**	**Moderate (n=111)**	**Severe (n=53)**	**Normal (n=105)**	**Total (n=329)**
Fever	18%(n=11)	25.0% (n=28)	31.5%(n=17)	17.9%(n=19)	22.7% (n=75)
Cough	65.6% (n=40)	81.3% (n=91)	81.5%(n=4)	61.3%%(n=65)	72.7% (n=240)
Respiratory distress	26.2% (n=16)	30.4% (n=34)	48.1% (n=26)	28.3%(n=30)	32.1% (n=106)
Sore throat	18% (n=11)	14.3%(n=16)	3.7%(n=2)	17.9%(n=19)	14.5%(n=48)
Malaise	45.9% (n=28)	32.1% (n=36)	40.7% (n=22)	29.2% (n=31)	35.5%(n=117)
Headache	8.2%(n=5)	8.9%(n=10)	13.0%(n=7)	10.4%(n=11)	10%(n=33)
Nausea/vomiting	6.6%(n=4)	4.5%(n=5)	13.0%(n=7)	1.9%(n=2)	5.5% (n=18)
Diarrhea	6.6 (n=4)	2.7%(n=3)	7.4%(n=4)	2.8%(n=3)	4.2%(n=14)
Myalgia	14.8% (n=9)	14.3% (n=16)	25.9% (n=14)	10.4% (n=11	15.2%(n=50)
Stomach pain	3.3%(n=2)	2.7%(n=3)	7.4%(n=4)	3.8%(n=4)	3.9%(n=13)
Loss of taste/smell	1.6%(n=1)	0.9%(n=1)	3.7%(n=2)	1.9%(n=2)	1.8%(n=6)
Comorbidities					
Cardiovascular disease	4.9%(n=3)	5.4%(n=6)	7.4%(n=4)	0.9%(n=1)	4.2%(n=14)
pulmonary disease	0	3.6%(n=4)	11.1%(n=6)	1.9%(n=2)	3.6%(n=12)
Diabetes	6.6%(n=4)	8.9%(n=10)	14.8%(n=8)	4.7%(n=5)	8.2%(n=27)
Hypertension	9.8% (n=6)	7.1%(n=8)	13%(n=7)	2.8(n=3)	7.3%(n=24)
Cancer	0	0.9%(n=1)	3.7%(n=2)	0.9%(n=1	1.2%(n=4)

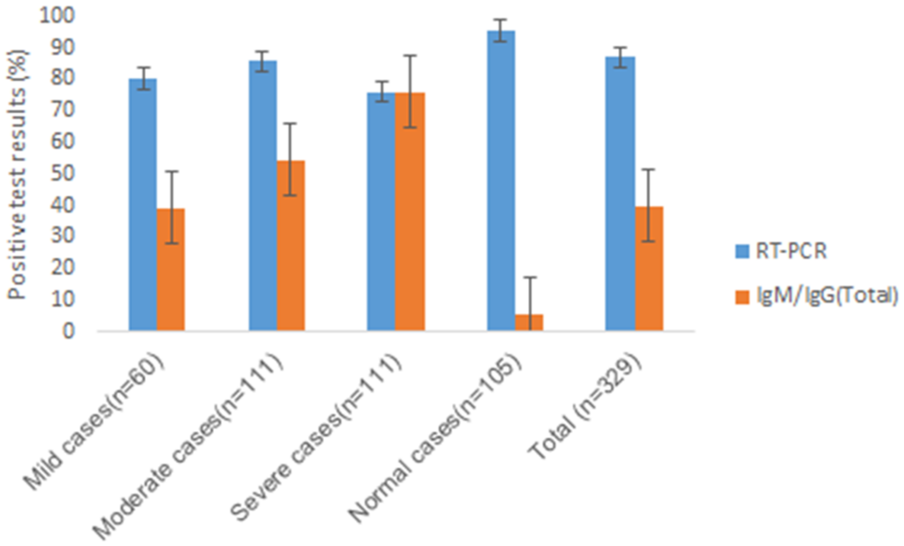

Figure 1. Positive percentages of RT-PCR and IgM-IgG tests by groups

Jurnal Teknologi Laboratorium
Vol.9, No.1, *Special Edition* 2020, pp. 103 – 114
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.29238/teknolabjournal.v9i1.230
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Article Review

On the novel coronavirus (COVID-19): a global pandemic

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HIGHLIGHTS

- COVID-19 has been declared a global health emergency.
- COVID-19 probably transmitted from bats or another host.
- Preventative measures are to be practiced.
- Efforts to develop and validate medications for COVID-19 are underway.

ARTICLE INFO

Article history
Received Date: July 27th, 2020
Revised Date: August 16th, 2020
Accepted Date: August 18th, 2020

Keywords
Zoonosis
COVID-19
Transmission
Respiratory Disease

ABSTRACT

Coronaviruses (COVs) are viruses transmitted through droplets of sputum from an infected person. Analyses identify COVs as zoonotic pathogens, possibly resulting from human-animal contact at animal markets. They share overlapping genetic characteristics with the avian influenza viruses from China. COVs released from humans through droplets of sputum and may land on various surfaces, which poses exposure risks; as studies have shown the virus can exist intact for a relatively long period of time (several days). The recent highly pathogenic COVs outbreak (COVID-19) emerged in Wuhan, China in 2019, include Severe Acute Respiratory Syndrome (SARs-COV). This highly transmittable disease causes pneumonia and other severe respiratory illnesses similar to SARS and MERS; it has a global mortality rate of about 6.13%. The virus has rapidly become a global pandemic, causing major global issues, including health, economic, and age-preference, among other issues. This text summarizes the nature of the emerging COVID-19 global pandemic while analyzing several factors concerning the etiology of the virus. This is done in an urgent effort to educate and provide relevant information about the virus.

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1. INTRODUCTION

Coronaviruses (COVs) belong to the Coronavirinae subfamily. COVs are single, positive stranded RNA viruses with genome ranging from 26 to 32 kilobases (kbs) in length. Corona means crown. The name, coronavirus, is derived from the appearance of the virion [1,2] which has a crown-like shape with spikes on the outer surface (Figure 1). Divided into low pathogenic (LP-COVs) and highly pathogenic (HP-COVs), six COVs are known to cause human diseases. LP-COVs are responsible for 10-30% of upper respiratory tract infections and caused minor respiratory diseases. Meanwhile, HP-COVs, which include Severe Acute Respiratory Syndrome (SARs-COV) and Middle East Respiratory Syndrome (MERS-COV), caused fatal pneumonia and infected lower respiratory airways. In 2002, SARs-COV appeared in China, infected 8,098 people, and caused 774 deaths.[3] This resulted in approximately a 9.6% mortality rate before it was controlled in 2003. The MERS-COV first appeared in Saudi Arabia; then, it surfaced in 26 other countries, and caused 2,494 reported cases. With 858 recorded deaths since 2012, MERS-COV had a 34.4% mortality rate.[4] Recently, a novel β-coronavirus (COVID-19) is shown to be highly transmittable and shares SARs-COV-like illness. Initial investigations showed that the virus emerged in Wuhan City, Hubei province in China (Table 1) in December 2019. As health officials were unable to contain the virus, it rapidly spread to all provinces in China and subsequently to multiple countries and territories (Figure 2). In late January 2020, the World Health Organization (WHO) declared the virus as a global pandemic.[5]

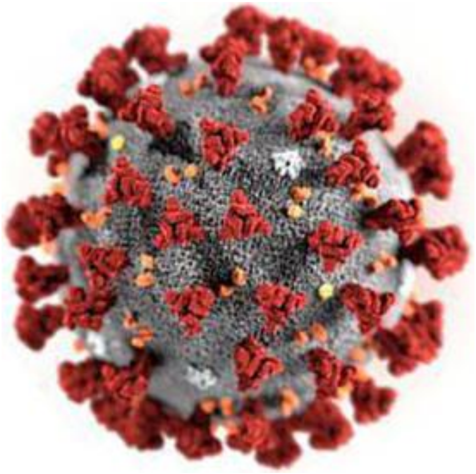

Figure 1. Microscopic illustration of the COVID-19 virion showing the spike ornaments on the outer surface.[1,2]

Many reports suggested that exposure to COVID-19 came from the Wuhan Seafood Market where wild animals are sold. Wild animals, such as bats, raccoons, snakes, bamboo rats, pangolins, and others, are suggested to be the original source of COVID-19. An intermediate source of origin and transfer to humans is not yet known. Altogether, these COVs are of serious concern to public health.[6] They lead to respiratory tract infections, resulting in pulmonary failure and fatality. In the case of COVID-19, the virus can survive and remain infectious for 2 hours to 9 days in various type of materials.[7,8] With an incubation period of 2-14 days after exposure (infection),[9,10] everyone is prone to the virus, while elderly individuals are more susceptible to experience severe illnesses, including death. Certain individuals are asymptomatic; they, along with individuals in which the virus is in the incubation period, are reported as the primary sources of infection and transmission. Transmission (direct and indirect)[8,11] can occur in various ways. This includes mucus and respiratory droplets from coughing and sneezing, which can all be suspended in air and on surfaces.

Table 1. A tabular comparison of coronaviruses outbreak outlying potential reservoirs, origins, and global data

	SARs-COV [3]	**MERS-COV** [4]	**COVID-19** [12]
Infected	8,098	2,494	6,057,853
Deaths	774	858	371,166
Mortality rate (%)	9.60	34.4	6.13
Potential Reservoirs	Bats; Civet cats	Dromedary camels	Bats; Pangolin
Date	Nov. 2002-July 2003	Sept. 2012-Nov. 2019	Dec. 2019-
Origin	Guangdong, China	Saudi Arabia	Wuhan, China
Affected countries	26	27	<120

All data presented in this text were collected from covidgraph.com and WHO Situation Report-133.[12] At the time of this writing, COVID-19, with a mortality rate of 6.13% – became a global pandemic affecting 6,057,853 individuals in more than 120 countries and territories (Figure 2). Currently, the United States (USA) has become the new epicenter of the virus with a mortality rate of about 5.48%.

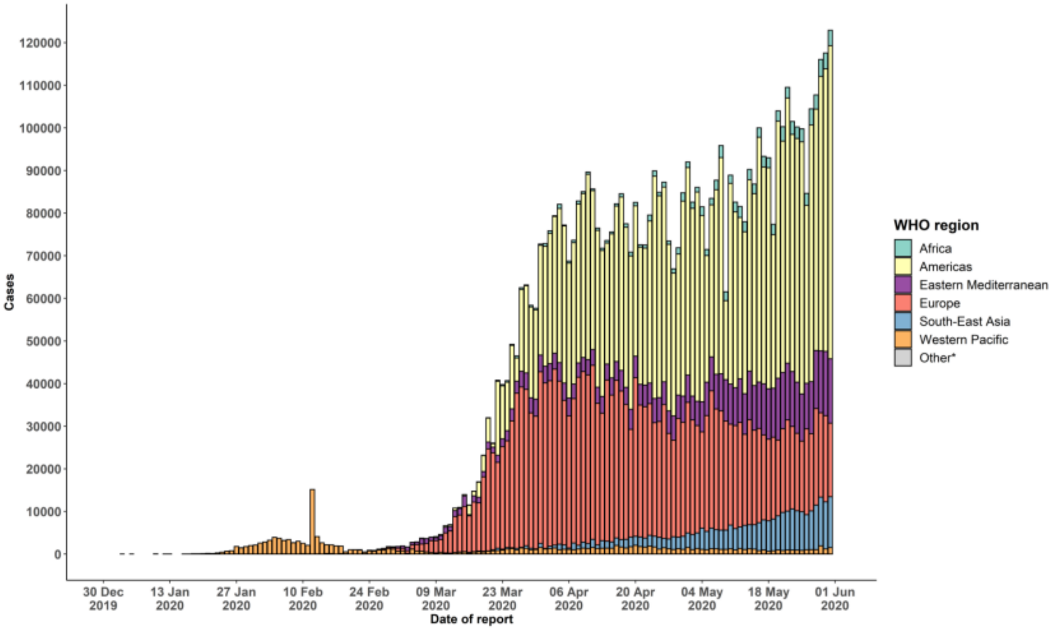

Figure 2. Geographical spread of COVID-19 showing reported cases as of 1 June 2020.[12]
For more details, see WHO COVID-19 Situation Report–133.

Among all reported case-fatality, New York, New Jersey, Illinois, California, and Massachusetts represent the top five states with the most fatalities. Altogether, the data shows that elderly individuals have a higher chance of infection. The mortality rate of COVID-19 (17-38%) is higher in elderly individuals with chronic diseases. Analyzing the data from New York state (Figure 3), the number of reported cases reached 375,133 and the death toll reached 24,133. Male represents 51.6% of the total cases while female represents 48.4%, however the death toll is much higher among male (60.9% death) compared to female (39.1%). Further analysis based on age groups shows the median infected age is 51 years old while individuals >75 are the most affected and vulnerable to COVID-19. Given the nature of the virus – highly contagious – it is important to protect these individuals from the virus by limiting direct interactions with and follow proper guidelines given by health officials. Currently, there are no available drug medications or vaccines to combat this pandemic. As transmission can occur among all age groups, the need to understand the nature of COVID-19 and its characteristics are critical in response to this global outbreak.

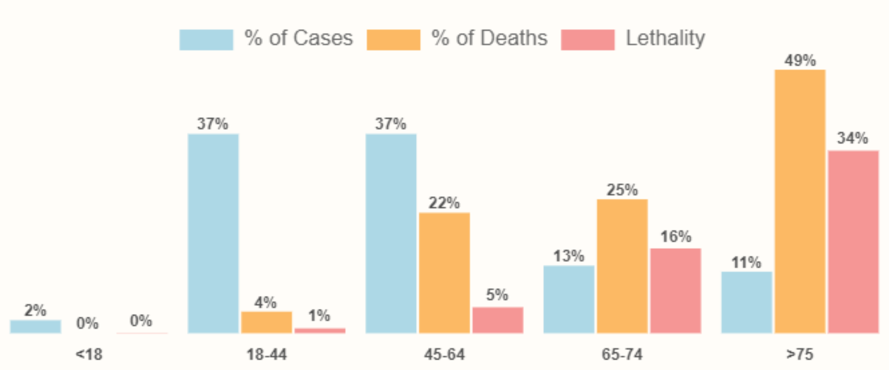

Figure 3. COVID-19 reported cases, deaths and lethality among various age groups in New York, USA.

2. REVIEW METHOD

All data presented in this text were collected using online resources from covidgraph.com, WHO Situation Reports, and U.S. Center for Disease Control and Prevention. A number of journal articles, news reports and papers available online were also used for sourcing information. All materials used for this publication are published in English. Searches were restricted to focus mainly on SARS-CoV-2, COVID-19, and a few articles searched with the keyword “coronavirus”. All data are properly cited throughout this publication. Knowing that data and information periodically are being made available, readers are encouraged to remain updated with more recent data and information following WHO, CDC, and public health officers.

3. RESULTS AND DISCUSSION

Zoonotic Pathogens

In light of zoonosis, a large number of diseases are due to human-animal contacts, causing local and global epidemy. A selected number of zoonotic diseases are briefly discussed in this text to further evaluate the consequences of zoonosis pathogens. Human-animal contact includes hunting, slaughtering, selling and cooking which pose a great risk of infection to a number of viruses– not only COVs. The handling and processing of animals –including wild animals, opens the door for more deadly pathogens that these animals may harbor. A chain of transmission can occur from human-animal contact and human-human transmission can follow. This is the case of many epidemics, including the avian influenza virus (i.e., H1N1) and numerous COVs (i.e., SARS, MERS). To minimize such epidemics, many researchers suggested not to aggregate various animal species, mammals, and birds in relatively closed environment (i.e., meat markets).[13,14,15]

The zoonotic origin of COVID-19 shares overlapping etiological characteristics with Ebola, as they are both transmitted from animal to human. Following recent studies, the full-length genome of COVID-19 was reported.[10] Its genome and spike glycoprotein showed 96.11% and 92.86% identity to *Rhinolophus affinis* bat coronavirus (BTA-COV) in Wuhan, respectively.[16,17] Bats, also known as flittermouse, with over 1,200 species, comprise about 20% of all mammalian species. They are classified under the order of *Chiroptera* and are found all across the world but Antarctica.[18] It is of interest to note that all continents except Antarctica have shown the presence of COVID-19.[19]

Bats are nocturnal. They can either be insectivores (insect-eater) or frugivores (fruit-eater), whereas other bats (i.e., vampire bats) practice hematophagia (feeding on blood). Surprisingly, bats provide numerous benefits to humans. For instance, their wastes, guano, are collected and used as fertilizer, since guano contains high levels of essential nutrients (nitrogen, phosphate and potassium) for plant growth. In addition, bats are a food source across Asia. However, they are natural reservoirs of various zoonotic pathogens.[20] Nearly 200 coronaviruses are identified in various bats species.[21] Many studies suggested that bats are a common reservoir for SARs-COV[22]; and the main bat reservoirs were identified in 2003.[23] Various bats species (i.e., *Taphozous perforatus*, *Rhinopoma hardwickii* and *Pipistrellus kuhlii*) are suggested to be the source of MERS-COV.[24] While not yet confirmed, studies suggest that the COVID-19 global outbreak was transmitted from bats to humans. This might occur directly or indirectly via an unknown, intermediate vector.[25,26] While more studies are needed for confirmation, a recent study showed Pangolin (armored anteater) as the intermediate host of COVID-19 virus.[27,28,29] Other studies suggested fish as the potential reservoir of infection from human-animal contact. Altogether, these studies demonstrated human-human transmission of COVID-19 through droplets from direct or indirect contact.[8,30,31] Developing tests at hospital settings showed that the virus is nosocomial, affecting 41% of patients based on incidence and transmission from asymptomatic carriers.[31,32,33,34,35,36,37]

Previously, the Asian palm civet cat (*Paradoxurus hermaphroditus*), a member of the *Viverridae* group, was reported as the reservoirs of SARs-COV from a wild animal market in Guangzhou, China.[38,39,40] The successful identification of this reservoir was pivotal in controlling the virus while suspending all trades of civet cats.[41] Therefore, the need to identify the reservoirs of the emerging COVID-19 outbreak is needed in order to take significant control of the disease and minimize mortality rate.

MERS-COV also has zoonotic origin. Numerous animals and birds were originally tested to identify MERS-COV's actual reservoirs. Results indicated dromedary camels as the actual reservoir (Table 1). Poultry markets in China were tested as the reservoirs for various type of influenza viruses. Learning from various studies concerning COVs and avian influenza, the authors suggested the following measured below in order to combat the virus and identify its reservoir. First, researchers should conduct serological testing of all animals sold at the Wuhan market prior to the widespread disease. This could provide information about the reservoirs and identify if other animals exposed to the virus have developed antibodies against COVID-19. Next, there is a need to investigate the seroconversion from any exposed animals. This is to confer protective immunity against the virus.

Preventative Measures

To decrease the global effect of COVID-19, government agencies, public health officials, and infection controls personnel need to urgently assess the nature of the virus while providing relevant information to the public. From previous experiences associated to MERS and SARs-COV, the World Health Organization (WHO) has been very critical in responding and recommending infection control interventions. This greatly helps to reduce risk of transmissions which include avoiding contact with affected individuals and wild animals, taking protective measures, and frequent handwashing. The WHO along with the US Centers for Disease Control and Prevention (CDC) have shared many preventative actions to minimize the risk of infection.[42,43] This includes various measures such as social distancing to slow down the rate of transmission (maintain 6 feet, 2 meters, away from each other), sneezing in clothing or disposable tissues, practicing cough etiquette (cover coughs), frequently decontaminating surfaces (i.e., door handles etc.), sheltering-in-place if required, and self-quarantining following domestic and international travels. This is in addition to not kissing (or other intimate social interactions), hugging, or handshaking. In consideration of these measures and the nature of the virus, many businesses are forced to be nonoperational, thus leading to global economic disaster, as the stock market has thus far shown.[44] These efforts are made to reduce the global impact of the virus, allow healthcare administrators and public health to better characterize the nature of the virus. This could greatly afford researchers to develop vaccines, therapeutics, and diagnostics measures in response to the virus in a timely manner. Health officials should constantly engage in circulating information, guidance and clarifying misinformation to the public. While the internet remains as the major source to relate and spread information, the public should be aware of fake news circulating the internet. The authors recommend individuals to follow credible sources for information (i.e., CDC, WHO, etc.).

Potential Treatments

Viruses bind to host receptors on target cell surface to generate infection. A recent study showed SARs-COV-2 (known as COVID-19) and SARs-COV use angiotensin-converting enzyme A (ACE-2) to gain entry into the cells.[45] ACE-2 can affect many tissues and organs in the human body since it is present in human's epithelia and small intestine including liver, lung, stomach, ileum, colon, and kidney.[46] This implies that coronaviruses can infect multiple organs (gastrointestinal and upper respiratory tracts). Identifying possible routes of COVID-19 infection could significantly have an impact on treatment of the virus. In this context, researchers have recently reported that the main target cell of COVID-19, AT2 cell is shown to express relatively low levels of ACE-2 in the lung.[45,47,48,49] This implies that COVID-19 may rely on co-receptors or other protein membranes to integrate into cell membrane, deposit its nuclear material, have that nuclear material integrated into host receptors on target cell surface, and have the host cell reproduce the viral particles until that host cell is dead and other cells are infected.[50] This is facilitated through the transmembrane protease serine 2 (TMPRSS2) for priming, where the virus is activated.[51] In this context, inhibition of TMPRSS2 could block cell entry by COVID-19, reduce the virus replication, and present a possible therapeutic pathways.

Currently, there is no effective treatment for COVID-19 and other COVs (i.e., MERS-COV); this is a significant obstacles facing the world. In the absence of known vaccines or medications, researchers are engaged in ongoing efforts to develop and validate medications to combat COVID-19 global pandemic. A number of vaccine candidates (~120) are now in clinical trials; and others are under testing. At the same time, researchers have focused on repurposing many drugs and traditional herbal medicines to combat the most severe cases of infection. Drug repurposing relies on the investigation of existing drugs for new therapeutic purposes.[52,53] This provides an effective way to rapidly recognize drugs with known side effects to be used while new drugs are under preparations.

Repurposing drugs such as hydroxychloroquine/chloroquine, used to prevent and treat malaria, in addition to autoimmune disease (rheumatoid arthritis, lupus, and porphyria cutanea tarda), is found to inhibit the replication of many DNA and RNA viruses– as in many coronaviruses.[54,55,56] The China National Center for Biotechnology Development (CNCBD) reported that infected patients (about 100) treated with chloroquine have showed improvements in their lung and decline in fever. In addition, these patients rapidly recovered when compared to the control groups. With the promising results from these studies, the Chinese Government proposed chloroquine as the front line drug treatment for the severe COVID-19 outbreak.[57,58,59,60] Ten clinical trials are underway to investigate the chloroquine as an anti-COVID-19 therapy.[61] While the drug is well known,[62,63] adverse side effects from COVID-19 patients treated with chloroquine need to be investigated. In the meantime, chloroquine remains as the available drug to fight the disease in China and other countries are suggested to use it. However, ongoing researches have shown that the use of hydroxychloroquine/chloroquine might be detrimental to COVID-19 infected patients.

In addition to chloroquine, teicoplanin can be an alternative drug for the treatment of COVID-19.[64] Teicoplanin has been found to significantly inhibit cellular entry of Ebola virus, SARs-COV and MERS-COV.[65] This antibiotic drug also shows efficacy against Staphylococcal infections, hepatitis C virus, HIV virus and influenza virus.[65,66] Further investigations are needed for the inclusion of teicoplanin as a potential repurposing drug for the treatment of COVID-19 global outbreak.

Remdesivir, a purine nucleoside analogue, is a broad-spectrum antiviral medicine developed by Gilead Sciences; it was previously used to treat Ebola and Marburg virus infection.[67] The medication was found to show antiviral activity against other single-stranded RNA viruses, such as respiratory syncytial virus and COVs.[68,69,70] Remdesivir is currently in clinical trials in Nebraska and China on hospitalized patients with COVID-19 to evaluate its safety and efficacy. Although this medication has been administered to patients, there is no available data on whether it can improve clinical outcomes.

Interferon Alfa-2B is an antiviral medicine used to treat hepatitis B and C, certain types of cancer, and genital warts. The medication was developed in Cuba; it is currently in clinical trials to treat COVID-19 patients in China, where the virus originally emerged.[71,72] Due to the absence of a general treatment, repurposing drugs such as those described above are suggested as potential treatments for patients infected with COVID-19, while researchers are investigating actual treatments.

Table 2. Medicinal Plants with Therapeutic Histories

Plants	**Bioactive/antiviral compounds**	**Therapeutics**
Zingiber officinale	Gingerol; Shogaols	Human respiratory syncytial virus
Allium sativum	Allicin	Influenza virus
Euphorbia hirta	Afzelin; Quercitin; Myricitrin; Rutin; Garlic acid; Caffeic acid	Asthma conjunctivitis
Curcuma longa	Curcumin	Dengue virus; Hepatitis C virus; Zika virus, Chikungunya virus
Olea europaea	Oleuropin	Herpes; mononucleosis; hepatitis virus; rotavirus; bovine rhinovirus; canine parvovirus; feline leukaemia virus; Respiratory syncytial virus; Parainfluenza type 3 virus
Aloe vera	Emodin; Chrysophanol; Aloe-emodin	Human respiratory syncytial virus
Allium Cepa	Quercetin; Isorhamnetin; Kaempferol; Myricetin	Human immunodeficiency virus (HIV); Herpes simplex virus type 1; Poliovirus type 1; Para-influenza virus type 3; Potato virus
Garcenea Kola	Kolaviron	Influenza virus; Hepatitis; Diarrhea; Laryngitis; Bronchitis; Gonorrhoea; Chest colds; Caughs
Echinacea	Caffeic acid	Colds; Influenza; Lung conditions; Candidiasis; Influenza A virus; Herpes simplex virus; Polio virus

Note: See reference [73] for a comprehensive review

In addition to repurposing drugs, various plants containing bioactive compounds have shown to be effective against previous coronaviruses. These plants include *Zingiber officinale*, *Allium cepa*, *Allium sativum*, *echinacea*, *euphorbia hirta*, *Garcenea kola*, *Curcuma longa*, *Aloe vera* and *olea europaea* (Table 2). The natural compounds from these plants could have potency to inhibit TMPRSS2, thus reducing the virus (COVID-19) replication. Research to better understand the mechanism of these herbal medicines are not yet fully available. However, herbal medicines have been produced in higher demands due to their use against COVID-19 (i.e., Madagascar). Countries such as China, Thailand, Bolivia, Tunisia, India, Haiti, Madagascar, and Nigeria have their respective traditional herbal medicine against the virus.[73,74,75,76,77,78,79] In a study reported by the National Health Commission of the People's Republic of China, of the 74,187 patients treated with traditional herbal medicines, nearly 90% of them have recovered. This further proves the potential use of herbal medicines to fight COVID-19 while given researchers more time to develop potential vaccines.

4. CONCLUSION

The novel coronavirus disease (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARs-COV-2). To date, the virus remains a global health issue, affecting 6,057,853 individuals with 371,166 confirmed deaths (6.13 % mortality rate) in more than 120 countries and territories. This emerging virus continues to cause a public health issue, economic disaster, physiological distress–including discrimination, and limit human activities.[80,81]

While the source of the virus and its immediate host remain controversial, researchers are engaged in ongoing efforts to develop and validate medications to combat the COVID-19 global pandemic. Due to the absence of a general treatment, many repurposing drugs and traditional herbal medicines are suggested as potential treatments for patients infected with COVID-19.

ACKNOWLEDGMENT

Thanks are due to Ms. Debbie Sanon, Ms. Ruthonce Stvil, and Dr. Rose Mary Stiffin for their contribution and feedback.

DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

FUNDING INFORMATION

This research did not receive any specific grant from public, government, commercial or non-profit funding agencies.

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