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REVIEW ARTICLE

HYPOTHESES FOR REACTIVATION RECURRENCE AND REINFECTION RECURRENCE OF COVID-19

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ABSTRACT

COVID-19 virus of the family *Coronaviridae*, is an enveloped virus with RNA nucleic acid. The virus spread rapidly from China and was declared a pandemic by the World Health Organization on March 11, 2020. Studies show that some patients who recovered from COVID-19 had a recurrence that arose concern among the scientists. The main purpose of this review is to provide potential hypotheses for the recurrence of COVID-19 infection in both clinical reactivation and reinfection. In reactivation recurrence, hypotheses such as hereditary immunodeficiency, the balance between angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme2 (ACE2), the presence of extracellular exosomes were presented, and in the field of reinfection recurrence, hypotheses such as a false primary RT-PCR test deals with positive, low load of COVID-19 virus, acquired immune system defects and mutations in viral RNA that alter viral epitopes were presented in this review. From the hypotheses presented in this paper, it can be concluded that an imbalance between ACE / AngII / AT1R and ACE2 / Ang1-7 / MasR and the defects in the immune system, can cause defects in the proliferation of NK cells and T lymphocytes, and exosomes contain viral mRNA ultimately leads to reactivation recurrence of the disease. Reinfection recurrence of the COVID-19 may be as a result of the primary false positive report in RT-PCR test, low viral load and inactivation of the immune system, defects in the acquired immune system, and mutations in virus RNA.

Keywords: COVID-19, reactivation, recurrence, reinfection, SARS-COV2

Abbreviations: ACE: angiotensin-converting enzyme, RT-PCR: reverse transcription polymerase chain reaction, CD: cluster differentiation

1. INTRODUCTION

Over the past two decades, coronaviruses have led to three major outbreaks, starting with SARS-CoV in 2002, then MERS-CoV in 2012, and most recently SARS-CoV-2 (COVID-19). Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. The disease spread rapidly from Wuhan, China; and was declared a pandemic by the World Health Organization on March 11, 2020. Due to the expansion of COVID-19 epidemy in different parts of the world, more

and more individuals are infected and of course, many people will be recovering from this viral infection (Abd El-Aziz and Stockand, 2020).

People with COVID-19 have a wide range of symptoms. Nearly all symptomatic COVID-19 patients experienced fever, cough or shortness of breath; however, a wide variety of other symptoms were also reported. The virus binds to angiotensin-converting receptors so it can affect any organ in the body. In critically ill patients, multiple organs are often affected (Ohadian, 2020). COVID-19 infection in patients can be grouped in any of the following stages:

Stage I: Asymptomatic incubation period with or without detectable virus

Stage II: Period of non-serious symptoms with detectable virus

Stage III: Period of severe respiratory symptoms and high viral load

One of the most important concerns during COVID-19 epidemy is the recurrence of infection. Unfortunately, there is inadequate information about reinfection (Roy, 2020). Elrashdy *et al.* (2020) reported that 9.1% of the discharged patients experienced reactivation of COVID-19 infection. Further, the clinical characteristics of patients with COVID-19 infection reactivation were similar to those who fully recovered from COVID-19 infection. This suggested that a proportion of affected patients are not immune to COVID-19 virus after recovery (Zheng *et al.*, 2020; Elrashdy *et al*., 2020).

There are two categories of patients who experience COVID-19 infection for the second time. The first category comprises of the patients who have only improved in terms of symptoms (reactivation group) and the second category comprise of the people who recovered from an infection, confirmed by a negative RT-PCR test (reinfection group). Based on the information generated on COVID-19 relapse, there are several principal hypotheses for reactivation and reinfection mechanisms (Alizargar, 2020).

The hypotheses for COVID-19 reactivation are:

i. Inherited or acquisitive immunodeficiency diseases
ii. Imbalance between angiotensin-converting enzyme (ACE) and (ACE2)
iii. The presence of extracellular exosomes

The hypotheses for COVID-19 reinfection are:

i. A false-positive primary RT-PCR test result;
ii. Low load of COVID-19 virus presented in the upper or lower respiratory system and they eliminated by the innate immune response;
iii. Adaptive immune deficiency causes a low count of memory B-cell or T-cell;
iv. Gene mutation in RNA which affects the viral epitopes

The main purpose of this review is to present hypotheses for recurrence of COVID-19 infection in the patients who had COVID-19 once before and also to discuss each of the hypotheses to elucidate the most likely causes of reactivation recurrence and reinfection recurrence of COVID-19 infection.

2. GENERAL STAGES OF COVID-19 VIRUS REPLICATION CYCLE

A. ***Attachment, penetration, and uncoating:*** The first step in viral infection is binding by reacting the virus with a specific receptor (ACE2) on the cell surface (Fig. 1). Each susceptible cell+ may have up to 100,000 receptor sites for a virus. The next step is penetration, in which COVID-19 virus is absorbed into the cell by endocytosis through ACE2 receptor. Then, it is the coating step, which occurs at the same time as the infiltration or shortly thereafter.

B. ***Genome expression and synthesis of virus components*:** After uncoating, the synthesis phase begins. Specific mRNA molecules whose resulting proteins are required to initiate replication must first be transcribed from the viral nucleic acid.

C. ***Formation and release of COVID-19 virus*:** Newly synthesized viral genomes combine with capsid polypeptides to create a new generation virus [Carroll *et al.*, 2015].

3. CORONAVIRUS REPLICATION CYCLE

The first step after uncoating the virus is to translate the virus genomic RNA to produce virus-specific RNA-dependent RNA polymerase. Viral polymerase transcribes a minus-strand complement, which

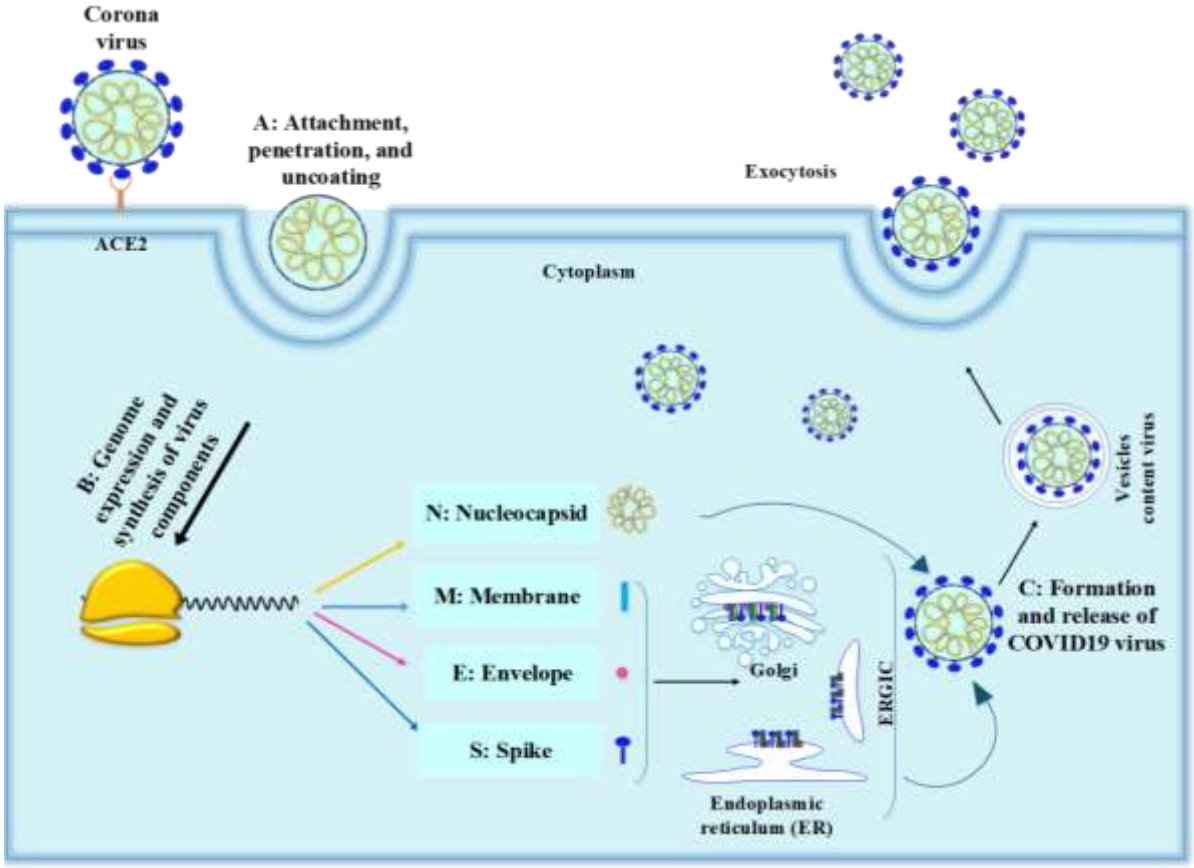

Fig. 1: General stages of COVID-19 virus replication cycle; A) The virus first binds to the ACE2 receptor, then penetration and uncoating occur. B) In host cells, genome expression and synthesis of virus components occur. C) The viral components assemble to form a complete virus and the COVID19 virus leaves the host cell.

acts as a template for a nested set of 5-7 sub-genomic mRNAs. Only 5´ terminal gene sequence is translated from each mRNA. Because each sub-genomic mRNA translates into a single polypeptide, polyprotein precursors are not common in coronavirus infections (Abd El-Aziz and Stockand, 2020). The genomic RNA encodes a large protein that is processed to produce viral RNA polymerase. New synthesized genomic RNA molecules in the cytoplasm interact with nucleocapsid proteins to form helical nucleocapsids. There is a preferred binding site for protein N in leader RNA.

Nucleocapsids germinate through the membranes of rough endoplasmic reticulum and Golgi apparatus in the areas where viral glycoproteins are present. Adult viruses may then be transmitted to the cell surface through vesicles to exit the cell, or they may wait in the cell for release (Fig. 2). Given the above, it is possible, the exosomes can bind to and enter other cells in the body through extra-exosomal tetraspanin receptors such as CD9, CD81 and CD63 (Hassanpour *et al.*, 2020; Crenshaw *et al.*, 2018).

The marker CD81 on the surface of exosomes is an immunoglobulin that can interact with CD36, which is present on many cells in the body. CD81 can also complex with CD19 and CD21 receptors on the surface of B lymphocytes (Hatano *et al.*, 2013; Crenshaw *et al.*, 2018; Calvo and Izquierdo, 2020; Kato *et al.*, 2020;). CD81 can bind to CD4 receptor on the surface of regulatory T cells and CD8 on the surface of cytotoxic T cells. This communication with the lymphocytes probably leads to the entry of exosomes into the lymphocytes. The entry of exosomes of viral mRNA content into T lymphocytes can lead to the infection of these lymphocytes and the production of a complete virus in these cells, and ultimately it is likely to lead to T lymphocyte apoptosis. T lymphocyte counts

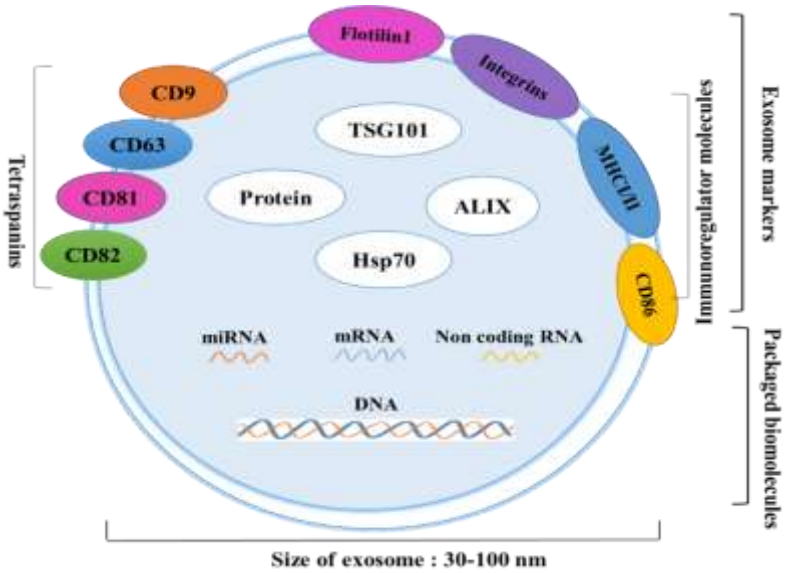

Fig. 2: Overview of exosomes, surface markers of exosomes including CD9, CD81, CD63, Flotillin1 and Integrin; Exosomes can target other cells in the body due to the presence of tetraspanins.

have been reported to decrease in patients with COVID-19; such a mechanism is suggested for the reduction of T lymphocytes in patients with COVID-19. Eventually, it leads to a defect in immune system and the body does not fully respond to viral infection, which can be a reason for the recurrence of disease (Fig. 3) (Flint *et al*., 1999; Petracca *et al*., 2000).

4. HYPOTHESES FOR REACTIVATION RECURRENCE OF COVID-19

4.1 Inherited or acquisitive immunodeficiency diseases

Among people infected with COVID-19, there are reports that some people have a reactivation recurrence of COVID-19, which may have several causes. Given that the recurrence rate of COVID-19 is not widespread in the population, it may be related to inherited immunodeficiency diseases. These inherited immunodeficiency diseases are uncommon and are sometimes associated with a severe onset and an early diagnosis. The presence of such changes may be one of the reasons for the recurrence of COVID-19. Inherited immune deficiencies abnormalities are uncommon and sometimes associated with serious conditions leading to the early diagnosis. Complement-related abnormalities, including deficiencies in the third component of complement (C3), cause abnormalities leading to problems in fighting febrile infections (Nori *et al.*, 2020). This may be one of the reasons for reactivation recurrence in patients who have had COVID-19 once (Turnpenny. 2020). There are wide range of inherited immunodeficiency diseases which are summarized in Table 1.

Table 1: Inherited immunodeficiency diseases

Inherited immunodeficiency disease	Inherited primary immune disorders	Innate immune Disorders	Humoral innate immune disorders	➢ Complement anomalies ➢ Defects in NF-kB signaling
			Cell-mediated innate immune disorders	➢ Chronic granulomatous disease ➢ Neutropenias ➢ Leukocyte adhesion deficiency
				➢ Candidiasis of autoimmune polyendocrinopathy ➢ Ectodermal dysplasia syndrome
		Acquired immune disorders	Acquired humoral immune abnormalities	➢ Bruton-type agammaglobulinemia ➢ Hyper IgM syndrome ➢ Hyper IgE syndrome ➢ Common variable immune deficiency (CVID)
				➢ Special acquired immune disorders mediated by cells ➢ Severe combined immune-deficiency (SCID)

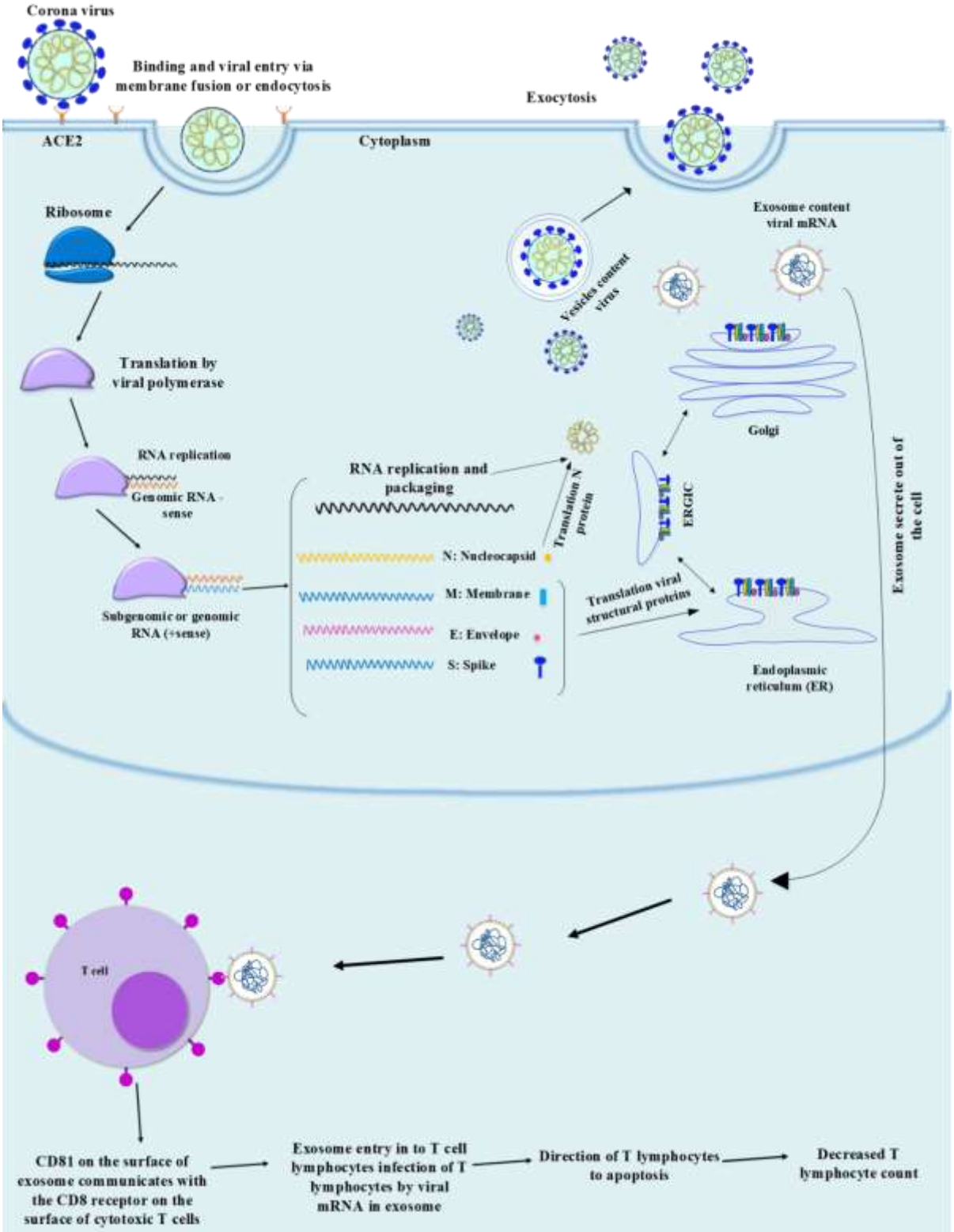

Fig. 3: Exosomes are potential mediators of viral infection, reinfection and reactivation; This figure shows the COVID19 virus replicating in host cell and the entry of viral components, including viral mRNA, into exosomes, secreting exosomes out of the cell, and binding to T lymphocytes by CD81 receptor.

4.2 ACE and ACE2 balance

ACE2 is the main receptor for COVID-19 virus in the body. ACE2 has an extracellular domain that is where the spike(s) protein of SARSCOV-2 binds and the virus enters the cell. ACE2 is an important component of the Renin-angiotensin-aldosterone system (RAS), which is an important regulator of blood pressure homeostasis. Renin is a protease secreted by the kidney that breaks down angiotensinogen into angiotensin I, and then breaks down into angiotensin II by ACE, which is secreted by the kidneys and lungs. Angiotensin II binds to the angiotensin I receptor (AT1) and causes vasoconstriction and the secretion of aldosterone and antidiuretic hormone and reabsorption, leading to high blood pressure and inflammation, fibrosis, and oxidative stress (Razeghian-Jahromi *et al.*, 2020; Sarzani *et al.*, 2020; Zemlin and Wiese, 2020).

ACE2 in RAS cleaves angiotensin I residue for the production angiotensin-(1-9) and an angiotensin II residue for production angiotensin-(1-7). Although ACE2 mRNA is found in almost all organs, its protein expression occurs mainly in lungs, heart, kidneys, and intestines. (Albini *et al*., 2020; Turner and Kodali, 2020; Kai and Kai, 2020).

There are two forms of ACE2 in the body, one in solution and the other consisting of a membrane anchor and an extracellular domain that binds to the COVID-19 virus. It has been suggested that the soluble form of ACE2 may inhibit the binding of the COVID-19 virus to the cell membrane surface (Behl *et al.*, 2020b; Zemlin and Wiese, 2020; Liu *et al*., 2019; Datta *et al.*, 2020; Vaduganathan *et al.*, 2020). When ACE2 is inactivated, it cannot convert angiotensin II to angiotensin-(1-7). Angiotensin II accumulates in the cell. Because ACE2 is a receptor for the COVID-19 virus in the body, by binding ACE2 to the COVID-19 virus, ACE2 activity is possibly reduced, and therefore virus-infected cells may not be able to convert angiotensin II to angiotensin-(1-7). Angiotensin II accumulates in the cell (Wang *et al.* 2020a; Zemlin and Behl, 2020).

The balance between ACE2/ Ang1-7/ MasR, known as the protective arm, and ACE/ AngII/ AT1R, known as the classic arm, is regulated by AMPK (Liu *et al.*, 2019b; Behl *et al*., 2020; Sarzani *et al*., 2020). When AMPK is active, ACE2 is increased and the NF-κB pathway is inhibited in the cell. During COVID-19 disease, angiotensin II levels increase, which inhibit AMPK activity via AT1R in kidney. When AMPK activity is inhibited by increased angiotensin II, it activates NF-κB pathway in cell, resulting in the production of inflammatory cytokines. Inhibition of AMPK activity may also lead to cardiac hypertrophy and down-regulation of mTOR2 (mTORC2) pathway (Yin *et al.*, 2016). Low regulation of mTOR2 pathway can lead to a defect in the proliferation of Natural killer cell (NK) cells, which, in turn, may lead to an increase in viral load in virus-infected cells and ultimately a defect in immune system. The main role of NK cells in the body is to have lethal activity against tumor cells and virus-infected cells, which secrete their intracellular enzymes into such cells, causing them to die. NK cells kill antibody- dependent cell-mediated cytotoxicity by cancer cells infected with the virus. Therefore, if the amount of these cells, which are a small group of lymphocytes in the body, decreases, it will probably increase the viral load in patients with COVID-19 (Fig. 4) (Abbas *et al.*, 2014). Accumulation of angiotensin II in the cell also leads to increased oxidative stress in the cell, which can damage the cell's mitochondria and induces cell apoptosis (Liu *et al*., 2019; Razeghian-Jahromi *et al*., 2020; Rossi *et al.*, 2020; Turner and Kodali, 2020).

4.3 Exosomes as potential mediators of viral infection, re-infection, and reactivation

Many viruses can enter extracellular vesicles or exosomes during synthesis and proliferation in the host cell. Exosomes are a group of extracellular vesicles that are between 30 and 100 nm in size and cannot accommodate particles larger than this range. The corona family of viruses is between 120-160 nm in size, so a complete viral particle cannot enter the exosomes. What enters into the exosomes is viral mRNA. The viral mRNA and proteins like HIV infection can be trapped inside exosomes (Sims *et al.*, 2018). The fact that such vesicles can be presented in host cells has been substantiated by postoperative histopathological analysis of samples from all patients with COVID-19 by light and electron microscopy (Carroll *et al.*, 2015; Chahar *et al.*, 2015).

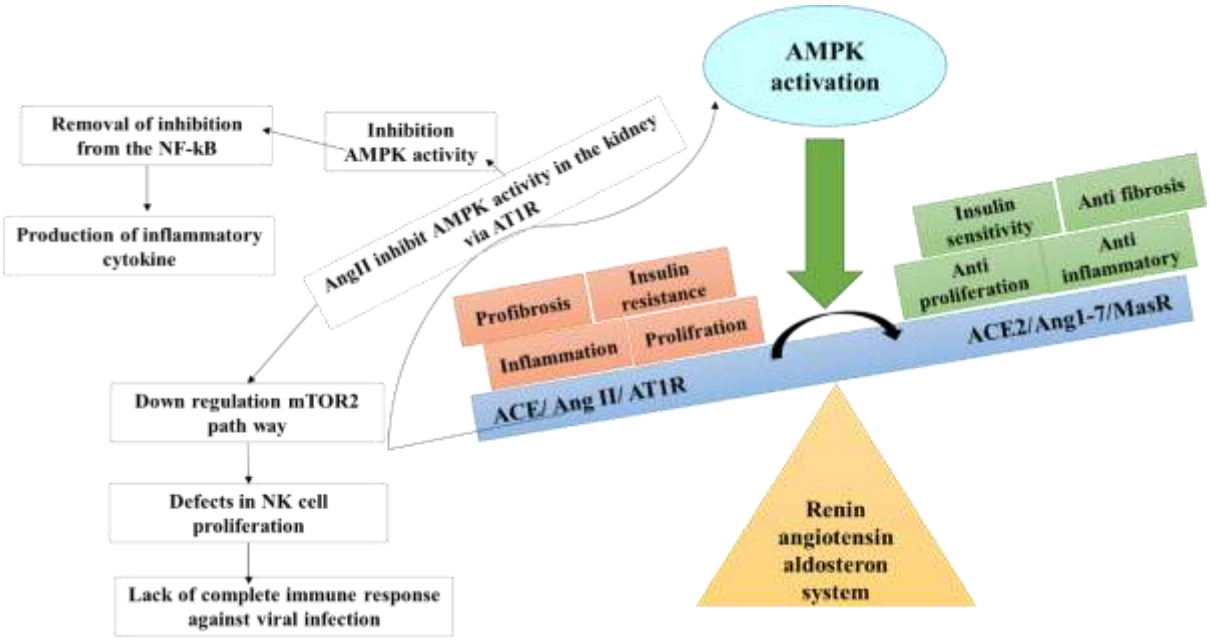

Fig. 4: ACE and ACE2 balance; The figure shows that when the COVID19 virus binds to ACE2 receptor, its function decreases and it cannot break down angiotensin II into angiotensin-(1-7). Thus, angiotensin II accumulates, which may inhibit the AMPK activity and lead to the activation of NF-κB pathway and production of inflammatory cytokines. On the other hand, it leads to the down-regulation of mTOR2 pathway. Down-regulation of mTOR2 pathway leads to decrease in NK cells and immunodeficiency and increase in viral load.

Exosomes have different biological roles that can be beneficial or harmful. These effects include immune system stimulation/tolerance, cell-cell communication, and resistance. Exosomes are known as the defense mechanism against the changes in extracellular environment caused by disease and stressors. Cell-derived exosomes are known to be efficient carriers of small RNAs (Emam *et al.*, 2018; Lin *et al.*, 2018; Wang *et al.*, 2020b; Kato *et al.*, 2020). Exosomes are protected from phagocytosis by mononuclear phagocytes due to their small size and can therefore transmit viral RNA to other cells. Exosomes have also been reported to target tissue or cells due to the presence of specific surface proteins such as tetraspanin (Fig. 2) (Emam *et al*., 2018; Behl *et al*., 2020a,b).

5. HYPOTHESES FOR REINFECTION RECURRENCE OF COVID-19

COVID-19 reinfection recurrence can occur for several reasons, include:

1- The primary RT-PCR test result is false-positive.
2- Low load of COVID-19 virus presented in higher or lower respiratory system and they eliminated by the innate immune response.
3- Adaptive immune deficiency causes a low number of (memory B-cell) or (memory T-cell).
4- Gene mutation in viral RNA in epitope-related area.

5.1 False-positive primary RT-PCR test

COVID-19 RT-PCR assays have different sensitivity and specificity. Best COVID-19 RT-PCR kits have a sensitivity and specificity of $\geq$ 95%. Hence, in best conditions about 5% of those who had false-positive primary reports can be infected by the COVID-19 virus in the future and misdiagnose it as COVID-19 reinfection (Osman *et al.*, 2020). PCR Ct-values are used as markers for the amount of virus RNA particles in a sample and determine the patient's infectivity. However, Ct-values correlate with the number of viral RNA particles so in different stages of infection Ct-values changes, in the late stages of infection Ct-values increase and sometimes go higher than thresholds and could be resolute as a false-negative report (Platten *et al.*, 2021). Also, different methods of swab

nasopharynx and oropharynx sampling for the COVID-19 test are performed in different laboratories. Unfortunately, there is no single gold standard assay exists for swab nasopharynx and oropharynx sampling, and this issue could result in the false-positives RT-RCR report.

5.2 Low load of COVID-19 virus

There is a semi-linear correlation between virus load and immune responses. The immune system is made up of two parts: the innate, (general) immune system and the adaptive (specialized) immune system. Innate immunity refers to nonspecific defense mechanisms that come into play immediately or within hours of the virus antigen's appearance in the body and provides an early line of defense against them (Wang *et al.*, 2020b).

In most infections, innate and adaptive immune mechanisms operate together and in sequence during host defense. But in some infection cases with low counts of the virus antigen's innate immunity can eliminate infection without adaptive immune help. In this situation, COVID-19 virus infection in the higher or lower respiratory system is eliminated only by innate immunity including macrophage, NK-cells, DC-cells, and neutrophils, and patients fully recovered but since B-cells and T-cells have not been involved, immune response ends without any antibody and memory cells for further immunity. Thus, these patients are not immune to the COVID-19 virus after recovery (Wang *et al.*, 2020b).

5.3 Adaptive immune deficiency

Primary immunodeficiency diseases are disorders in which part of the body's immune system is missing or does not function properly. These disorders can be divided into two groups:

1. Those fewer common conditions with defects in the innate immune system, a system of cells and mechanisms that defend the host from infection in a non-specific manner.
2. Those conditions are due to defects of the adaptive immune system in which defense is carried out in a more specific manner by T-cells and antibody-producing B-cells.

Patients with defects of adaptive immune system are diagnosed with COVID-19 infection reactivation. Since these illnesses are rare and only recently identified, the long-term outlook has not yet been established. Too many factors are involved in primary immunodeficiency diseases, so there is considerable variability in the severity of their response to COVID-19 infection (Ahmadpoor and Rostaing, 2020).

5.4 Gene mutation in viral RNA in epitope-related area

RNA viruses are known to have higher mutation rates than DNA viruses but only products of epitope genes are recognized by the adaptive immune system. Mutation in viral RNA in the epitope gene could result in a new variant of the virus. Recovered COVID-19 patients are not immune to the new variant of the COVID-19 virus after recovery. The spike protein of the COVID-19 virus has been undergoing mutations and is highly glycosylated. It is critically important to investigate the biological significance of these mutations (Fig. 5) [Dawood 2020]. For example, four variants of the COVID-19 virus including A475V, L452R, V483A, and F490L became resistant to some neutralizing antibodies (Li *et al.*, 2020; Wang *et al.*, 2021). These findings are important in the development of vaccines and therapeutic antibodies (Dawood, 2020; Li *et al.*, 2020).

6. CONCLUSION

From the hypotheses presented in the area of reactivation recurrence, it appears that a defect in immune system is the main key factor in the reactivation recurrence of COVID-19. There is also an imbalance between ACE/ AngII/ AT1R and ACE2/ Ang1-7/ MasR which can occur for several reasons and the exosomes which contain viral mRNA may be the possible reasons for the reactivation recurrence of COVID-19 patients. From the hypotheses presented in the area of reinfection recurrence, it appears that the mutation in genomic RNA of COVID-19 virus causes a change in the epitope region that creates a new variant which is the key factor in reinfection recurrence of COVID-19.

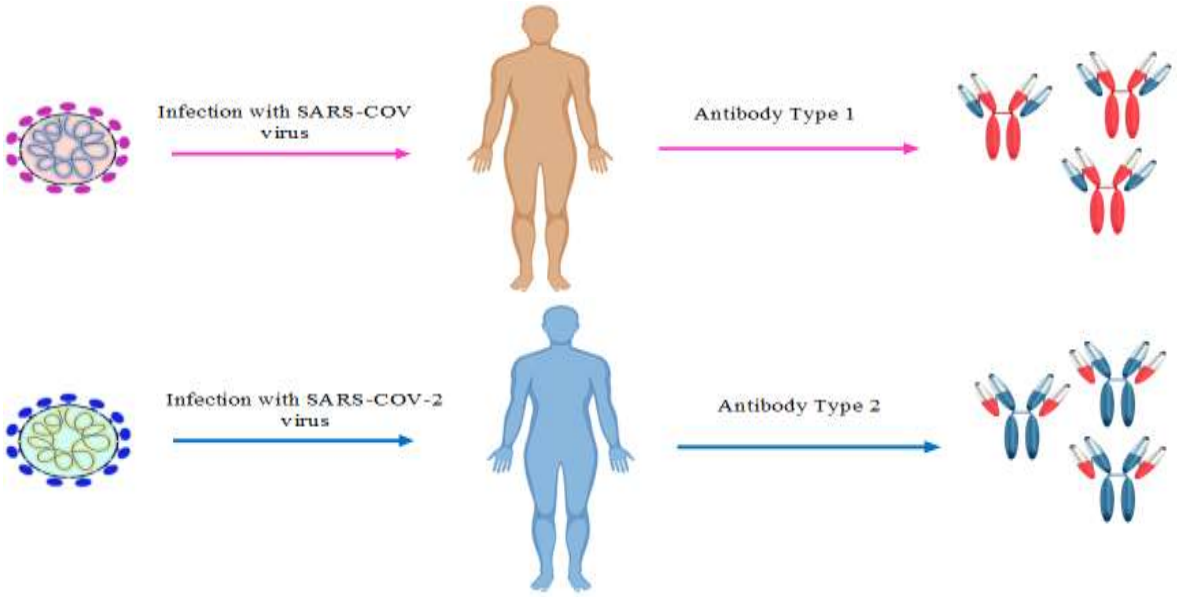

Fig. 5: SARS-CoV1 and SARS-CoV2 antigen can induce immune system to produce two different antibodies; This figure shows that getting sick with different variants of the SARS virus due to differences in the type of antigen can cause the body to produce different antibodies. It can be concluded that getting sick with one variant of the SARS virus does not create immunity for another type.

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Conflict of interest: The authors declare that they have no conflict of interests.

Contributors: All the authors are contributed in the preparation of the article including data collection, writing and editing.

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