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FREQUENTLY ASKED QUESTIONS OF NOVEL CORONAVIRUS: A REVIEW OF THE EVIDENCE

Mahmudah I.[1], Waskito L.A.[1], Miftahussurur M.[1,2]*

[1] Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia
[2] Gastroentero-Hepatology Division, Department of Internal Medicine, Universitas Airlangga, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

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Abstract

The corona virus outbreak has become a global pandemic. This situation triggers a sense of crisis in the community. Massive flow of information makes people confused due to the information might be incorrect. Therefore, summary in a review to answers some questions that commonly asked among community is necessary. Information related to sunbathing habits during the daytime is becoming popular, vitamin D is believed by the public to come from sun exposure. However, excessive ultra violet light from sun exposure could be dangerous. Therefore, it needs to consider several things in applying sunbathing habits. The controversy over the outcome of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers has an adverse effect on coronavirus infection. In contrary, recent studies of the drug have a protective effect against lung tissue damage caused by this virus infection. Therefore, it not necessarily to stop this medication. In addition, there were findings that a high viral titer of coronavirus was found in asymptomatic or pre-symptomatic patients. Therefore, it could contribute to high transmission rates in the community. It is necessary to do precaution in order to stop the transmission. The existence of massive information flow though needs to filter information, based on scientific to prevent the misleading information. Break the chain of infection with appropriate diagnostic tests then isolate the positive person is the best way to resolve this pandemic.

Keywords: *COVID-19, SARS-CoV-19, Indonesia, FAQ.*

Introduction

In the last two decades, there have been two major pandemics caused by coronaviruses, in 2003 there was severe acute respiratory syndrome (SARS) and the middle east respiratory syndrome (MERS) in 2012 [*Raoult D et al., 2020*]. In the end of 2019, the world was surprised by the emersion of a new virus caused pneumonia which was at reported by the Chinese Government on December 31, 2019. This new emerge disease first hit Wuhan, a city in the center of China. The death cases from this new novel viral infection were first reported in Wuhan China on 9th January 2020. The virus was then known as new species of the coronavirus family, which is well known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease is known as Coronavirus Disease (COVID-19) [*Raoult D et al., 2020*]. This new virus has attribute to spread quickly and by now had reached more than 150 countries. WHO stated that this COVID-19 is a global pandemic on March 11, 2020 [*Morens D et al., 2009*]. In Indonesia, COVID-19 cases were first reported on March 2, 2020 from Jakarta, in 2 women aged 64 and 31 years.

During the outbreak of this virus, there were several unclear information about COVID-19 circulating in the community. Since this disease is massively spread worldwide, there are several assumptions how actually this virus is transmitted among human, and what the best way to prevent this transmission. Is wearing mask, physical distancing was the best way to break the transmission chain? In addition, a relative cheaper option of immunoassay-based test to screen infected individual is still debatable. Indeed, a caution is necessary to interpret the result of this immunoassay-based test. As this viral infection which most likely to be a self-limiting disease, reinforcing the immune system is one way to prevent contracting the COVID-19. Sunbathing was reported may enhance immunity by producing vitamin D to give a beneficial in the role of immunity [*Maruotti N, Cantatore F, 2010*]. However, sunbathing also has an adverse effect on triggers the development of skin cancer due to UV radiation [*D'Orazio J et al., 2013*]. Therefore, it is important

Address for Correspondence:
Muhammad Miftahussurur, MD, PhD
Gastroentero-Hepatology Division, Department of Internal Medicine, Dr. Soetomo General Academic Hospital, Surabaya 60286, Indonesia Jalan Mayjend Prof. Dr. Moestopo No 6-8 Surabaya 60286, Indonesia
Tel: +6231-502-3865; Fax: +6231-502-3865
E-mail: muhammad-m@fk.unair.ac.id

to understand how this virus actually spread, what test is adequate to determine infected individual and how actually we can do to prevent the transmission.

It is familiar that SARS-CoV 2 used angiotensin converting enzyme 2 (ACE2) receptor which is one of target therapy for hypertension patients [*Zhang H et al., 2020*]. Hypertension itself remains a problem in Indonesia with prevalence of 33.4 % (95 % CI: 32.7-34.0) [*Peltzer K, Pengpid S, 2018*]. Therefore, it makes anxiety in patients with hypertension whose been controlled with anti-hypertensive drug therapy group ACE inhibitors (ACEI) or Angiotensin 2 Receptor Blockers (ARB). In addition, smokers were generally considered a group with a high risk of becoming infected with lung disease. According to a study, smokers usually suffer from a more severe and prolonged form of the disease, which then causes COVID-19, compared to the nonsmokers group [*Russo P et al., 2020; Zhou F et al., 2020*]. However, the nicotine contained in cigarettes was recently known to have a therapeutic effect for COVID-19 [*Wittebole X et al., 2007; Mabley J et al., 2011*].

The global case fatality rate of COVID-19 was approximately 1.78 % (95% CI: 1.34-2.22) [*Maitra S et al., 2020*], depends on the how fast is detected and treated and comorbid condition. Delayed disease discovery may increase the case fatality rate. Therefore, the effective drugs are needed in the community. The discovery of a treatment that was developing at this pandemic was chloroquine (and hydroxychloroquine) can also be used also for COVID-19 therapy. This drug has been known as a malaria drugs [*Meo S et al., 2020*]. However, this drug that has not been used for a long time because of malaria parasite resistance to this drug in Indonesia [*Sutanto I et al., 2010*]. It was still often used in autoimmune disease [*Mehra M et al., 2020*].

Those issues are most commonly asked in Indonesia, in the general community or even among medical practitioners. Here, we summarize the current understanding regarding most frequently asked questions about COVID-19. It is necessary to underline that the answers to the questions below were temporary answers that may change any time along with the development of the latest scientific evidence related to COVID-19.

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

Is the sunbathing has benefit to prevent COVID-19?

Sunlight is source of light, heat and energy essential for life on the earth. It consists of various types of light, ultraviolet (UV) light is one of them. UV has multiple and complex benefit for human health. One of the UV benefit for human that it mediates natural synthesis of endorphins and vitamin D [*Trummer C et al., 2016*]. Atrophy, wrinkles, pigment changes, and malignancies, such as squamous cell carcinoma (SCC) and malignant melanoma (MM) are skin disorders influenced by the role of UV as risk factors [*González Maglio D et al., 2016*].

UV fall in the midst of the wavelengths of visible light and gamma radiation (approx. 100 *nm*-400 *nm*) [*Ramanathan K et al., 2014*]. Based on the electro physical properties, UV light divided into UV-A, -B and -C photons, with UV-A has the longest wavelengths (315-400 *nm*), UV-C has the shortest wavelengths (200-280 *nm*) and UV-B is falling in between UV-A and UV-C. Based on those different wavelengths, there are diverse effects on cell biology of the skin along with the immune system [*Maruotti N, Cantatore F, 2010; D'Orazio J et al., 2013*]. Sunlight reaching earth's surface was dominated by UV-A (95-99%) and UV-B (1-5%) because the atmospheric ozone layer absorbs UV-C [*D'Orazio J et al., 2013*]. The low amount UV-B reaching earth's surface mostly due to the 99% of UV-B radiation is absorbed by the ozone layer [*D'Orazio J et al., 2013; Wacker M, Holick M, 2013*].

Vitamin D in humans is obtained orally through food and synthesized in the skin. UV-B radiation exposure in the skin triggers the synthesis of vitamin D. During sunlight exposure, radiation of UV-B is absorbed by the skin and transformed 7-dehydrocholesterol to pre-vitamin D_3 and will be finalized as a vitamin D3 [*Holick M et al., 1981*]. Phosphate and calcium metabolism are useful for the maintenance of metabolic functions is also obtained from UV-B radiation which is absorbed by Pre-vitamin D3 and vitamin D3 and converted into various of the two main photoproducts; lumisterol 3 and tachysterol 3[*Holick M, 1994*]. Enhancing calcium homeostasis and improving bone health are also functions of vitamin D [*Maruotti N, Cantatore F, 2010*].

The kidneys and liver metabolize Vitamin D in into 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D as the major circulating and biologically active form, respectively. Many organs are able to produce 1,25-dihydroxyvitamin and have a vitamin D receptor. Therefore, several of biological pathways and association studies related to vitamin D deficiency are influenced by 1,25-dihydroxyvitamin D with increased risk for disease of cardiovascular, cancers, autoimmune diseases, schizophrenia, diabetes mellitus type 2, and infectious disease [*Hoel D et al., 2016*].

B-, T-, and antigen presenting-cells as immune cells express vitamin D receptors, so vitamin D can modulate

innate and adaptive immune responses. Many studies showed auto-immune diseases is associated with vitamin D deficiency although an increasing infection susceptibility [*González Maglio D et al., 2016*]. Report between 1988 and 1994 using nearly 19,000 subjects showed individuals with <30 *ng/ml* (low) vitamin D level were more likely to be infected with upper respiratory infections than those with adequate levels [*Ginde A et al., 2009*]. Other prospective study, double blind placebo study showed that administration of vitamin D resulted in a statistically significant decrease was generated in incidence of influenza infection [*Urashima M et al., 2010*].

In vivo studies showed the important role of vitamin D in immune response [*Liu P, 2006; Liu P et al., 2009; Greiller C, Martineau A, 2015*]. Vitamin D receptor (VDR) and the CYP27B1 enzyme expressions by most immune cells has extensive and varied effects on the immune system. The increase of the 1-α-hydroxylase and the VDR expressions is provoked by toll like receptor binding [*Liu P, 2006*]. Then, induce binding of the 1,25 D-VDR-RXR heterodimers to the VDREs of the genes for cathelicidin, beta defensin 4 and subsequent transcription of these proteins. Transcription of cathelicidin is absolutely dependent on sufficient 1,25-hydroxyvitamin D [*Liu P, 2006*]. It is now clear that beta defensin 4 transcription requires NF-κB binding to appropriate response elements on the beta defensin 4 RNA [*Liu P et al., 2009*]. Translocation of NF-κB to its binding site is generated via toll like receptor 2-1 signaling facilitating the IL-1 receptor [*Liu P et al., 2009*]. Therefore, the effects of vitamin D metabolites in the pathogenesis of viral or bacterial in the adhesion to the respiratory epithelial cells.

Synthesis of sunlight that induced vitamin D is exceedingly influenced by many factors, including season, time, latitude, altitude, air pollution, sunscreen utilization, skin pigmentation, and aging [*Maruotti N, Cantatore F, 2010*]. Study in Alaska showed in early morning before 10 am and late afternoon after 3 pm any vitamin D_3 can be released in the skin [*Kenny D et al., 2004*]. Sunlight exposure at that time will produce vitamin D, which can lasts twice as long in the blood compared to the digested vitamins [*Haddad J et al., 1993; Kenny D et al., 2004*]. A clinical study from Sweden comparing full body irradiation with UV-B lamps 3 times a week for 6 weeks to a vitamin D_3 daily supplement of 1,600 *IU* daily for 6 weeks found UV-B therapy to be more effective in increasing serum into 25-hydroxyvitamin D concentration [*Bogh M et al., 2012*]. However, various studies on the effect of light exposure UV-B to a concentration of vitamin D, generally performed on white populations and uses artificial UV-B rays. UV-B rays from sunlight in the tropical country, including Indonesia showed the highest intensity in the sun exposure between 11 am and 11 pm [*Setiati S, 2008*]. However, there are several factors that can decrease vitamin D_3 production in the skin, including increased skin pigmentation, aging, and the topical application of a sunscreen [*Bogh M et al., 2012; D'Orazio J et al., 2013*].

Vitamin D levels in the form of 25-hydroxyvitamin D are maintained by the body at least 30 *ng/mL* [*Hoel DG et al., 2016*]. Large capacity of vitamin D is produced through the skin, 15,000-20,000 *IU* of vitamin D is equivalent to 1 erythema minimum (MED) is one-time exposure to sunlight throughout the body. Therefore, the body surface needs to be exposed to 0.5 MED (50%) to produce 4000 *IU* of vitamin D a day. Body surface exposed to 0.5 MED with a duration of 2-3 times a week is equivalent to consuming 2000 *IU* of vitamin D a day to reach a minimum level of 30 *ng/mL* in the blood [*Hoel D et al., 2016*]. The exposure time required at an intensity of 1 MED/hour is 1/4 x 60 minutes or equal to 15 minutes [*Setiati S, 2008*]. Therefore, duration needed to sunbathe in Indonesia at an optimal hour is about 7.5 minutes between 11 a.m. to 1 p.m. 2-3 times a week but it is still debatable because MED among individuals was varies especially depend on the skin pigmentation.

As mention above, sunlight exposure especially those that are sufficiently able to provide vitamin D has an impact on the immune system which can prevent various infections, may including the COVID-19. However, there was no research evidence about the benefits for killing the SARS-CoV-2. In addition, there are several considerations need to addressed prior sunbathing, including increasing risk for skin cancer, possibility of heat-stroke and dehydration after the sunbathing.

How effective was the use of rapid immunoassay-based tests in Indonesia?

Polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assay (ELISA) and rapid diagnostic test (RDT) or lateral colloidal gold immunochromatography are some of the methods used to detect SARS-CoV-2 [*Green K et al, 2019*]. Currently, the frequently use two types of examination tests for COVID-19 infection, including RDT and PCR. Indonesian government using RDT for screening method [*Intan G, 2020*].

Direct detection by the presence of virus via quantitative reverse transcriptase (RT-qPCR) is the recommended test for detecting SARS-CoV-2. Therefore, it is considered the best method for diagnosing COVID-19. Collection of specimens was taken from nose, throat of both using either swabs. Test recommendations to detect the most sensitive SARS-CoV-2 with samples collected from both upper and lower respiratory and Broncho alveolar lavage fluid (BAL). This recent study reported that more RNA from SARS-CoV-2 was detected via nasopharyngeal swab (NP) than an oropharyngeal (OP) swab, 63% and 32%, respec-

tively. [*Ramanathan K et al., 2020*]. Purulent sputum production due to pneumonias viruses is typically not produced. Therefore, the NP specimen collection method is usually used in daily. The US CDC also recommends only the NP swab [*CDCP, 2020*]. However, sputum collection and especially via bronchoscopy is a procedure that requires trained staff, high-cost equipment and has the potential to increase a safety risk for healthcare workers through the production of aerosol droplets. Therefore, BAL was not used routinely. It should be noted that a patient with pneumonia should not be assumed that each of these (e.g., NP swab specimen, sputum, BAL) will have an equal chance of detecting SARS-CoV-2; the detection rate in each specimen type varies in every patient and may change in individual clinical condition. Thus far, the gold standard diagnosis method for COVID-19 is the detection of nucleic acid by NP (nasal) and OP (throat) swab sampling or other respiratory tract samplings with RT-qPCR and further confirmed by sequencing.

RDT or lateral flow assays use the same mechanism usually used for pregnancy tests. This particular method detects the antibody of virus from patient blood (e.g. vein or from a small finger prick) indicating the COVID-19 infection. It yields to the detection of antibody response instead of the virus itself. There are several factors increasing the performance of antibody-based test, including time of the test (how long from the symptoms), age and patients' immunology status. Early studies suggest that most patients seroconvert approximately 7 to 21 days after exposure to the virus, although some patients develop antibodies more rapidly [*Bastos M et al., 2020; Patel R et al., 2020*]. It may yield a negative or non-reactive result if tested too early. In addition, recent systematic reviews and meta-analysis studies on accuracy of COVID-19 serological diagnostic tests showed the biochemical technique sensitivity of enzyme-linked Immunosorbent Assay (ELISA), lateral flow immunoassay and chemiluminescent immunoassay were 84.3% (95% CI:75.6-90.9%), 66.0% (95% CI:49.3%-79.3%) and 97.8% (95% CI:46.2%-100%) respectively [*Bastos M et al., 2020*]. Therefore, a caution is necessary to interpret the result of the RDT.

There are several brands of RDT were circulating in Indonesia. Each brand may have different their own quality control prior distribution of the products. This different leads to major problem due to it may yield a different result. Many countries have returned rapid test products to factories due to the tests was not work properly or fake [*Grady D, 2020*]. Therefore, WHO has issued a list of NRAs that have been received through independent quality control conducted by other countries.

RDT is one option to detect COVID-19 in the community. However, since COVID-19 is a global pandemic with massively spread among community, the best approach is to detect the sick person using standard diagnose method. Indeed, it need a huge amount of resources to test all of "person at risk" and isolate them. However, it is currently the best option to slow down or even stop the pandemic. In addition, RDT accuracy is very dependent to the patients' clinical condition, time of test, target population and quality control of the RDT itself. There was no validation study conducted to the RDT that circulating in Indonesia. Therefore, it is necessary to conduct a validation study for the available RDT. As for usage, RDT is more suitable for screening instead of diagnostic.

How to understand silent spreaders in COVID-19 transmission?

The SARS-CoV 2 has a feature of quickly spread among community with almost 80% of the infected individuals are asymptomatic [*Rothe C et al., 2020*]. This leads to a difficulty to detect infected person unless they showed a *cardinal sign* of COVID-19 and confirmed by PCR test. In addition, asymptomatic person doesn't know if they were asymptomatic carriers of SARS-CoV-2. Finding and testing in persons who had close contact with the patient in some cases confirmed asymptomatic carriers [*Pan X et al., 2020*]. The asymptomatic carrier may have a main contribution to the quickly spread of the SARS-CoV 2.

Pre-symptomatic people are a group of people who have been infected. However, do not yet show symptoms. SARS-CoV 2 transmission occurred predominantly after several days of illness and was associated with modest viral loads in the respiratory tract at the onset of the disease, with viral loads peaking about 14 days after symptom onset [*WHO, 2020*]. A study from 23 January to 16 March in Singapore involving 243 cases of COVID-19 identified seven groups of cases where secondary case explanations occurred pre-symptomatic transmission [*Wei W et al., 2020*]. Pre-symptomatic transmission was performed on contact tracing including the period before the onset of symptoms. These findings suggest even limiting the symptomatic person only may not enough to control the pandemic, since the asymptomatic individual may transmit the virus as well [*Kimball A et al., 2020*].

High viral titers of SARS-CoV-2 are reported in the saliva of COVID-19 patients. These high viral titers are just as high in asymptomatic or pre-symptomatic patients [*Maclntyre C et al., 2009*]. Many COVID-19 patients are asymptomatic, and nearly all have a pre-symptomatic incubation period ranging from 2 to 15 days, with a median length of 5.1 days [*WHO, 2020*]. The early course of infection as a route of transmission was also found in previous reports [*Zou L et al., 2017*]. Therefore, attention to asymptomatic patients and during the incubation period needs attention to prevent people from being infected.

Do mask really reduce coronavirus spread?

The route of transmission of SARS-CoV-2 is likely via small droplets that are ejected when speaking, coughing or sneezing [*Duguid J, 1946*]. The most common droplet size threshold has a minimum at 5 *μm* to 10 *μm* [*Morawska L et al., 2009*]. Recent analysis shows that speaking can be an important vector of transmission, transmission with a higher viral load is associated with an increase in the number and size of droplets created with louder speech [*Howard J et al., 2020*].

Recommendations on masks in the community vary across countries during the COVID-19 pandemic. In many Asian countries, face mask is used as hygienic habit. In contrast in European and North American countries as something only people who are unwell induced stigmatization and racial aggravations [*Feng S et al., 2020*]. The use of face masks in general, including healthy people, can prevent discrimination individuals who wear masks when feel unhealthy [*Feng S et al., 2020*]. A prospective study using a cluster-randomized trial comparing surgical masks, P2 masks that were not properly tested, and no masks in prevention of influenza like illness (ILI) in households suggests that face masks should have some effect on viral transmission such as distraction on hand-nose contact [*Maclntyre C et al., 2009*].

Based on their filtering capability, mask can be made of different design and materials. N95 or FFP_2 respirators and face masks (surgical mask) are examples of personal protective equipment used to protect the user from airborne particles and from fluids that contaminate the face. N95 (the American standard) was equivalent with FFP_2 (the Europe standard) respirators are recommended for health workers conducting aerosol-generating procedures during clinical care of COVID-19 patients [*Howard J et al., 2020*]. It has been shown that N95 or FFP_2 respirators and surgical mask perform well as personal protective equipment and can become a scarce resource during a pandemic. Therefore, cloth fabric face mask come into place to reduce the demand of N95 or surgical mask as general personal protective equipment. Particle sizes for speech are on the order of 1 *μm* while typical definitions of droplet size are 5 *μm*- 10 *μm* [*Davies A et al., 2013*]. Generally, available household materials have a filtration rate between 49% and 86% for 0.02 *μm* exhaled particles whereas surgical masks filtered 89% of those particles [*Davies A et al., 2013*]. In a laboratory setting, household materials had 3% to 60% filtration rate for particles in the relevant size range, finding them comparable to some surgical masks [*Rengasamy S et al., 2010*]. In summary, the filtration capacity in the droplet size involved in household masks is laboratory proven to be effective at blocking droplets from the user [*Papineni R, Rosenthal F, 1997*].

Since the COVID-19 pandemic spread so quickly around the globe and the cure of COVID-19 has not been discovered yet, the best strategy to overcome is by preventing to infect more people. Due to evidence shows COVID-19 can be transmitted before symptoms onset, the use of face masks in community can reduce the level of community transmission if everyone including people who are infected but without symptoms (asymptomatic) wear a face mask.

Is it necessary to stop taking ACEI or ARB during the Covid-19 Pandemic in hypertensive patients?

It is well known that SARS-CoV 2 interact with ACE receptor in human respiratory tract. In the human physiology, ACE has a major function to regulate body fluid, blood electrolyte and blood pressure. Currently, ACE inhibitor and angiotensin receptor blockers (ARB) drugs are commonly as therapeutic agent in the supervision of hypertension, myocardial infarction in post treatment, heart failure and to prevent the progression of diabetes-related kidney disease rapidly [*Rico-Mesa J et al., 2020*]. Since there is an intersect of receptor between COVID-19 and cardiovascular disease, it is interesting to understand the relationship between those diseases.

Physiologically, plasma sodium concentration is maintained by the renin-angiotensin-aldosterone (RAAS) system by the way of feedback from baroreceptors, blood pressure, and sodium as well as potassium levels. Initially, the metabolism of angiotensinogen to angiotensin I was played by renin released from the kidneys. Furthermore, the lungs and kidneys produce ACE, which changes angiotensin I to angiotensin II. Angiotensin II stimulates the cardiovascular response, vasoconstriction, aldosterone production and antidiuretic hormone, which lead increases the volume of body fluids through sodium, potassium, and water resorption resulting an increase of blood pressure [*Sparks M et al., 2014*]. The homolog of the ACE2 receptor is ACE, a transmembrane aminopeptidase that express in many organs such as lungs, artery, kidney, and heart [*Kuba K et al., 2010*]. Reversal of RAAS activation due to ACE2 receptors by lowering angiotensin II and increase angiotensin 1/7 [*Sparks M et al., 2014; Rico-Mesa J et al., 2020*]. Angiotensin 1/7 acts on the receptor to have a vasodilation effect [*Sparks M et al., 2014*]. Thus, the role of ACE2 and ACE was opposite in regulating blood pressure (Figure 1).

SARS epidemic in 2002-2003 found that the SARS-CoV virus can enter host cells mediated through ACE2 receptors [*Patel V et al., 2014*]. ACE2 bonds in humans have a strong affinity with the surface of the SARS-CoV virus as shown from crystal analysis studies and biochemical interactions on virus spike protein interactions [*Li F, 2005*]. These bonds helped the SARS-CoV virus enter host cell. SARS CoV virus had 76.5% similarity in amino acid sequence with SARS-CoV 2 [*Xu X et al., 2020*]. A study showed that SARS-CoV2 virus was more

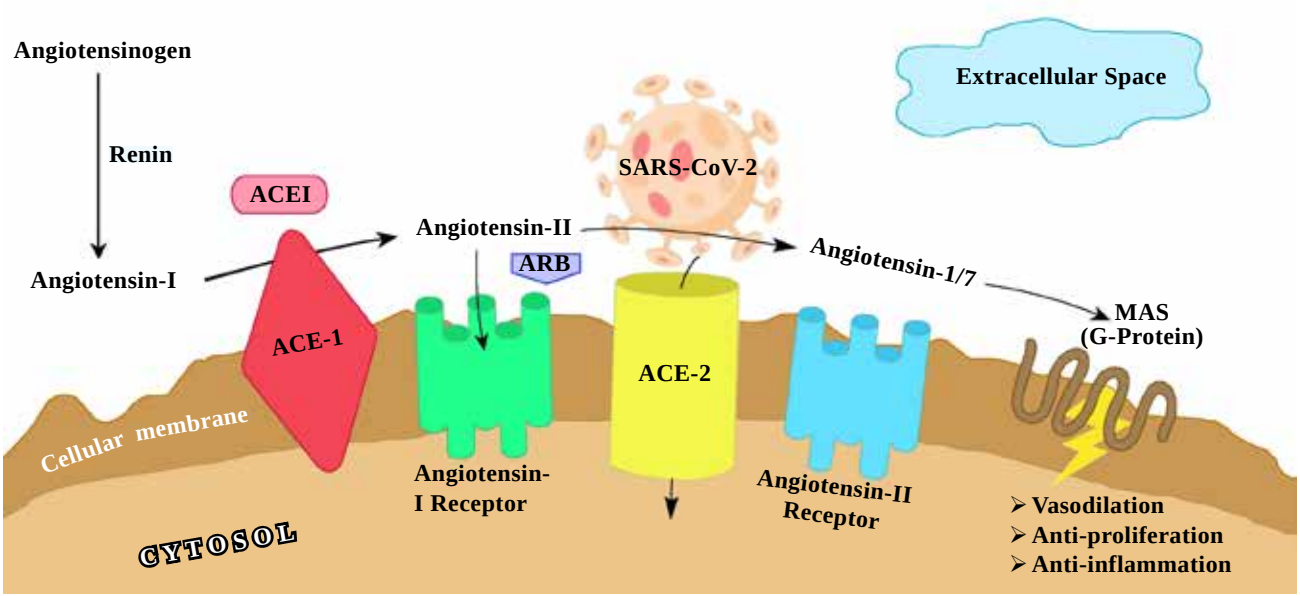

Figure 1. *RAAS pathway showing mechanism of action ACEI/ARB and SARS-CoV-2 infectious mechanism by ACE2 receptors*

efficient at recognizing ACE2 receptors in humans [*Wan Y et al., 2020*]. Therefore, it has higher human to human transmission rate than SARS as we are experiencing now.

The important factor in infectivity is strongly suspected by the interaction between SARS-CoV and ACE2, considering the SARS-CoV affinity for ACE2 receptors was high. ACE inhibition by ACE inhibitor reduce angiotensin I levels causing negative feedback; hence it may increase the number of ACE2 receptors to interact due to low amount of available angiotensin I [*Rico-Mesa J et al., 2020*]. Therefore, it may increase SARS-CoV 2 binding sites. Increasing binding sites is observed among patients with diabetes and/or hypertension, due to they are taking ACE inhibitor or ARB regularly [*Fang L et al., 2020*]. In addition, there were findings patients using ARB especially Olmesartan had an upregulation of ACE2, showed by an increasing of ACE2 secretion in urinary secretions [*Furuhashi M et al., 2015*]. In concordance, another study showed that Lisinopril could increase ACE2 levels by 5-fold and losartan increased ACE2 levels by 3-fold [*Ferrario C et al., 2005*]. These findings suggest the use of ACE inhibitor and ARB may increase risk for severe COVID-19 infection. However, despite the hypothesis of an increase in ACE2, until now there is no correlation showed between usage of ACE inhibitor and ARB to the mortality or morbidity among COVID-19 patients [*Rico-Mesa J et al., 2020*].

One study analyzed association between discontinuation of ACE inhibitor and ARB among 112 COVID-19 patients showed poor outcomes in cardiovascular mortality. This poor outcome mostly due to lactic acidosis, thrombotic states and fulminant inflammation [*Rico-Mesa J et al., 2020*]. This finding suggests the usage of ACE inhibitor and ARB in COVID-19 patients with cardiovascular disease did not affect the COVID-19 progression. Therefore, several related professional organizations such as the Heart Failure Society of America, the American College of Cardiology, and the American Heart Association still recommend to not stop the use of ACE inhibitor and ARB drugs in COVID-19 patients who have used it before, due to the benefits of the drug were greater for preventing complications due to uncontrolled hypertension [*Bozkurt B et al., 2020; Guan W et al., 2020*]. In addition, ASEAN Federation of Cardiology (AFC) and Indonesian Heart Association (PERKI) also rejected that reasoning of the harmful of ACEI or ARB pertinent to COVID-19 infection [*Santoso A, Kiat N, 2020*]. Therefore, those drugs still important for patient with cardiovascular disease and it not necessarily to stop the usage in this COVID-19 pandemic.

Does smoking protect us against coronavirus?

There was a lot of presumption about the smoking effects on COVID-19. The nicotine, primary components of tobacco smoke, may increase the risk of COVID-19 [*Russo P et al., 2020; Vardavas C, Nikitara K, 2020*]. Recent study reported that current smokers and those with chronic obstructive pulmonary disease has a higher expression of the ACE-2 epithelial cells [*Leung J et al., 2020*]. As SARS-CoV 2 use ACE2 receptor as binding site, the up-regulation of ACE-2 caused by smoking could be harm for COVID-19 patients [*Cai G, 2020*]. However, many studies showed different result that seems up-regulation of ACE2 may become protective against disease severity [*Reynolds H et al., 2020*].

Angiotensin II side effects are neutralized by ACE2 by cutting angiotensin I and angiotensin II to angiotensin

based on the renin-angiotensin-aldosterone axis. Recently, expression of ACE2 decreased in the lungs and other tissues is found in smoking and nicotine [*Farsalinos K et al., 2020; Zhang H et al., 2020*]. Nicotine modify the of the equilibrium RAAS by upregulating the destructive ACE/angiotensin (ANG)-II/ANG II type 1 receptor axis and downregulating the compensatory ACE2/ANG-(1-7)/Mas receptor axis, contributing to the development of cardiovascular and pulmonary diseases [*Oakes J et al., 2018*]. A study showed ACE2 protects the development of ARDS in mice [*Guo Y et al., 2020*]. The recently observed up-regulation of ACE2 may be provoked as a defense way to neutralize the effects of angiotensin II in smokers [*Farsalinos K et al., 2020*]. Experimental data showed that organ damage and disease severity can be due to the continuous replication and infection of SARS-CoV-2 causing up-regulation of ACE2 regulation [*Reynolds H et al., 2020*]. Therefore, increasing of ACE2 does not due define an increase in disease susceptibility or severity and may in fact be worthwhile. In addition, there are conflicting findings about smoking in the literature, reports that smoking and nicotine reduce ACE2 were widely published before the COVID-19 pandemic [*Durmus N, Grunig G, 2018; Oakes J et al., 2018*]. Whereas, several studies published during the pandemic reported upregulation of ACE2 [*Cai G, 2020; Farsalinos K et al., 2020; Russo P et al., 2020*]. Therefore, it can be assumed that there are no strong conclusions concerning the result of nicotine or smoking on ACE2.

Recent study showed a series of 150 cases in COVID-19 patients, a high of cytokines IL-6 are predictors of mortality [*Ruan Q et al., 2020*]. In concordance, result also was found in study using 191 patients that increased IL-6 was found to be increased in patients who did not survive compared to patients who survived [*Zhou F et al., 2020*]. However, releasing a large of cytokine IL-6 in SARS-CoV-2 infection is activated by the innate immune system, which causes increased vascular permeability resulting in the process of alteration of fluids and blood cells to the alveoli, resulting in clinical appearance of dyspnea and respiratory failure [*Green K et al., 2019; Farsalinos K et al., 2020*]. The infection response that develops into ARDS was caused by an increase in the release of pro-inflammatory cytokines known as the cytokine storm phenomenon or cytokine release syndrome [*Jonge W, Ulloa L, 2007; Farsalinos K et al., 2020*]. Therefore, cytokine suppression effects are needed to against cytokine-mediated diseases, including endotoxemia and septicemia, which lead organ damage and death.

Nicotine agonists with cholinergic that regulate immunity through inflammatory responses and anti-inflammatory pathways [*Tracey K, 2010*]. In vitro studies conducted on animal models, acute respiratory distress syndrome can be prevented and expression of TNF in airway epithelial cells can be suppressed by nicotine [*Mabley J et al., 2011*]. In addition, in vivo studies conducted in humans unprotected to endotoxins, nicotine also showed anti-inflammatory effect [W*ittebole X et al., 2007*]. IL-6, IL-1 and TNF as pro-inflammatory cytokines are inhibited production but IL-10 as anti-inflammatory cytokines is not inhibited [*Ulloa L, 2005*].

Hypotheses regarding nicotine potential based on RAAS complex interactions and immunomodulatory effects can be explained. Measurable evidence regarding the risk of smokers with SARS-CoV-2 infection in some literature has not been proven. Thus, a population-based study is required to answer it. Clinical trials regarding cytokine storm as a therapeutic target are still being developed in COVID-19 patients. Recently, the US FDA approved a phase III clinical trial of IL-6 inhibitors as a reduction in the intensity of cytokine storms in nicotine utilization [Fa*rsalinos K et al., 2020*].

Will the antimalarial drug effective to combat COVID-19?

Nowadays, there was no definite and recommended therapy for COVID-19 due to the disease was still new. However, all antivirals used in COVID-19 therapy in almost all countries were still in trial and error. Like in other part of the world, there were no definitive guidelines to cure COVID-19 in Indonesia. As the COVID-19 outbreak began in China, Indonesia tried to refer to China regarding the drugs used, including Chloroquine. Chloroquine is well-known drug to treat malaria as an anti-plasmodium. Autoimmune diseases, including rheumatoid arthritis and lupus erythematosus also use chloroquine as therapy agent; hence it might have a potential to be a broad-spectrum antiviral drug [*Meo S et al., 2020*]. Increased pH of endosomes for viral fusion into cells and inhibition of cellular receptor glycosylation is known to be a way to inhibit SARS-CoV infection by Chloroquine [*Guo Y et al., 2020; Zhou F et al., 2020*]. A study of more than 100 COVID-19 patients showed usage of Chloroquine decrease the pneumonia exacerbations, long of symptoms and accelerated viral clearance without serious side effects [*Gao J et al., 2020; Mehra M et al., 2020*]. In addition, in vitro study showed hydroxychloroquine in the SARS outbreak was previously inform to have anti-SARS-CoV [*Madrid P et al., 2016; Retallack H et al., 2016*].

Hydroxychloroquine was a chloroquine analogue that reduces concern about drug interactions [*Jallouli M et al., 2015*]. In addition, hydroxychloroquine showed a stronger and less toxic. A study proves hydroxychloroquine has stronger antiviral activity shown by the EC_{50} value for hydroxychloroquine was smaller than the EC_{50} value for chloroquine [Li *C et al., 2017; Yao X et al., 2020*]. Hydroxychloroquine is a more soluble and safer metabolite of chloroquine, which causes less side effects [*Yao X et al.,*

Table 1

Studies report of hydrocloroquine and chloroquine in COVID-19 treatment

Type of study	Strategies	Intervention	Outcome	Ref
Clinical trial sample	30 patients	Hydrochloroquin, 400 *mg* per day for 5 days	• 86.7% of cases negative for virus nucleic acid on throat swabs specimen • Radiological progression in 33.3% cases	[*Doudier B, Courjon J, 2020*]
Clinical trial sample	100 patients	Chloroquine phosphate, dose not mentioned	• Chloroquine phosphate was important effect of clinical outcome and viral clearance • Exacerbation of pneumonia, improvement of lung image on radiological examination, negative virus examination and limitation of disease progression can be inhibited by chloroquine phosphate.	[*Gao J et al., 2020*]
Clinical trial sample	20 patients	Hydrochloroquin, 600 *mg* daily combined with azithromycin	• Hydrochloroquin was associated with the decrease and disappearance viral load of SARS-CoV-2 and azithromycin strengthens this effect	[*Doudier B, Courjon J, 2020*]
Expert Study Group Discussion	Expert consensus	Multicenter collaboration group	• It was recommended that chloroquine phosphate 500 mg twice per day for 10 years acceptable safety in treating COVID-19 associated pneumonia.	[*Meo SA et al., 2020*]

2020]. Indeed, both have immunomodulatory effects by suppressing the immune response which is resulted by SARS-CoV 2 infection and reducing the cytokine storm of IL-6 and IL-10; hence it may prevent the multi organ failure [*Huang C et al., 2020; Schrezenmeier E, Dörner T, 2020*]. Therefore, some consensus and research outcomes include Chloroquine and/or hydroxychloroquine as a drug-of-choice in preventing and treating COVID-19 (Table 1).

Current research data showed chloroquine-based therapy has promising result to combat COVID-19. Nonetheless, age, disease severity and clinical presentation are important to consider regarding the benefits of using chloroquine [*Touret F, de Lamballerie X, 2020*]. If clinical data confirm the biological results, chloroquine and hydroxychloroquine may be used in prophylaxis as well as curative treatment for individuals exposed to SARS-CoV-2 [*Meo S et al., 2020*]. Therefore, in future assessments of chloroquine dosage trials include high-quality clinical and safety data are required.

Conclusion

The COVID-19 is not only global health pandemic, but also multi sectoral issue. A massive sense of crisis towards COVID-19 leads to massive information flow among community which may leads to misleading information. Scientific paper should be become a basis for every people to answer the commonly asked question among society. With the current state of pandemic which the finish line seems to be long way ahead, the best way to encounter the pandemic is by massive appropriate diagnostic test then isolate them; hence it may break the infection chain.

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REFERENCES

1. *Bastos ML, Tavaziva G, Abidi SK, Campbell JR, Haraoui LP., et al.* Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. BMJ. 2020; 370: 2516
2. *Bogh MK, Gullstrand J, Svensson A, Ljunggren B, Dorkhan M.* Narrowband ultraviolet B three times per week is more effective in treating vitamin D deficiency than 1600 IU oral vitamin D3 per day: A randomized clinical trial. British Journal of Dermatology. 2012; 167(3): 625-630
3. *Bozkurt B, Kovacs R, Harrington B.* ACC/AHA Statement Addresses Concerns Re: Using RAAS Antagonists in COVID-19. J Card Fail. 2020; 26(5): 370

4. *Cai G.* Bulk and single-cell transcriptomics identify tobacco-use disparity in lung gene expression of ACE2, the receptor of 2019-nCov. medRxiv 2020. p. 2020.02.05.20020107. doi: 10.1101/2020.02.05.20020107
5. *CDCP* (Centers for Disease Control and Prevention I. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. www.cdc.gov. 2020 Available at: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html (Accessed: 20 April 2020)
6. *D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott, T.* UV radiation and the skin. International Journal of Molecular Sciences. 2013; 14(6): 12222-12248
7. *Davies A, Thompson KA, Giri K, Kafatos G, Walker J, Bennett A.* Testing the efficacy of homemade masks: would they protect in an influenza pandemic? Disaster medicine and public health preparedness. 2013; 7(4): 413-418
8. *Doudier B, Courjon J.* Hydroxychloroquine and azithromycin as a treatment of COVID-19; results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020; 56(1): 105949
9. *Duguid JP.* The size and the duration of air-carriage of respiratory droplets and droplet-nuclei. Journal of Hygiene. 1946; 44(6): 471-479
10. *Durmus N, Grunig G.* Air Pollution Exposure with Fine Dust Responses in the Pulmonary Vasculature and the Right Heart. Ann Am Thorac Soc. 2018; 15(2): S126
11. *Fang L, Karakiulakis G, Roth M.* Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? The Lancet Respiratory Medicine Elsevier Ltd. 2020; 8(4): e21
12. *Farsalinos K, Barbouni A, Niaura R.* Systematic review of the prevalence of current smoking among hospitalized COVID-19 patients in China: could nicotine be a therapeutic option? Intern Emerg Med. 2020; 15(5): 845-852
13. *Feng S, Shen C, Xia N, Song W, Fan M, Cowling BJ.* Rational use of face masks in the COVID-19 pandemic', The Lancet Respiratory Medicine. Elsevier Ltd. 2020; 2(20): 2019-2020
14. *Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB., et al.* Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation. 2005; 111(20): 2605-2610
15. *Furuhashi M, Moniwa N, Mita T, Fuseya T, Ishimura S., et al.* Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. American Journal of Hypertension. 2014; 28(1): 15-21
16. *Gao J, Tian Z, Yang X.* Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 2020; 14(1): 72-73
17. *Ginde AA, Mansbach JM, Camargo CA.* Association between Serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine. 2009; 169(4): 384-390
18. *González Maglio DH, Paz ML, Leoni J.* Sunlight Effects on Immune System: Is There Something Else in addition to UV-Induced Immunosuppression? Biomed Res Int. 2016; 2016: 1934518
19. *Grady D.* Coronavirus Test Kits Sent to States Are Flawed, C.D.C. Says, The New York Times. Available at: https://www.nytimes.com/2020/02/12/health/coronavirus-test-kits-cdc.html (Accessed: 20 April 2020)
20. *Green K, Graziadio S, Turner P, Fanshawe T, Allen J.* What tests could potentially be used for the screening, diagnosis and monitoring of COVID-19 and what are their advantages and disadvantages? 2 NIHR Newcastle In Vitro Diagnostics Co-operative Newcastle upon Tyne NHS Hospitals Foundation Trust, Newcast'. 2019
21. *Greiller CL, Martineau AR.* Modulation of the immune response to respiratory viruses by vitamin D. Nutrients. 2015; 7(6): 4240-4270
22. *Guan W, Ni Z, Hu Y, Liang W, Ou C, He J., et al.* Clinical characteristics of coronavirus disease 2019 in China. New England Journal of Medicine. 2020; 382(18): 1708-1720
23. *Guo YR, Cao QD, Hong ZS, Tan YY, Chen SD., et al.* The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Mil Med Res. 2020; 7(1): 11
24. *Haddad JG, Matsuoka LY, Hollis BW, Hu YZ, Wortsman J.* Human Plasma Transport of Vitamin D after Its Endogenous Synthesis. J Clin Invest. 1993; 91(6): 2552-2555
25. *Hoel DG, Berwick M, de Gruijl FR, Holick MF.* The risks and benefits of sun exposure. Dermato-Endocrinology. 2016; 8(1): e1248325
26. *Holick MF, MacLaughlin JA, Doppelt SH.* Regulation of cutaneous previtamin D3 photosynthesis in man: Skin pigment is not an essential regulator. Science. 1981; 211(4482): 590-593
27. *Holick MF.* Conferencia del Premio McCollum, 1994: Vitamina D: nuevos horizontes para el siglo XXI. The American Journal of Clinical Nutrition. 1994; 60(4): 619-630

28. *Howard J, Huang A, Li Z, Tufekci Z, Zdimal V., et al.* Face Masks Against COVID-19: An Evidence Review. Preprints. 2020, 2020040203 (doi: 10.20944/preprints202004.0203.v1)
29. *Huang C, Wang Y, Li X, Ren L, Zhao J., et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020; 395(10223): 497-506
30. *Intan G* [Jokowi Instructs Mass Holds Corona Rapid Test] [Published in Bahasa]. www.voaindonesia.com. Available at: https://www.voaindonesia.com/a/jokowi-instruksikan-gelar-tes-cepat-corona-secara-massal/5335428.html (Accessed: 20 April 2020)
31. *Jallouli M, Galicier L, Zahr N, Aumaître O, Francès C., et al.* Determinants of hydroxychloroquine blood concentration variations in systemic lupus erythematosus. Arthritis and Rheumatology. 2015; 67(8): 2176-2184
32. *Jonge WJ, Ulloa L.* The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. British Journal of Pharmacology, 2007; 151(7): 915-929
33. *Kenny DE, O'Hara TM, Chen TC, Lu Z, Tian X, Holick MF.* Vitamin D content in Alaskan Arctic zooplankton, fishes, and marine mammals', Zoo Biology. 2004; 23(1): 33-43
34. *Kimbal A, Hatfield KM, Arons M, James A, Taylor J., et al.* Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility – King County, Washington, March 2020. Morbidity and Mortality Weekly Report. 2020; 69(13): 377-381
35. *Kuba K, Imai Y, Ohto-Nakanishi T, Penninger, JM., et al.* Trilogy of ACE2: A peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. Pharmacology and Therapeutics. Elsevier Inc. 2010; 128(1): 119-128
36. *Leung JM, Yang CX, Sin DD.* COVID-19 and Nicotine as a Mediator of ACE-2. Eur Respir J. 2020 Jun; 55(6): 2001261
37. *Li C, Zhu X, Ji X, Quanquin N, Deng YQ. Tian, M. et al.* Chloroquine, a FDA-approved Drug, Prevents Zika Virus Infection and its Associated Congenital Microcephaly in Mice. EBio Medicine. Elsevier B.V. 2017; 24: 189-194
38. *Li F.* Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005; 309(5742): 1864-1868
39. *Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M., et al.* Convergence of IL-1β and VDR activation pathways in human TLR2/1-induced antimicrobial responses. PLoS ONE. 2009; 4(6): e5810
40. *Liu PT.* Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006; 311(5768): 1770-1773
41. *Mabley J, Gordon S, Pacher P.* Nicotine exerts an anti-inflammatory effect in a murine model of acute lung injury'. Inflammation. 2011; 34(4): 231-237
42. *Maclntyre CR, Cauchemez S, Dwyer DE, Seale H, Cheung P., et al.* Face mask use and control of respiratory virus transmission in households. Emerging Infectious Diseases. 2009; 15(2): 233-241
43. *Madrid PB, Panchal RG, Warren TK, Shurtleff AC, Endsley AN., et al.* Evaluation of Ebola Virus Inhibitors for Drug Repurposing. ACS Infectious Diseases. 2016; 1(7): 317-326
44. *Maitra S, Biswas M, Bhattacharjee S.* Case-fatality rate in COVID- 19 patients: A meta-analysis of publicly accessible database. medRxiv. 2020; 020.04.09.20059683. doi: 10.1101/2020.04.09.20059683
45. *Maruotti N, Cantatore FP.* Vitamin D and the immune system. Journal of Rheumatology. 2010; 37(3): 491-495
46. *Mehra MR, Desai SS, Ruschitzka F, Pate AP* Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet (London, England). Elsevier Ltd. 2020; 6736(20): 1-10
47. *Meo SA, Klonoff DC, Akram J.* Efficacy of chloroquine and hydroxychloroquine in the treatment of COVID-19. European review for medical and pharmacological sciences. 2020; 24(8): 4539-4547
48. *Morawska L, Johnson GR, Ristovski ZD, Hargreaves M, Mengersen K., et al.* Size distribution and sites of origin of droplets expelled from the human respiratory tract during expiratory activities. Journal of Aerosol Science. 2009; 40(3): 256-269
49. *Morens DM, Folkers G.K, Fauci AS.* What Is a Pandemic? The Journal of Infectious Diseases. 2009; 200(7): 1018-1021
50. *Oakes JM, Fuchs RM, Gardner JD, Lazartigues E, Yue X.* Nicotine and the Renin-Angiotensin System. Am J Physiol Regul Integr Comp Physiol. 2018; 315(5): R895-R906
51. *Pan X, Chen D, Xia Y, Wu X, Li T., et al.* Asymptomatic cases in a family cluster with SARS-CoV-2 infection', The Lancet Infectious Diseases. Elsevier Ltd. 2020; 20(4): 410-411
52. *Papineni RS, Rosenthal FS.* The size distribution of droplets in the exhaled breath of healthy human subjects. Journal of Aerosol Medicine: Deposition, Clearance, and Effects in the Lung. 1997; 10(2): 105-116

53. *Patel R, Babady E, Theel ES, Storch GA, Pinsky BA., et al.* Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: Value of Diagnostic Testing for SARS–CoV-2/ COVID-19. mBio. 2020; 11(2): e00722-20

54. *Patel VB, Clarke N, Wang Z, Fan D, Parajuli N., et al.* Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: A positive feedback mechanism in the RAS. Journal of Molecular and Cellular Cardiology. Elsevier Ltd. 2014; 66: 167-176

55. *Peltzer K, Pengpid S.* The Prevalence and Social Determinants of Hypertension among Adults in Indonesia: A Cross-Sectional Population-Based National Survey. International Journal of Hypertension. Int J Hypertens. 2018; 2018: 5610725

56. *Ramanathan K, Antognini D, Combes A, Paden M, Zakhary B., et al.* Planning and provision of ECMO services for severe ARDS during the COVID-19 pandemic and other outbreaks of emerging infectious diseases. Lancet Respir Med. https://doi.org/10.1016/ s2213-2600(20)30121-1. https://doi.org/.

57. *Raoult D, Zumla A, Locatelli F, Ippolito G, Kroemer G.* Coronavirus infections: Epidemiological, clinical and immunological features and hypotheses. Cell Stress. 2020; 4(4): 66-75

58. *Rengasamy S, Eimer B, Shaffer RE.* Simple respiratory protection - Evaluation of the filtration performance of cloth masks and common fabric materials against 20-1000 nm size particles. Annals of Occupational Hygiene. 2010; 54(7): 789-798

59. *Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT., et al.* Zika virus cell tropism in the developing human brain and inhibition by azithromycin. Proceedings of the National Academy of Sciences of the United States of America. 2016; 113(50): 14408-14413

60. *Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Iturrate E., et al.* Renin-Angiotensin-Aldosterone System Inhibitors and Risk of Covid-19. N Engl J Med. 2020; 382(25): 2441-2448

61. *Rico-Mesa JS, White A, Anderson AS.* Outcomes in Patients with COVID-19 Infection Taking ACEI/ ARB. Current Cardiology Reports. 2020; 22(5): 20-23

62. *Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G., et al.* Transmission of 2019-NCOV infection from an asymptomatic contact in Germany. New England Journal of Medicine. 2020; 382(10): 970-971

63. *Ruan Q, Yang K, Wang W, Jiang L, Song J.* Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine. Springer Berlin Heidelberg. 2020; 46(5): 846-848

64. *Russo P, Bonassi S, Giacconi R, Malavolta M, Tomino C, Maggi F.* COVID-19 and Smoking. Is Nicotine the Hidden Link? The European respiratory journal. 2020; 55: 2001116. doi: 10.1183/13993003.01116-2020.

65. *Santoso A, Kiat NW.* AFC AFC Position Statement on ACE-I and ARBs use related to COVID-19 outbreak. Indonesian Heart Association. 2020. http:// www.inaheart.org/. Available at: http://www.inaheart.org/news_and_events/news/2020/3/20/afc_ statement_19march2020

66. *Schrezenmeier E, Dörner T.* Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nature Reviews Rheumatology. 2020; 16(3):155-166

67. *Setiati S.* [The Effect of Sun's Ultraviolet B Rays on the Concentration of Vitamin D and Parathyroid Hormone in Indonesian Elderly Women] [Published in Bahasa]. Kesehatan. 2008; 2(UV B): 1-7

68. *Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM.* Classical renin-angiotensin system in kidney physiology. Comprehensive Physiology. 2014; 3: 1201-1228

69. *Sutanto I, Endawati D, Ling L, Laihad F, Setiabudy R, Baird JK.* Evaluation of chloroquine therapy for vivax and falciparum malaria in southern Sumatra, western Indonesia. Malaria Journal. 2010; 9(1): 11-15

70. *Touret F, de Lamballerie X.* Of chloroquine and COVID-19. Antiviral Research Elsevier. 2020; 177: 104762

71. *Tracey KJ.* Physiology and immunology of the cholinergic anti-inflammatory pathway. Journal of Japanese Association for Acute Medicine, 2010; 21(2): 230-244

72. *Trummer C, Pandis M, Verheyen N, Grübler M, Gaksch M., et al.* Beneficial effects of UV-radiation: Vitamin D and beyond. International Journal of Environmental Research and Public Health. 2016; 13(10): 1-16

73. *Ulloa L.* The vagus nerve and the nicotinic anti-inflammatory pathway. Nature Reviews Drug Discovery. 2005; 4(8): 673-684

74. *Urashima M, Segawa T, Okazak M, Kurihara M, Wada Y, Ida H.* Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. American Journal of Clinical Nutrition. 2010; 91(5): 1255-1260

75. *Vardavas CI, Nikitara K.* COVID-19 and smoking: A systematic review of the evidence. Tobacco Induced Diseases. 2020; 18: 1-4

76. *Wacker M, Holick MF.* A global perspective for health. Dermato-Endocrinology. 2013; 5: 51-108

77. *Wan Y, Shang J, Graham R, Bari RS, Li F.* Receptor Recognition by the Novel Coronavirus from Wuhan: An Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. Journal of Virology. 2020; 94(7): e00127-20

78. *Wei WE, Li Z, Chiew CJ, Yong SE, Toh MP, Lee VJ.* Presymptomatic Transmission of SARS-CoV-2-Singapore. Morbidity and mortality weekly report. 2020; 69(14): 411-415

79. *WHO*. Coronavirus disease 2019 (COVID-19) Situation Report – 73. www.who.int. 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200402-sitrep-73-Covid-19.pdf (Accessed: 22 April 2020)

80. *Wittebole X, Hahm S, Coyle SM, Kumar A, Calvano SE., et al.* Nicotine exposure alters in vivo human responses to endotoxin. Clinical and Experimental Immunology. 2007;147(1): 28-34

81. *Xu X, Chen P, Wang J, Feng J, Zhou H., et al.* Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Science China Life Sciences. 2020; 63(3) :457-460

82. *Yao X, Ye F, Zhang M, Cui C, Huang B., et al.* In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome. Coronavirus 2 (SARS-CoV-2). Clin Infect Dis. 2020; 71(15):732-739

83. *Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS., et al.* Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Medicine Springer Berlin Heidelberg. 2020; 46(4): 586-590

84. *Zhou F, Yu T, Du R, Fan G, Liu Y., et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet Elsevier Ltd. 2020; 395(10229): 1054-1062

85. *Zou L, Ruan F.,Huang M, Liang L, Huang H, Hong Z., et al.* SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients. N Engl J Med. 2020; 382(12): 1177-1179

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ANTIBODY RESPONSE TO SARS-COV-2 WITHIN HEALTHCARE WORKERS: A SINGLE-CENTER STUDY IN KAZAKHSTAN

Tajibayev T.K., Sagatov I.Y.*, Bekmuhamedova A.Y., Medeubekov U.S., Baimakhanov B.B.

JSC "National Scientific Center of Surgery named after A.N. Syzganov", Almaty, Kazakhstan

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Abstract

The first severe acute respiratory syndrome coronavirus 2 outbreak was reported in December 2019, and the virus has rapidly spread worldwide. The antibody response in infected patients remains largely unknown, and the clinical value of antibody testing has not been fully demonstrated. In the period from the end of June to July in Kazakhstan, there was a sharp increase in the incidence.

Present study aimed to evaluate the incidence rate and the development of immune response among healthcare workers during the height of the severe acute respiratory syndrome coronavirus 2 pandemic in Kazakhstan.

The prospective study was carried out on the basis of the Syzganov National Scientific Center of Surgery in the period from July 2020 to August 2020. The project involved 248 people.

In total, there were 50 men and 198 women under study, aged 21 to 80 years, with the average age of men being 43.3 ± 0.2 years, women - 37.4 ± 0.3 years. The total percentage of antibody detection was 11.7% (29 participants). The largest percentage of the development of an immune response was among junior medical personnel IgG was detected in 10 (4%)Immunoglobulin M (IgM) in 1 (0.4%), while among the middle and senior medical personnel IgG and IgM was 7 (2.8%) and 1 (0.4%), respectively. Antibodies were not detected among paraclinical and technical personnel. Of the 29 seropositive results, IgG was detected in 24, IgM in 3, and IgG + IgM in 2, respectively.

The presence of immune response among medical personnel, a significant percentage of infection was in clinical personnel compared to paraclinical and technical personnel. The majority of subjects with a seropositive result had an asymptomatic course.

Keywords: *healthcare workers, COVID-19, severe acute respiratory syndrome coronavirus 2, IgM, IgG, Kazakhstan.*

Introduction

The first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak was reported in December 2019, and the virus has rapidly spread worldwide and become a pandemic within 3 months [*Zhu N et al., 2020*].

Severe cases of COVID-19 might eventually develop acute respiratory distress syndrome (ARDS), septic shock, multiple organ failure, bleeding, and coagulation dysfunction [*Chen N et al., 2020; Huang C et al., 2020*] and is featured by pneumonia, lymphopenia, exhausted lymphocytes, and elevated serum levels of proinflammatory cytokines characterized as a cytokine storm [*Zhuang M et al., 2020*]. Two previously identified coronaviruses, SARS-CoV and MERS-CoV (Middle East respiratory syndrome coronavirus), caused severe pneumonia but, unlike SARS-CoV-2, exhibited only limited person-to-person spread, resulting in dramatically lower numbers of confirmed cases (8,100 and 2,500, respectively) [*Sariol A, Perlman S, 2020*].

The novel coronavirus SARS-CoV-2 is a newly emerging virus. The antibody response in infected patients remains largely unknown, and the clinical value of antibody testing has not been fully demonstrated [*Mangalmurti N, Hunter A, 2020*].

At the time of submission of the article (October 18, 2020), 39 596 858 confirmed cases of COVID-19, including 1 107 374 deaths, were registered worldwide at WHO (2020).

Address for Correspondence:

Inkar Y. Sagatov, MD, PhD
JSC "National Scientific Center of Surgery"
62 Zheltoksan Street, Almaty 050004, Kazakhstan
Tel: +7 708 155 15 77
E-mail: inkar_sagatov@mail.ru

Kazakhstan is also one of the countries severely affected by COVID-19. In the period from the end of June to July in Kazakhstan, there was a sharp increase in the incidence, moreover, today Kazakhstan is in the "red" zone of most countries of the world due to the disappointing epidemiological situation. According to the official portal of Covid-19 in Kazakhstan [*MH RK, 2020*], at the time of submission of the article (October 18, 2020) the number of registered cases of SARS-COV-2 reached more than 109 thousand, as well as pneumonia with signs of coronavirus infection in more than 35 thousand cases.

A great public response in the country was the incidence of the dramatic number of infected healthcare workers in infection hospitals, as well as provisional hospitals, which covered the entire Republic. Moreover, institutions that were not engaged in the diagnosis and treatment of patients with COVID-19 (Scientific centers, non-infection multidisciplinary hospitals, non-infection private medical centers), but in which a large number of infected among the medical staff were detected, were quarantined. The reason for this crisis in the Healthcare system was many factors that are clarifying up to this time. According to Wang J. and co-authors, the increase in awareness of personal protection, sufficient personal protective equipment, and proper preparedness and response would play an important role in lowering the risk of infection for healthcare workers [*Wang J et al., 2020*].

Currently, the real time Reverse transcription polymerase chain reaction (RT-PCR) assay is the gold-standard method to diagnose SARS-CoV-2. However, false-negative cases have been reported due to problems with sample collection and transportation, RNA extraction, enzyme inhibitors, and the RT-PCR method [*Yang Y et al., 2020*]. By contrast, conventional serological assays, such as the enzyme-linked immunoassay (ELISA) for specific IgM and IgG antibodies, have a high-throughput advantage, and they avoid false-negative cases that occur with the RT-PCR method [*Xiao S et al., 2020*]

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

Thus, we initiated a study to determine the percentage of morbidity among medical institutions in institutions not related to the provision of medical services to patients with COVID-19.

Present study aimed to evaluate the incidence rate and the development of immune response among healthcare workers during the height of the SARS-COV-2 pandemic in Kazakhstan.

MATERIAL AND METHODS

The prospective study was carried out on the basis of the Syzganov National Scientific Center of Surgery in the period from July 2020 to August 2020. The project involved 248 people, consisting of junior (medical orderly), middle (nurses, laboratory assistants, pharmacists) and senior medical staff (doctors), as well as paraclinical (social workers, medical registrars, managers, scientific and educational department, administration) and technical staff (technical department, security, catering workers). Before the start of the study, a questionnaire was conducted for the presence of current or past symptoms (fever, cough, weakness, rapid breathing), as well as the presence of significant comorbidities (diabetes mellitus, cardiovascular or cerebrovascular pathology, chronic respiratory diseases, oncology).

The serum was collected at distinctive time points, and SARS-CoV-2–specific antibodies were detected using "New Coronavirus (2019-nCoV) Antibody Detection Kit" (Innovita, China). By enzyme-linked immunosorbent assay to assess the presence of an immune antibodies IgG, IgM was performed. Upon detection of the acute phase of the virus within medical stuff went into quarantine for a period of 2 weeks, followed by repeated testing.

The study protocol was approved by our Institutional Local Research Ethics Committee (26/07/2020), and the study protocol was developed by conforming with the ethical standards of the Declaration of Helsinki (1964). All participants in the study submitted informed consent.

RESULTS

In total, there were 50 men and 198 women under study, aged 21 to 80 years, with the average age of men being 43.3 ± 0.2 years, women - 37.4 ± 0.3 years. The total percentage of antibody detection was 11.7% (29 participants). The largest percentage of the development of an immune response was among junior medical personnel IgG was detected in 10 (4%) IgM in 1 (0.4%), while among the middle and senior medical personnel IgG and IgM was 7 (2.8%) and 1 (0.4%), respectively. However, in two cases, one each in senior and middle medical personnel, there was the presence of IgG + IgM antibodies. An-

Table 1

The presence of an immune response among different categories of medical workers

Category	N=248	IgG n (%)	IgM n (%)	IgG+IgM n (%)
Junior medical stuff	68	10 (4.0%)	1 (0.4%)	0 (0%)
Middle medical stuff	102	7 (2.8%)	1 (0.4%)	1 (0.4%)
Senior medical stuff	63	7 (2.8%)	1 (0.4%)	1 (0.4%)
Paraclinical personnel	10	0 (0%)	0 (0%)	0 (0%)
Technical and other personnel	5	0 (0%)	0 (0%)	0 (0%)

tibodies were not detected among paraclinical and technical personnel (Table 1).

During the study, a significant number (n = 18; 62%) of medical personnel with a seropositive result had no symptoms. Of the 29 seropositive results, IgG was detected in 24, IgM in 3, and IgG + IgM in 2, respectively. IgG antibodies were detected in 4 patients with significant comorbidities. The detailed structure of seropositive exposure can be examined in table 2.

Table 2

Features of subjects with a seropositive result

	N=29	IgG	IgM	IgG+IgM
Male	8 (27.5%)	6 (20.6%)	2 (6.9%)	0 (0%)
Female	21 (72.5%)	18 (79.4%)	1 (3.4%)	2 (6.9%)
Presence of current symptoms	3 (10.3%)	0 (0%)	2 (6.9%)	1 (3.4%)
Presence of past symptoms	8 (27.5%)	7 (24.1%)	1 (3.4%)	0 (0%)
Asymptomatic	18 (62.0%)	17 (58.6%)	0 (0%)	1 (3.4%)
Presence of significant comorbidities	4 (13.7%)	4 (13.8%)	0 (0%)	0 (0%)

Conclusion

Thus, in the course of studying the presence of immune response among medical personnel, a significant percentage of infection was in clinical personnel compared to paraclinical and technical personnel, of which junior medical personnel had the highest number of antibodies. This is due to the fact that clinical personnel are in direct contact with patients. The majority of subjects with a seropositive result had an asymptomatic course. Further research in this field is needed.

REFERENCES

1. *Chen N, Zhou M, Dong X, Qu J, Gong F., et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395(10223): 507□513
2. *Declaration of Helsinki*. Human Experimentation: Code of Ethics of the World Medical Association. BMJ. 1964; 2(5402): 177-177
3. *Huang C, Wang Y, Li X, Ren L, Zhao J., et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223): 497-506
4. *Mangalmurti N, Hunter AC.* Cytokine Storms: Understanding COVID-19. 2020; 53(1): 19-25
5. *MH RK*. Official information of Covid-19 in Kazakhstan, hotline of the ministry of health of the republic of Kazakhstan (MH RK). 2020. www.coronavirus2020.kz
6. *Sariol A, Perlman S.* Lessons for COVID-19 immunity from other coronavirus infections. Immunity. 2020; 53(2): 248-263
7. *Wang J, Zhou M, Liu F*. Reasons for healthcare workers becoming infected with novel coronavirus disease 2019 (COVID-19) in China. J Hosp Infect. 2020; 105(1): 100-101
8. *World Health Organization (WHO)*. Coronavirus disease (COVID-19) outbreak. 2020. Available at: https://www.who.int/emergencies/
9. *Xiao SY, Wu Y, Liu H*. Evolving status of the 2019 novel coronavirus infection: proposal of conventional serologic assays for disease diagnosis and infection monitoring. J Med Virol. 2020; 92: 464-467
10. *Yang Y, Shen C, Wang F, Yuan J, Li J., et al.* Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv2020: 2020.02.11.20021493
11. *Zhu N, Zhang D, Wang W, Li XY, Song J., et al.* A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; 382(8): 727□733
12. *Zhuang MW, Cheng Y, Zhang J, Jiang XM, Wang Li., et al.* Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. Journal of Medical Virology. 2020; 92(11): doi:10.1002/jmv.26139