Oxidative Stress Induced Thrombosis and Hypercholesterolemic Condition in COVID-19 Infection

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons)

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

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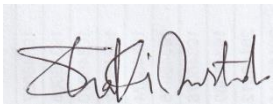
Supervisor:  31.12.2020

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Ethics Statement

This study does not involve any animal or human trial.

Abstract

The unprecedented emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has put an enormous challenge to the healthcare system worldwide due to the lack of specific treatment options. Several studies found that patients with COVID-19 infection are developing thrombosis. There is a desperate need of understanding the mechanism by which COVID-19 infection is causing the generation of such thrombotic events. In this review, we suggest that excessive production of ROS induces endothelial cell dysfunction which results the release of von Willebrand factor. High von Willebrand factor leads to thrombus production. We also argue that hypercholesterolemia is a life-threatening comorbidity as it can influence thrombus formation by overwhelming production of OxLDL in the presence of COVID-19 infection. Lastly, we present drugs that are suitable in these conditions and their possible drug-drug interaction with investigational antiviral agents used against COVID-19 infection.

Keywords: COVID-19; ROS; thrombosis; von Willebrand Factor; hypercholesterolemia; OxLDL.

Dedication

Dedicated to my parents and grandparents

Acknowledgement

I would like to begin by thanking the almighty Allah (SWT) for blessing me with the patience, guidance and support needed to complete my thesis. Also, I am grateful of my parents and grandparents for supporting me throughout. It is because of their prayers and love; I was able to come this far.

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List of Acronyms

COVID-19	Coronavirus Disease of 2019
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
ROS	Reactive Oxygen Species
CAC	COVID-19 Associated Coagulopathy
ACE	Angiotensin Converting Enzyme
vWF	Von Willebrand Factor
WPB	Weibel- Palade Bodies
ICU	Intensive Care Unit
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
OxLDL	Oxidized Low Density Lipoprotein
TC	Total Cholesterol
NETs	Neutrophil Extracellular Traps
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
TLR	Toll Like Receptor

Chapter 1

Introduction

An unprecedented outbreak of novel Corona virus disease was first diagnosed in the city of Wuhan, Hubei Province, China on December 8, 2019 (Wu & McGoogan, 2020). After that, on January 30, 2020, the World Health Organization (WHO) declared the novel Coronavirus disease as a Public Health Emergency on International Concern (World Health Organization, 2020). Following this, on March 11, 2020, WHO declared the novel Coronavirus as a pandemic (Cucinotta & Vanelli, 2020). In a span of 6 months, the virus spread all over the world in more than 216 countries. According to WHO Coronavirus Disease (COVID-19) Dashboard, till September 25, 2020, there has been 32,029,704 confirmed cases worldwide. At present, the USA holds the highest number of confirmed cases of 15,987,906 (WHO, 2020). According to World Health Organization (WHO), SARS-CoV-2 or COVID-19 can spread by mouth and respiratory droplets, such as coughing, sneezing, talking or singing can transmit the virus. Moreover, the virus can enter the body through mouth, nose and eyes. It is recommended that people should keep their hands clean and remain cautious while touching their face with hands (WHO, 2020).

Coronavirus is a type of virus that belongs to the family Coronavirdiae, subfamily Coronavirinae and order Nidovirales. This virus has four genera known as, Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Deltacoronavirus (δCoV), and Gammacoronavirus (γCoV) (Jasper Fuk Woo Chan et al., 2020). Furthermore, Alphacoronavirus (αCoV), Betacoronavirus (βCoV) has the ability to infect human (Jasper F.W. Chan et al., 2015). Before the emergence of the Covid-19, there was 6 known coronaviruses with the capability of infecting human, there are HCoV-229E, HKU-NL63, HCoV-OC43, HCoV-HKU1, SARS-CoV and MERS-CoV(Woo et al., 2005). All the Coronaviruses are known to be the positive stranded RNA virus

(de Haan, Kuo, Masters, Vennema, & Rottier, 1998) with genome lengths of 26.4 – 31.7 kb (Woo, Huang, Lau, & Yuen, 2010). The diameter of Coronavirus virions is 125nm. A unique morphological feature of these viral particles are the club shaped projections which demonstrates a crown like appearance hence gives the name, Coronavirus. The Coronavirus has four structural proteins, the spike (S), the envelop (E), nucleocapsid (N) and membrane (M) proteins (Fehr & Perlman, 2015). The spike (S) protein (150 kDa) resides in the surface of the viral particle and helps in attachment and entry into the host cell (Beniac, Andonov, Grudeski, & Booth, 2006). The S protein has two domains, S1 and S2, where the S1 helps the viral particle to attach with the host cell receptors and S2 help in the blending of the viral particle with the host cell membrane (DEGROOT RJ et al., 1987). The structural protein that gives the virus its shape is the membrane protein (M) (Nal et al., 2005). The M protein is a 25-30 kDa protein with three transmembrane domain (Filion, 1984). One of the structurer proteins, the E protein, an 8-12 kDa protein, helps in the assembly and release of the viral particles (Nieto-Torres et al., 2014; Rabaan et al., 2020). Moreover, the N protein is responsible for the encapsulation of the genetic material inside the viral particle. This protein has 2 domains, N-terminal domain and C-terminal domain. These domains binds with RNA (Chang et al., 2006). Additionally, the N protein remains helical shape in the dormant state and remains spherical shape inside the viral particle (Rabaan et al., 2020). Two Coronavirus, HCoV-OC43 and HCoV-HKU1 contains genes that codes for another structural protein, named hemagglutinin-esterase (HE) protein. They help in the viral entry into the host cell (Klausegger et al., 1999).

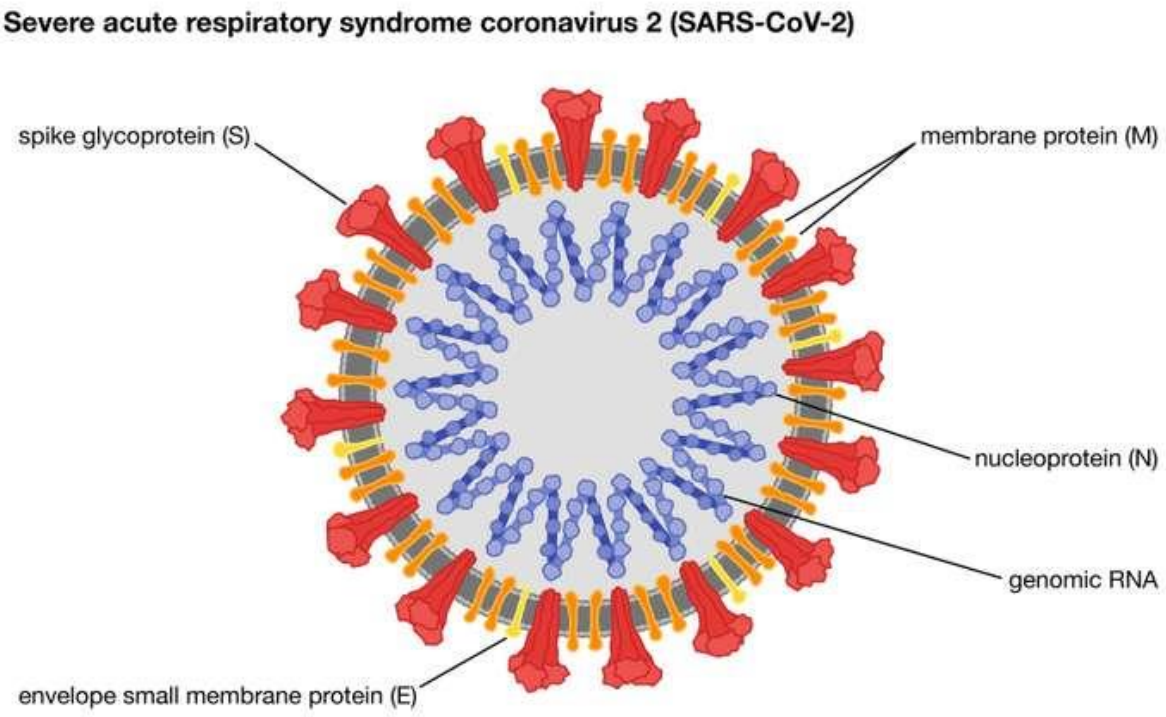

Figure 1: Diagram of SARS-CoV-2(Encyclopædia Britannica, 2020)

The Alphacoronavirus (αCoV) is responsible for the common cold in human. However the Betacoronavirus (βCoV), such as SARS-CoV, MERS-CoV and COVID-19 causes severe respiratory complications (Yuki, Fujiogi, & Koutsogiannaki, 2020). The host cell invation of COVID-19 is somewhat similar to SARS-CoV as both of their target receptor is ACE2 (Angiotensin Converting Enzyme type 2) receptor (Letko, Marzi, & Munster, 2020). An analysis of scRNA-seq datasets showed that lung, upper respiratory track, ileum, heart, and kidney has the most ACE2 receptor expression (Zou et al., 2020). Firstly, the S1 domain of COVID-19 virus binds with the ACE2 receptors. After that, the proteolytic cleavage occurs in the S2 domain in order to get fuse with the host cell and facilitate the viral entry (Letko et al., 2020). In comparison to SARS-CoV, the S1/S2 of COVID-19 gets completely cleaved in host cell invasion (Walls et al., 2020). The S1/S2 is cleaved by TMPRSS2 (trans- membrane protease serine 2) (Ou et al., 2020).

In the initial period of the pandemic, the virus was thought to be responsible for diseases of the respiratory tract. However, as days are passing and new studies are coming with new findings on the disease manifestations; several reports suggest that patients are suffering from pneumonia (Ye, Zhang, Wang, Huang, & Song, 2020), dyspnea as well as ARDS (X. Zhang, Zhang, Li, & Niu, 2020) due to COVID-19 infection as COVID-19 binds with ACE2 receptor to enter the host cell and affect the renin-angiotensin-aldosterone system (South, Diz, & Chappell, 2020). However, the mechanism by which the virus manifests diverse clinical conditions is not yet fully understood. Furthermore, many research articles provided data that COVD-19 infected patients were having high levels of D-dimer, a biomarker to detect the presence of thrombus. There have been suggestions that hyper-inflammatory state due to cytokine storm may stimulate the formation of thrombosis in COVID-19 infected patients (Henry, Vikse, Benoit, Favaloro, & Lippi, 2020). Although, the exact mechanism of the formation of thrombus is not yet established, many new hypothesis are suggested. Moreover, it has been seen that COVID-19 positive patients are showing high von Willebrand factor (Escher, Breakey, & Lämmle, 2020; Goshua et al., 2020; Huisman, Beun, Sikma, Westerink, & Kusadasi, 2020; Ladikou et al., 2020; Panigada et al., 2020), a multimeric protein that effectively work on activating platelet and increase the activity of factor VIII (Rusu & Minshall, 2018). In Italy, a cohort study was done on 3988 COVID-19 infected patients. The study showed that 67.4% of the subjects had at least one comorbidity. Furthermore, after calculation of mortality rate, it showed that patients with hypercholesterolemia as comorbidity had 3rd highest mortality rate (22.4 per 1000 patients-days) (Grasselli et al., 2020). Also, hypercholesterolemia is responsible for the development of cardiac related diseases, such as atherosclerosis (WILSON & LERMAN, 2001) and myocardial infraction (Pluijmert et al., 2019). On the other hand, several studies reported that cardiovascular diseases (CVD) were one of the prominent comorbidities in COVID-19 infections (Evans et al., 2020;

Nikpouraghdam et al., 2020; B. Wang, Li, Lu, & Huang, 2020). There has been a retrospective cohort study on patients with familial hypercholesterolemia, and it showed that these patients have higher tendencies of developing atherosclerotic cardiovascular disease (ASCVD) and coronary heart disease (CHD) (Masana et al., 2019). As hypercholesterolemia leads to atherosclerotic cardiovascular disease (ASCVD) and CVD is a common comorbid in COVID-19 infected patients, the effects of being a hypercholesterolemia patient in COVID-19 infection needs to be investigated.

Altogether, in this article, we provide hypothesison the development of thrombotic events in COVID-19 infected patients are due to high von Willebrand factor release because of oxidative stress induced endothelial cell dysfunction. Furthermore, we speculate that hypercholesterolemia can be a life threatening comorbid. We also present drugs that are suitable in these conditions and their possible drug-drug interaction with investigational antiviral agents used against COVID-19 infection.

Chapter 2

Background Information

2.1 Hypercholesterolemia

2.1.1 About Hypercholesterolemia

Hypercholesterolemia is a condition in which the cholesterol level in blood is significantly high. Hypercholesterolemia is one of the prominent risk factors which causes atherosclerosis and its related complexities (Borghi, 2016). According to American Heart Association, high level of LDL (Low density lipoprotein) cholesterol in blood leads to the condition known as

hypercholesterolemia (American Heart Association, 2017). Cholesterol in blood are of two types, HDL (High density lipoprotein) and LDL (Low density lipoprotein). HDL cholesterol is also known as good cholesterol where it is taken up by liver and do not cause harm. However, LDL cholesterol is not taken up by liver. Also, LDL cholesterol travel through blood circulation and form plaques which leads to atherosclerosis and cardiovascular complexities. Thus, LDL cholesterol is known as the bad cholesterol (Centers for Disease Control and Prevention, 2017). The development of atherosclerosis depends on the concentration of LDL cholesterol in blood. The concentration of LDL and HDL cholesterol higher than 190 mg/dl and lower than 40 mg/dl puts a subject in high risk category respectively(Hao & Friedman, 2014).

2.1.2 Cholesterol Generation

Cholesterol is produced through mevalonate pathway in the body. At firstly, three acetyl-CoA units form HMG-CoA (3-hydroxy-3-methylglutaryl-CoA). Then HMG-CoA is reduced to

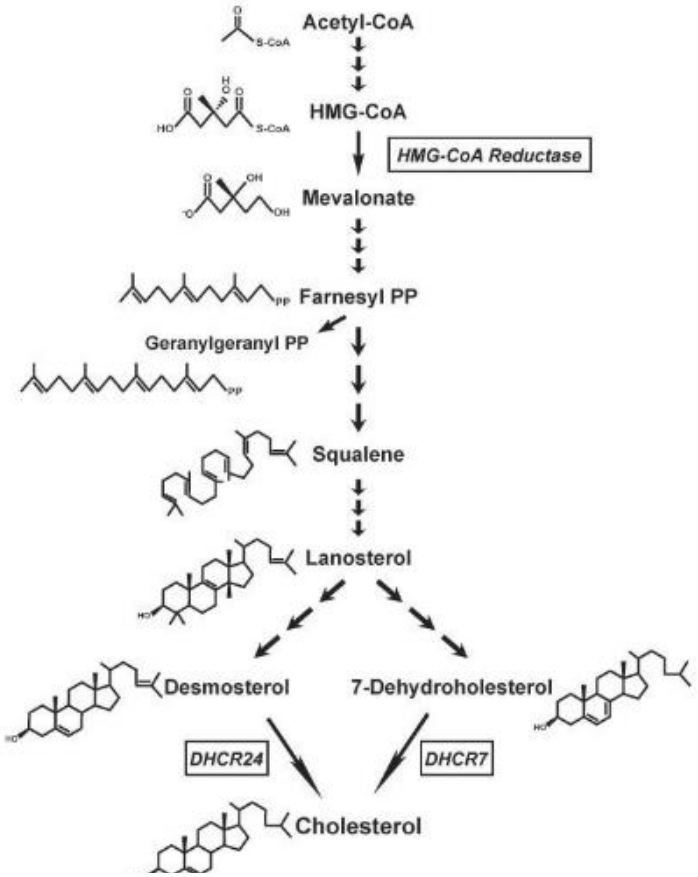

Figure 2:Cholesterol Generation Flowchart (Cortes et al., 2014).

form mevalonate with the help of HMG-CoA reductase. This HMG-CoA reductase works as the target for the reduction of cholesterol formation in body (Cortes et al., 2014). After the formation of mevalonate, sterol and nonsterol isoprenoids are formed. Furthermore, this steroid isoprenoid is the cholesterol and nonsterol isoprenoids are the dolichols and ubiquinone(Ward, Watts, & Eckel, 2019).

2.2 Thrombosis

2.2.1 About Thrombosis

Blood clotting is a part of our general body function. When there is a damage in the blood vessels, bleeding occurs which may lead to several health hazards. To counter this, the body has its own defense mechanism known as blood clotting. In the vascular damage site of blood vessel, formation of blood clot occurs by the aggregation of platelets in fibrin to prevent bleeding (D. Nguyen & Coull, 2017). However, when the blood clotting mechanism is over productive, it commence the event known as thrombosis (Furie & Furie, 2008). The formation of thrombosis generally means excessive blood clotting which in turn hampers normal blood flow through blood vessels (D. Nguyen & Coull, 2017). Due to sever injury, fractured bone, autoimmunity, certain medication as well as defect in gene may lead to thrombosis (Johns Hopkins Medicine, n.d.). There are several types of thrombosis, such as deep vein thrombosis (DVT) (Thachil, 2014), pulmonary embolism (PE) (Di Nisio, van Es, & Büller, 2016), portal vein thrombosis (Intagliata, Caldwell, & Tripodi, 2019), renal vein thrombosis (Asghar et al., 2007) as well as atherothrombosis (Sloop, Weidman, & St. Cyr, 2017). In the developed countries, thrombosis holds the position of being the most common cause of death (Mackman, 2008). Furthermore, venous thrombosis is the second and third most leading cause of death in patient with cancer (Furie & Furie, 2008) and cardio vascular complications (Mackman, 2008).

Due to the development of thrombosis, it can cause myocardial infraction, stroke, shortness of breath (Johns Hopkins Medicine, n.d.). Depending on the cause of disease and location of thrombus formation, the patient management varies drastically (D. Nguyen & Coull, 2017). Till 2016, there have been cases of pulmonary embolism in 29 – 48 per 100,000 person per year and cases of deep vein thrombosis in 45 – 117 per 100,000 person per year (Heit, Spencer, & White, 2016). In DVT, blood clot appears in the veins usually located in the legs. Furthermore, some common symptoms are, the leg starts to swell, get red or bluish in color, experience of severe pain and warmth (Thachil, 2014). The blood clot, fat particle or even tumor, that travel through blood circulation and big enough to obstruct blood flow is refer to as embolism. When the blood flow in the lung arteries are hampered by such obstruction, it is known as pulmonary embolism. This obstruction of circulation may occur not only by blood clot, but also tumor or fat (Essien, Rali, & Mathai, 2019). Shortness of breath, frequent coughing, bleeding while coughing and sever chest pain are some of the common symptoms of pulmonary embolism (Aurora Health Care, n.d.). Together deep vein thrombosis and pulmonary embolism is known as venous thromboembolism (VTE) (Essien et al., 2019). On the other hand, in the blood arteries, the breakdown or atherosclerotic plaques with outward thrombosis following obstruction of blood flow (Dziedzic, Machowski, Oleszczak-Kostyra, & Dąbrowski, 2018) is known as atherothrombosis (Viles-Gonzalez, Fuster, & Badimon, 2004). According to the location of the formation of atherothrombosis, coronary artery disease, stroke, transient ischemic attack and peripheral arterial disease can develop (Viles-Gonzalez et al., 2004). Pulmonary embolism (PE) may also responsible for myocardial infraction (MI). A case was reported on 41-year-old woman with severe chest pain. The electrocardiogram showed ST-segment-elevation myocardial infraction (MI). The transesophageal cardiogram showed a large pulmonary thrombus on the right side of the lung as well as presence of patent foramen ovale (PFO). With this findings, the report concluded that paradoxical embolism was the cause

of the myocardial infraction (MI) (Kikuni, Silance, Debbas, & Unger, 2019). Paradoxical embolism is the condition where the thrombus developed in the veins travel to the arteries through cardiac or pulmonary shunt (Windecker, Stortecky, & Meier, 2014). However, Centers for Disease Control and Prevention provided a statement that deep vein thrombosis (DVT) does not cause myocardial infraction (MI) (Centers for Disease Control and Prevention, 2020).

2.2.2 Physiology of Thrombus Formation

In the formation of thrombus in the bold vessels, both platelet activation and fibrin formation are required (Swieringa, Spronk, Heemskerk, & van der Meijden, 2018). In fibrin activation, two pathways, intrinsic and extrinsic pathway occurs. Furthermore, both of the pathways lead to the activation of factor X to factor Xa, one of the blood clotting factors. After that, factor Xa helps to activate factor II, prothrombin to factor IIa, thrombin. Which ultimately helps in both platelet activation and activates fibrinogen to fibrin. This fibrin, a protein forms a mesh like structure to hold and further accumulate platelets and other blood cells (Chaudhry & Babiker, 2018). However, there is another component in the subendothelial region named collagen (Xu & Shi, 2014). This component is essential in initiation platelet activation (Furie & Furie, 2008). After vascular injury, collagen gets exposed in the blood lumen. Then a receptor in the platelet, glycoprotein VI binds with collagen. Again, platelet glycoprotein Ib-V-IX interacts with von Willebrand factor, a protein embedded in collagen. This interaction helps in platelet activation (Furie & Furie, 2008). After initial activation of platelet causes change in shape of the cell and releases ADP as well as thromboxane A2. After that ADP then binds with $P2Y_{12}$ receptors on other platelet's surfaces to cause further platelet activation. Furthermore, the thromboxane A2 released by platelet on initial activation goes and binds with thromboxane-prostanoid (TP) receptor on the surface of other platelets to activate them (Dorsam & Kunapuli, 2004). In case of atherothrombosis, the formation of thrombus is caused by the disruption of atherosclerotic plaque and plaque erosion in the arteries. In the atherosclerotic plaque region, there is an

increased expression of coagulating factor III, tissue factor (TF). This tissue factor then interacts with factor VII initiating the intrinsic pathway of blood coagulation (Yamashita & Asada, 2015). This eventually leads to a cascade of reactions to form fibrin and thus thrombus forms (Furie & Furie, 2008). In addition to that, platelet activation and accumulation is essential in the thrombus formation (Tomaiuolo, Brass, & Stalker, 2017). As previously discussed, von Willebrand factor binds with platelet glycoprotein Ib-V-IX in activating platelet aggregation (Furie & Furie, 2008). There has been found that, patients of acute myocardial infraction had very high amount of von Willebrand factor in their coronary arteries (Yamashita et al., 2006). Thus, can be said that atherosclerotic plaque rupture and increased level of von Willebrand factor in coronary artery may lead to atherothrombosis. However, there is another mechanism of platelet activation, platelet activation through ROS (reactive oxygen species) production (Fuentes, Gibbins, Holbrook, & Palomo, 2018). ROS are produced as a consequence of imbalance in antioxidant system in the body (Incalza et al., 2018). Through this imbalance, the expression of NADPH oxidase increases in platelets. This enzyme, NADPH oxidase stimulates the oxidation and produce ROS (Brandes, Weissmann, & Schröder, 2014). Elevation of ROS induces oxidative stress (Incalza et al., 2018). Furthermore, elevation of oxidative stress leads to vascular endothelial dysfunction (Incalza et al., 2018). This phenomenon leads to the exposure of collagen and thus leads to the interaction of von Willebrand factor and platelet glycoprotein Ib-V-IX. After that, platelet aggregation stimulates leading to the formation of thrombosis (Fuentes et al., 2018)

Table 1: Clotting Factors (Lab Tests Online AU, n.d.)

Factor	**Name of the factor**
I	Fibrinogen
II	Prothrombin
III	Tissue factor or thromboplastin

IV	Calcium
V	Proaccelerin (Labile factor)
VII	Proconvertin (Stable factor)
VIII	Antihaemophilic factor A, Antihaemophilic globulin
IX	Antihaemophilic factor B, Plasma thromboplastin component, Christmas factor
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent, Haemophilia C, Rosenthal syndrome
XII	Hageman factor
XIII	Fibrin stabilising factor,Laki-Lorand factor

Chapter 3

COVID-19 on Renin-Angiotensin-Aldosterone System

RAAS or renin angiotensin aldosterone system is responsible for the regulation of the arterial blood pressure by contracting and dilating blood vessels. Although, this RAA system involves renin, angiotensin and aldosterone system (Fountain & Lappin, 2018), we focused more on angiotensin in relation to COVID-19. Angiotensin I, a protein molecule that binds ACE receptor type one or ACE-I receptor. Upon binding with ACE-I, it converts into angiotensin II which is responsible for the vasoconstriction, pro-inflammation as well as pro-oxidation effect. However, to maintain the homeostasis, angiotensin II binds with ACE-II receptor and coverts into angiotensin 1-7 which binds with MasR receptor which ultimately gives vasodilation, anti-

inflammation and anti-oxidant activity (Henry et al., 2020). This review focused on the oxidative activities with regards to RAAS. The novel coronavirus, SARS-CoV-2, also known as COVID-19 exerts its effect through ACE II receptor. The virus, COVID-19 binds with ACE II receptor on the membrane of target cell and it enters the host target cell. This binding of COVID-19 with ACE II elevates the level of angiotensin II. That eventually upregulated the activity of angiotensin II (Alexandre, Cracowski, Richard, & Bouhanick, 2020).

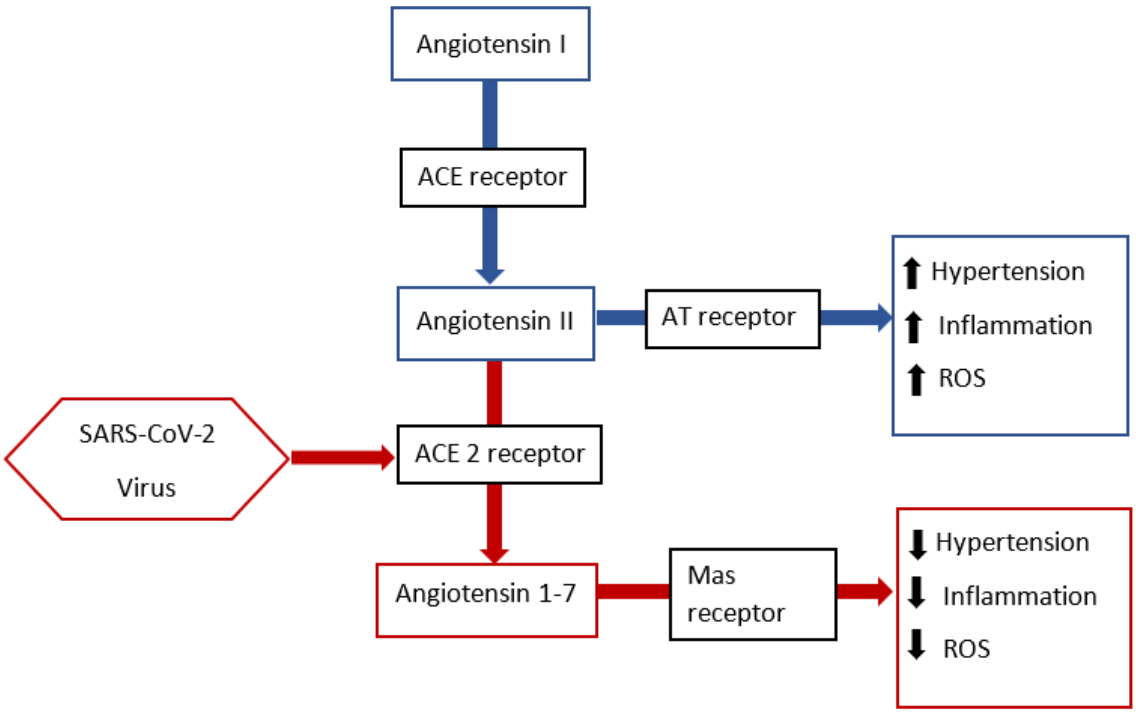

Figure 3: The effects of SARS-CoV-2 on Renin-Angiotensin-Aldosterone system. The red colored boxes and arrows signify the events that are hampered by SARS-CoV-2.

Chapter 4

Association of Thrombosis and Hypercholesterolemia with COVID-19

4.1 COVID-19 and ROS (Reactive Oxygen Species) Production

Upon binding with ACE-II receptors, downregulation of angiotensin 1-7 occurs (Alexandre et al., 2020). Angiotensin 1-7 is a protein molecule that have vasodilatory, ani-inflammatory as well as anti-oxidant properties (Fountain & Lappin, 2018). A group of enzymes, NADPH oxidase, converts oxygen molecule to superoxide. There are five homologues, Nox1, Nox2, Nox3, Nox4 and Nox5. These homologues work effectively in formation of super-oxides (Garrido & Griendling, 2009).In the body, the formation of superoxide, hydrogen peroxide and hydroxy free radicals occur successively in mitochondria. These super-oxides, hydrogen peroxide and hydroxy free radicals are known as reactive oxygen species or ROS (Phaniendra, Jestadi, & Periyasamy, 2015). When body have ROS level more than baseline level, it is known as oxidative stress. Oxidative stress often generates when the balance between ROS production and antioxidant production is not maintained. One of the agonists that stimulates the production of ROS is angiotensin II (Madamanchi, Hakim, & Runge, 2005). Usually, the angiotensin II activates Nox1, Nox2 and Nox4 in the vascular region. However, angiotensin 1-7 potentially inhibit the effect of NADPH oxidase enzyme by reducing the expression of p22phox (Lovren et al., 2008) and further prevents the production of reactive oxygen species (Lu et al., 2017; Mordwinkin et al., 2012; Shi et al., 2015; L. L. Zhang et al., 2016). As COVID-19 bind with ACE-II receptors, it upregulates the level of angiotensin II (Alexandre et al., 2020). In COVID-19 patients, the upregulation of angiotensin II and downregulation of angiotensin 1-7 occurs may be the potential cause of the over production of ROS. After the viral entry, the innate immunity gets into action. Upon the activation of innate immunity, at the site of infection, cells release cytokines and chemokines. In a retrospective analysis showed that COVID-19 patients

had high levels of IL-1β, IL-1RA, IL-7, IL-8, IL-10, IFN-ɣ, monocyte chemoattractant peptide (MCP)-1, macrophage inflammatory protein (MIP)-1A, MIP-1B, granulocyte-colonystimulating factor (G-CSF), and tumor necrosis factor-alpha (TNF-α) in their blood plasma (C. Huang et al., 2020). IL-8, TNF-α, IFN type I and II attracts neutrophils in the site of infection (Selders, Fetz, Radic, & Bowlin, 2017). Several studies reported that COVID-19 infected patients have high levels of neutrophil (Barnes et al., 2020; Didangelos, 2020; Hemmat et al., 2020; Y. Zhao et al., 2020). Neutrophils can also generate reactive oxygen species through the activation of NADPH oxidase in order to kill the pathogens (G. T. Nguyen, Green, & Mecsas, 2017). Thus, high levels of neutrophils can also be responsible for the elevation of the overall ROS production in COVID-19 infection.

4.2 Epidemiological Studies on CAC (COVID-19 Associated Coagulopathy)

Infections can also induce thrombosis in the body where pathogens increase the expression of tissue factors on the immune cells (Connors & Levy, 2020). There have been numerous reports that showed elevated changes in coagulation pattern in patients affected with novel coronavirus 2019 infection. CAC or COVID-19 associated coagulopathy is the term defines the alteration or initiation of coagulation pattern in patients with COVID-19 infection (Connors & Levy, 2020). One of the early reports from China on 99 hospitalized COVID-19 infected patients in Wuhan showed high D-dimer level (36% of the patients), high activated partial thromboplastin time (6% of the patients), high level of prothrombin (5% of the patients) in blood (Chen et al., 2020). Also, there was a report on three COVID-19 infected patients in ICU facility. All three of them developed large pulmonary embolism (Pishgahi et al., 2020). Furthermore, another clinical report on 138 COVID-19 infected patients from Wuhan hospital showed high levels of prothrombin. In the same report, among the ICU (Intensive Care Unit) patients, 26% of them showed high D-dimer level (D. Wang et al., 2020). In another study with 191 hospitalized COVID-19 infected patients from Wuhan, 54 died and the authors concluded that the older age,

high Sequential Organ Failure Assessment (SOFA) score, high D-dimer level (more than 1 μg/mL on admission) were the cause of death (Ramanathan et al., 2020). A study was conducted on 183 COVID -19 infected patients with pneumonia, which is termed as novel coronavirus pneumonia (NCP) in Tongji Hospital, Wuhan and reported that the dead patients had very high D-dimer level, high fibrin degradation product level, increased prothrombin time and high activated partial thromboplastin time. They also found that 15 (71.4%) of the dead patients had disseminated intravascular coagulation. These reports confirm that there must be some link between COVID-19 infection and coagulation (Tang, Li, Wang, & Sun, 2020). A study on lung autopsy specimens from seven COVID-19 infected and seven influenza (H1N1) infected patients was studied. Also, ten non-infected specimens were used as control. The finding was that the levels of CD4 positive T cells were much higher in lungs of COVID-19 infected than influenza infected patients. Furthermore, a multiplex analysis using NanoString Technologies showed that expression of 79 inflammation related genes in COVID-19 infected lung samples and only 2 in influenza infected lung samples. Moreover, 57.14% of both the COVID-19 and influenza infected lung samples had thrombus formation in the pulmonary arteries with diameter of 1 mm to 2 mm. However, none of the thrombus obstructed blood flow. In the COVID-19 infected lung samples, the presence of alveolar capillary microthrombi was 9 times more than influenza infected lung samples (Ackermann et al., 2020). Again, a study was conducted in China on 1099 patients with COVID-19 infection from 31 provinces, they concluded that 40% of the patients were at a high risk of developing venous thromboembolism (T. Wang et al., 2020). A study was conducted on 184 ICU patients with established COVID-19 pneumonia. It showed that 31% of the patients had developed thrombotic complications, 27% of them developed venous thromboembolism and 3.7% of them arterial thrombotic complications (Klok et al., 2020). The authors concluded that every COVID-19 infected critically conditioned patients must get anti-thrombotic treatment with considering the

thrombotic complications (Klok et al., 2020). These reports provide us with assumptions that COVID-19 infection has something to do with the development of thrombosis. However, the question remains on the which mechanism the development of these thrombotic events is occurring. Carsana et al. conducted analysis on lung samples collected from 38 patients who died from COVID-19 infection and found that 33 of the lung samples had developed platelet-fibrin thrombi (Carsana et al., 2020). With these in mind, there are some suggestions can be taken in consideration on how thrombosis is developing in COVID-19 infected patients, such as polyphosphate generated by microorganisms induce platelet activation (Smith et al., 2006), complementary system also induces platelet activation and fibrin deposition (Subramaniam et al., 2017). Again, neutrophil extracellular trap also contributes in the formation of thrombin (Noubouossie, Reeves, Strahl, & Key, 2019). A study was conducted on 24 ICU patients infected with COVID-19 infection. They studied the blood sample from the patients and they concluded that hypercoagulability was associated with sever inflammatory state (Panigada et al., 2020).

4.3 Oxidative Stress andEndothelial Dysfunction

For usual endothelial function as well as maintaining endothelial homeostasis, nitric oxide (NO) is a key molecule. Nitric oxide synthase (NOS), an enzyme that oxidize L-arginine and produce nitric oxide (NO) (Loscalzo, 2002). Nitric oxide has various functions in maintaining the vascular homeostasis, such as vasodilation, decreasing platelet aggregation, decreasing neutrophil and leukocyte adhesion, reducing cytokine production as well as they are believed to reduce the production of reactive oxygen species by administering high level of NO (Tousoulis, Kampoli, Tentolouris Nikolaos Papageorgiou, & Stefanadis, 2011). ROS decreases the bioavailability of NO and initiate the endothelial dysfunction. There are two main mechanisms by which NO activity is reduced. As ROS are very reactive, thus the first one is the reaction of super oxide with nitric oxide and produce per-oxynitrite (OONO-) inactivating

NO (Loscalzo, 2002). Secondly, when NOS is unable to bind with its cofactor or there is an insufficient L-arginine available, NOS reduces oxygen and produce superoxide anion, an ROS which results in the reduction of NO bioavailability (Luo, Lei, Qin, & Xia, 2014). This reduction of NO leads to the disturbance in vascular homeostasis, hence affects the endothelial integrity. This is how endothelial dysfunction occurs (Loscalzo, 2002; Tousoulis et al., 2011). Upon endothelial dysfunction, Weibel Palade bodies (WPB), the storage granules found in endothelial cell region, releases von Willebrand factor and P-selectin (Kaufman, Sanvictores, & Costanza, 2020). WPB also stores interleukin-8 (IL-8), eotaxin-3, endothelin-1, and angiopoietin-2(Rondaij, Bierings, Kragt, Van Mourik, & Voorberg, 2006). As previously discussed, von Willebrand factor works on platelet activation through binding with glycoprotein Ibα (GpIbα)(Rusu & Minshall, 2018). Furthermore, von Willebrand factor also increases the half-life of factor VIII which works on intrinsic pathway of thrombus formation (Rusu & Minshall, 2018).

4.4 von Willebrand Factor Levels in COVID-19 Patients

A large glycoprotein with multiple peptide chains named von Willebrand factor, which is observed in blood plasma, subendothelial matrix, Weibel-Palade bodies and platelet α-granules(Sadler, 1998). Interestingly, a case report on a 72-year-old COVID-19 infected ICU patient was published. The case showed that the patient had D-dimer levels of 0.69 mg/l on the day of admission and it increased to 20.63 mg/l on day 21 of admission. Also, on day 21, the level of von Willebrand factor was 555% where the normal range is 42%-136%. The activity of von Willebrand factor was 520% with normal range of 43%-168%. Also, the patient had increased clotting factor VIII activity to 369% with normal range of 55%-164% (Escher et al., 2020). Another study was conducted on 24 COVID-19 positive patients. The mortality was 16.7% and patients admitted in ICU was 75%. The study demonstrated that the median von Willebrand factor was 350% and median factor VIII level was 279 u/dl. The study concluded

that increased von Willebrand factor and factor VIII are responsible for the high coagulopathy in COVID-19 patients (Ladikou et al., 2020). Furthermore, in a previous study conducted by Panigada et al., showed that 11 patients COVID-19 infection had increased von Willebrand factor and factor VIII with mean score of 529 U/dl and 297 U/dl respectively (Panigada et al., 2020). In a study with 12 COVID-19 infected patients, von Willebrand factor antigen level was 4.08 IU/ml with reference range of 0.60-1.80 IU/ml. The study also showed that the factor VIII level was 4.30 IU/ml with reference range of 0.60-1.50 IU/ml. Furthermore, the level of ADAMTS13(a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) was 0.48 IU/ml with reference range of 0.61-1.31 IU/ml (Huisman et al., 2020). Also, in a single-centre cross-sectional study with 68 COVID-19 infected patients were done where 48 were ICU patients and 20 were non-ICU patients. The objective of the study was to observe the related biomarkers in endothelial cell and platelet activation. One of their findings was von Willebrand factor antigen was 565% in ICU patients and 278% in non-ICU patients with $p<0.0001$, which means statistically significance (Goshua et al., 2020). These findings are supporting the association of von Willebrand factor on thrombosis generation in COVID-19 infected patients. Thus, a possible potential hypothesis on thrombosis generation in COVID-19 patients can be; COVID-19 generate oxidative stress in the body by inhibiting the formation of angiotensin-1,7 or up regulating the levels of angiotensin-II (Pai, Lo, Hsu, Peng, & Wang, 2017; Rico-Mesa, White, & Anderson, 2020). Angiotensin-1,7 regulates NADPH oxidase enzyme, an enzyme that helps in the formation of superoxide (Pai et al., 2017). Furthermore, generation of oxidative stress is associated with endothelial dysfunction (Incalza et al., 2018) by decreasing the bioavailability of NO (Loscalzo, 2002). Endothelial dysfunction is associated with the release of von Willebrand factor in the blood (Rusu & Minshall, 2018). Endothelial dysfunction then leads to the activation of platelet by von Willebrand factor in the blood

coagulation system. That in turn initiates coagulation cascade (Malerba et al., 2017; Rusu & Minshall, 2018).

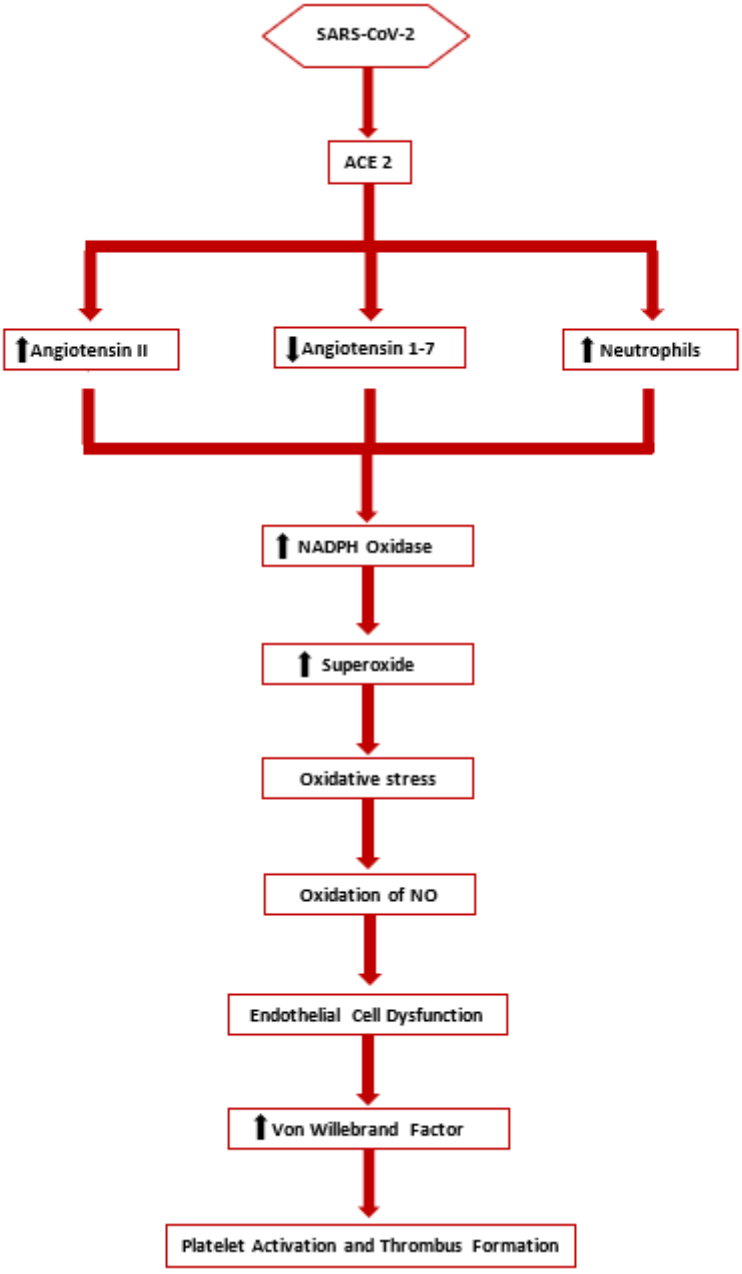

Figure 4: SARS-CoV-2 on thrombus formation. SARS-CoV-2 virus binds with ACE2 receptors that will in turn increase oxidative stress. Oxidative stress will commence endothelial dysfunction. These events will increase platelet activation and thrombus formation.

4.5 Cholesterol Levels of COVID-19 Patients

There were few studies on COVID-19 infection conducted and found that there was a decrease in the levels of cholesterol in COVID-19 infected patients. A retrospective longitudinal analysis on 21 COVID-19 infected patients was done. Their LDL, HDL and total cholesterol (TC) levels were measured. The study showed that patients had decreased in LDL, HDL and TC levels than prior to COVID-19 infection. The patients had an elevation of LDL and TC levels after recovery whereas the HDL level remain decreased (Fan et al., 2020). Also, there was a study on 597 COVID-19 infected patients, the study showed low level of LDL and TC level in mild, severe and critical level. Also, decreased in HDL levels were seen in critical patients (X. Wei et al., 2020). Another study with 2629 confirmed COVID-19 patients revealed that there was low level of both LDL and HDL in blood (W. Huang et al., 2020). Furthermore, a study was conducted on 97 COVID-19 infected patients. All the patients were hospitalized and found that HDL levels decreased with disease progression. They also determined that ApoA1, CD3+T%, and CD8+T% levels were also reduced in COVID-19 infected patients (Nie et al., 2020). Again, a study was done to determine the lipid concentration in the blood. The study had 861 subjects with COVID-19 infection and 1108 healthy controls. The level of HDL and triglyceride in the blood was low in COVID-19 patients compared to the control group (C. Wei et al., 2020). Moreover, with 71 confirmed COVID-19 infected patients and 80 controls, a study was done to determine the lipid profile of COVID-19 infected patients in Wenzhou, China. They found that COVID-19 infected patients had low levels of HDL, LDL and TC compared to the control group with $p<0.001$ (Hu, Chen, Wu, He, & Ye, 2020). Fan et al. and C. Wei et al. stated in their study that liver damage might be a possible reason for having low cholesterol level (Fan et al., 2020; X. Wei et al., 2020). Furthermore, there was a study on 19 COVID-19 patients with pneumonia and 15 non COVID-19 patients with pneumonia. The study found that there was elevation on AST (aspartate aminotransferase) and ALT (alanine

aminotransferase) levels in COVID-19 infected patients. Although the p value was 0.005 and 0.03 respectively (D. Zhao et al., 2020). Thus, it can be hypothesized that liver damage might be the cause of this lowering of cholesterol. Also, liver damage can occur due to the elevation of reactive oxygen species induced by COVID-19 infection.

4.6 Viral Entry and Cholesterol Levels

On the other hand, according to several articles, viral entry into the host cell is facilitated by high cholesterol level. Human immunodeficiency virus (HIV) (Raulin, 2002), transmissible gastroenteritis virus (TGEV)(Ren, Glende, Yin, Schwegmann-Wessels, & Herrler, 2008), flavivirus (Osuna-Ramos, Reyes-Ruiz, & Del Ángel, 2018), rubella virus (Otsuki et al., 2017) as well as borna disease virus (BDV) (Clemente, de Parseval, Perez, & de la Torre, 2009) have been reported to have high cellular entry in the host cell in the presence of high level of cholesterol. Moreover, there was a research conducted to observe the SARS-CoV in the membrane binding with different cholesterol level. The study provided evidence that there was high affinity of SARS-CoV towards the cells with high level of cholesterol (Meher, Bhattacharjya, & Chakraborty, 2019). Also, there was another study which worked on porcine delta coronavirus (PDCoV). The study found that low levels of membrane cholesterol decreases the viral infection and, high levels of membrane cholesterol increases the viral infection (Jeon & Lee, 2018).As previous data on COVID-19 infected patients with reduced cholesterol level and evidences on SARS-CoV and porcine delta coronavirus (PDCoV) that elevation of cholesterol increases viral entry, we can hypothesize that high levels of cholesterol might also facilitate the COVID-19 entry into the host cell.

4.7Hypercholesterolemic Patients with COVID-19

If we consider the patients with hypercholesterolemia, these patients have high levels of total cholesterol as well as LDL cholesterol (Egan, Li, Qanungo, & Wolfman, 2013). As previously

discussed, upon the infection from COVID-19, patients generate reactive oxygen species (Ntyonga-Pono, 2020). On the other hand, LDL are oxidized in presence of free radicals, hence reactive oxygen species (Parthasarathy, Raghavamenon, Garelnabi, & Santanam, 2010). So, patients with hypercholesterolemia can develop more oxidative LDL (OxLDL) level after COVID-19 infection. Again, Fan et al. and C. Wei et al. discussed that measuring OxLDL level was a necessary in their study (Fan et al., 2020; X. Wei et al., 2020). In COVID-19 infection, the patients were found to have high levels of neutrophils and macrophages because of innate immunity (Prompetchara, Ketloy, & Palaga, 2020). A lot of evidences suggest that OxLDL in the presence of neutrophils and/or macrophages generates reactive oxygen species and neutrophil extracellular traps (NETs). NETs or neutrophil extracellular traps are network of fiber like structure composed of histone and nucleic acid (DNA) (Brinkmann & Zychlinsky, 2012). In a study, in vitro observations were done to explore the effect of OxLDL on NETs formation. Neutrophil cells were incubated with phorbol 12-myristate 13-acetate (PMA) in the presence of both OxLDL and non-oxidized LDL. The formation of NETs was accelerated in the presence of OxLDL compare to non-oxidized LDL. Furthermore, in vivo observations were also done in the same study on human aortic endothelial cell culture. The formation of NETs was increased in the presence of both oxidized LDL and non-oxidized LDL. However, in presence of oxidized LDL, the formation of NETs was significantly increased compare to non-oxidized LDL (Obama et al., 2019). Again, another study was also done to observe the effect of OxLDL on NETs formation. In the study NETs formation was seen as a result of OxLDL with PMNs. Also, in the same study NADPH oxidase enzyme inhibitor significantly reduced the formation of NETs. Thus, the study concluded that NADPH oxidase enzyme is the major player in the formation of OxLDL induced NETs formation from PMNs (Awasthi et al., 2016). Moreover, Y. G. Zhang et al. performed a study to observe the role of exosome secreted from macrophages induced by OxLDL in the formation of atherosclerosis. In the mice, the

macrophages induced by OxLDL with neutrophils, generated high level of ROS and there was NETs formation also observed. The development of atherosclerosis was also detected. Thus, it is clear that OxLDL induced macrophages with neutrophils will generate high ROS level and NETs which will result in atherosclerosis formation (Y. G. Zhang et al., 2019). A study confirmed that macrophages treated with OxLDL will increase the formation of ROS by up-regulating the expression of p47phox. The p47phox is a regulatory subunit that upon phosphorylation activates NADPH oxidase enzyme that is the key player in the elevation of ROS (J. Wang et al., 2018). It is clear that hypercholesterolemic patients with COVID-19 infection will have high ROS levels, hence high OxLDL level. Also, with high macrophage and neutrophil level, these patients will generate oxidative stress as well as NETs. On the other hand, COVID-19 through renin-angiotensin-aldosterone system, decreases the level of angiotensin 1-7 that effectively work against NADPH oxidase enzyme. Thus, we can postulate that patients with hypercholesterolemia are vulnerable in terms of COVID-19 infection.

Here, we can elucidate the fact that increased generation of reactive oxygen species, hence oxidative stress is the main potential factor in terms of developing thrombus in COVID-19 patient. As explained, patients with hypercholesterolemia can produce more reactive oxygen species in COVID-19 infection with high OxLDL and neutrophils. With that in mind, we can come to a conclusion that patients with hypercholesterolemia are more susceptible to get thrombotic events as well as organ failure, such as liver damage which might be responsible for the lowered cholesterol level.

Chapter 5

Drug Options

5.1 Statins

5.1.1 About Statins

The class of drug used to reduce the formation of cholesterol are HMG-CoA reductase inhibitors, statins. Statins have been in the market for many years and effectively worked in the treatment of hypercholesterolemia. This treatment helped to reduce the cardiovascular complications related to high level of cholesterol in blood. Statin monotherapy is well tolerated in patients (Bellosta & Corsini, 2018). However, patients using other drugs with statin usually suffer from side effects. One of the most highly reported side effects of statin is statin-associated muscle symptoms (SAMS). This is considered as the leading cause of statin discontinuation (Stroes et al., 2015). In UK, there are five statin drugs available in the market, atorvastatin, fluvastatin, pravastatin, rosuvastatin and simvastatin (NHS, 2016).

5.1.2 Immunomodulatory Effects of Statins

Despite having cholesterol lowering effects, statins also have immunomodulatory effects. Several disease model showed that statin have immunosuppressive effect due to the blockage of mevalonate pathway (Zeiser, 2018). It has been proposed that statin as a potential addition in treatment measures against COVID-19 infection because of its immunosuppressive effect (Castiglione, Chiriacò, Emdin, Taddei, & Vergaro, 2020). TLR (Toll-like receptors), a group of proteins that works on recognition of pathogens. Animal study provided evidence that TLR interacts with SARS-CoV-1 and upregulate the gene expression of MyD88 (Myeloid differentiation primary response 88). This upregulation causes the formation of pro-inflammatory factor, NF-κB(Totura et al., 2015). Another animal study showed that inhibition

of the pro-inflammatory factor NF-κB led to high rate of survival in mice against SARS-CoV-1 infection (DeDiego et al., 2014). Atorvastatin showed a potential mitigation of NF-κB activation (Chansrichavala, Chantharaksri, Sritara, & Chaiyaroj, 2009). Also, in COVID-19 patients, it was claimed that the infection leads to endotheliitis(Varga et al., 2020). An treatment measure of statin with ARB (Angiotensin receptor blocker) was taken to prevent endotheliitis during Ebola outbreak in west Africa (Fedson & Rordam, 2015). The author proposed similar treatment approach for patients with COVID-19 infection (Fedson, Opal, & Rordam, 2020).

5.1.3 Statins and Drug-Drug Interaction with Investigational Anti-viral agents for COVID-19

Statins, such as lovastatin, simvastatin and atorvastatin use cytochrome P3A4 (CYP3A4) to get metabolized in liver. On the other hand, cobicistat and ritonavir, drugs that are used against COVID-19 works by inhibiting CYP3A. Considering this, it can be said that use of cobicistat and ritonavir would increase the plasma drug concentration of statins, which in turn may facilitate SAMS and liver toxicities. It has been theorized that statins such as lovastatin, simvastatin and atorvastatin should not be taken with cobicistat or ritonavir (Castiglione et al., 2020).

5.1.4 Recommendations on Statin Use

Use of statin solely against COVID-19 infection is not yet established. Evidences from clinical trials are required to determine the actual benefits of statin and its safety profile in patients with COVID-19 infection (K. C. H. Lee, Sewa, & Phua, 2020). An observational study was performed in 8910 patients over 11 countries and concluded that use of statin may have some relationship with the reduction of death associated with COVID-19. They also pointed out that randomized control trial is required to establish the activity of statin in COVID-19 patients

(Mehra, Desai, Kuy, Henry, & Patel, 2020). It has been proposed not to use statin on the purpose of treating COVID-19 infection. However, infected patients already in statin medication should continue ("ESC Guidance for the Diagnosis and Management of CV Disease during the COVID-19 Pandemic," 2020). A retrospective cohort study was done in South Korea to assess the relation between COVID-19 related mortality and statin medication. In the study, it showed the reduction of hazard ratio, HR=0.637 with 95% confidence interval of 0.425-0.953 and P=0.0283. It clearly showed the reduction of mortality of COVID-19 infected patients using statin medication(H.-Y. Lee et al., 2021). Rossi, Talarico, Coppi, and Boriani conducted a study which showed the reduction of mortality in COVID-19 infected patients with simvastatin and atorvastatin medication (Rossi, Talarico, Coppi, & Boriani, 2020). As a well-established cholesterol lowering agent with anti-inflammatory effects, this class of drug can be a treatment measure for hypercholesterolemic patients with COVID-19 infection. Several clinical trials are ongoing on stating for COVID-19 infection treatment measurement.

5.2 Antithrombotic Agents

5.2.1 CAC (COVID-19 Associated Coagulopathy) and Antithrombotic Agents

It is getting clear that the use of antithrombotic drugs is necessary in terms of patients suffering from COVID-19 associated coagulopathy (CAC). A study was conducted to evaluate the relationship between in-hospital administration of anticoagulants with the survival of COVID-19 infected patients. The study showed that in 395 mechanically ventilated patients, those who received anticoagulants had mortality of 29.1% with median survival of 21 days. However, those who did not take anticoagulants, there was 62.7% mortality with median survival of 9 days (Paranjpe et al., 2020). Another study was done on 449 COVID-19 infected patients. From

them, 99 patients took low molecular weight heparin for 7 days. A multivariate analysis shown patients who took low molecular weight heparin had better prognosis compare to those who did not received low molecular weight heparin (Tang, Bai, et al., 2020). An observational study was conducted in five Italian hospitals on 192 patients with confirmed COVID-19 infection. Among them, 38% of the patients had acute respiratory distress syndrome (ARDS). The authors concluded that, the anticoagulants and antiplatelet agents did not provide any beneficiary effects on COVID-19 infected patients. However, they also stated that, patients who received anticoagulant and antiplatelet agents had co-morbidities (Russo et al., 2020).

5.2.2 Antiplatelet Agents on COVID-19 and its Possible Drug-Drug Interaction with Investigational Antiviral Agents

Ticagrelor, an antiplatelet agent work by inhibiting $P2Y_{12}$ receptor (Cattaneo, 2015). COVID-19 infection associated pneumonia or novel coronavirus pneumonia cases shows significant coagulation abnormalities (Tang, Li, et al., 2020). Also, in pneumonia patients, ticagrelor can reduce thromboinflammatory markers effectively (Sexton et al., 2018). Thus, it is believed that ticagrelor can have beneficiary effect on COVID-19 infected patients (Akşit, Kırılmaz, Gazi, & Aydın, 2020). Ticagrelor metabolism occur in the liver with the help of CYP3A enzymes (Adamski et al., 2018). Aspirin, one of the most widely used NSAIDs. Aspirin inhibits the cyclooxygenase pathway, resulting in the inhibition of both COX-1 and COX-2 isoenzymes. By inhibiting the cyclooxygenase pathway, it also inhibits the synthesis of prostaglandins and thromboxane A2 which results in an antithrombotic effect (Mohamed-Hussein, Aly, & Ibrahim, 2020). A plaque reduction assay was done on aspirin. The study showed that aspirin had antiviral activity against influenza A H1N1 virus, an RNA virus (Glatthaar-Saalmüller, Mair, & Saalmüller, 2017). So, it is believed that aspirin can be used against COVID-19. Canadian Pharmacist Association provided an article on their website on the use of NSAIDs against COVID-19 infection. They said that NSAIDs can be used but further randomized

clinical trials are needed (Canadian Pharmacists Association, 2020). Antiplatelet drugs, $P2Y_{12}$ receptor inhibitors, such as clopidogrel and ticagrelor used in thrombotic patients can have some interactions with investigational antiviral agents that are used in COVID-19 infected patients. Clopidogrel is a prodrug that require to transform into its active form to give its pharmacological activity. To get the active metabolite, the CYP2C9, CYP2C19 and CYP3A4 are required (Sangkuhl, Klein, & Altman, 2010). By using antivirals that can inhibit the CYP3A4 (Yeh et al., 2006), will prevent the formation of the active metabolite of this drug. This phenomenon will decrease the level of active form of this drug. So, caution should be taken in case of using these drugs in covid-19 positive patients. Ticagrelor, when administered, transforms into its active metabolite AR-C124910XX with the help of CYP3A4 (Stezzi, Liberti, & Peart, 2012). With the inhibition of this CYP3A4 may decrease the level of active metabolite hence, decreasing the activity of the drug. So, co-administering with antivirals should take in considerations. There has not been any interaction profiling found on aspirin with the antiviral drugs used in COVID-19 infection (Bikdeli et al., 2020).

5.2.3 Anticoagulant Agents on COVID-19

Heparin, one of the most widely used anticoagulants. It is sulfated polysaccharide. There are two major types of heparin used as anticoagulants. Intravenously administered unfractionated heparin (UH) and subcutaneously administered low molecular weight heparin (LMWH) (Onishi, St Ange, Dordick, & Linhardt, 2016). A study in Tongji Hospital comparing the mortality of 449 COVID-19 infected patients with and without the use of heparin was conducted. The study conducted that treatment with low molecular weight heparin had better disease prognosis compare to non-users (Tang, Bai, et al., 2020). A randomized controlled trial will take place to compare the safety profile of high and low dosages of low molecular weight heparin (Marietta et al., 2020). There was a case reported of a COVID-19 infected patient. The patient was 72 years old, after six days of admission the patient was sent to ICU. The d-dimer

level of that patient was increasing continuously over time. On day four, the d-dimer level went up to 2.55 mg/l from 0.69 mg/l. On day 21, it was 20.63 mg/l. After day 21, they started treatment with high dose unfractionated heparin. On day 24, the d-dimer lever came down to 6.26 mg/l and after day 29 the level was 1.94 mg/l (Escher et al., 2020). Warfarin, vitamin K antagonist, an anticoagulant works by inhibiting the synthesis several clotting factors in the body. Vitamin K helps in the synthesis of factor VIIa, IXa, Xa and thrombin. The metabolism of warfarin is done by CYP2C9 (Onishi et al., 2016). Another class of anticoagulants are direct oral anticoagulants (DOAC), such as apixaban, edoxaban and rivaroxaban. They work by directly inhibiting the activated clotting factor Xa(Schwarb & Tsakiris, 2016). The metabolism of apixaban is done through CYP3A4 and it is a substrate of P-glycoprotein. However, edoxaban is hardly metabolized by CYP450 isoenzymes, but mainly metabolism occur through P-glycoprotein efflux transporter mechanism. Rivaroxaban, on the other hand, is metabolized through CYP3A4 and P-glycoprotein (Schwarb & Tsakiris, 2016).

5.2.4 Possible Drug-Drug Interaction Between Anticoagulant Agents and Investigational Antiviral Agents for COVID-19

Vitamin K antagonist or warfarin is a potential anticoagulant drug for coagulopathy. Warfarin is metabolized by the CYP2C9 (Onishi et al., 2016). There was a pharmacokinetic study conducted on 14 volunteers with lopinavir/ritonavir therapy. The study showed that there was an increase in activity of CYP2C9 by 29% and decrease in activity of CYP3A was by 77% with lopinavir/ritonavir therapy (Yeh et al., 2006). Thus, increase in the activation of CYP2C9 will decrease the plasma concentration of warfarin. So, dose adjustment is required in case of warfarin therapy with lopinavir/ritonavir with consideration of INR value (Bikdeli et al., 2020). There is no established interaction observed between warfarin and other investigational antiviral drugs against COVID-19 infection, such as lopinavir, ritonavir andremdesivir. There is another class of effective anticoagulants (DOAC), the direct oral anticoagulants, such as

apixaban, edoxaban, rivaroxaban and betrixaban. These drugs metabolize through p-glycoprotein and/or CYP3A4 pathway (Schwarb & Tsakiris, 2016). As mentioned before, antiviral agents can decrease the activity of CYP3A. Caution should be taken while taking these drugs together. There was a study conducted on COVID-19 infected

Table 2:Drugs with Possible Durg-Dug Interactions with Investigational Anti-viral Agents Against COVID-19.

Drug Class	**Drug**	**Antiviral Agent**	**Possible Drug-Drug Interaction**
Statin	Lovastatin, Simvastatin	Ritonavir, Lopinavir, Remdesivir	Increase the plasma drug concentration of statins
Antiplatelet	Ticagrelor, Clopidogrel		Theoretically decrease the plasma drug concentration of ticagrelor and clopidogrel. However, no evidence found on such interaction
Anticoagulant	Warfarin		Theoretically decrease the plasma drug concentration of warfarin. However, no evidence found on such interaction
Direct Oral Anticoagulant (DOAC)	Apixaban, Rivaroxaban		Increase the plasma drug concentration. Evidences suggest not to take DOAC with the investigational antiviral agents against COVID-19 infection

patients. 32 of the patients were on direct oral anticoagulants before getting infected with COVID-19. 20 of them discontinued the therapy of DOAC and 12 continued DOAC therapy. 8 of the 12 patients were on lopinavir/ritonavir and rest 4 were on darunavir/ritonavir antiviral treatment. The c-trough level was averagely 6.14 higher than before starting the antiviral therapy. The article concluded by providing a recommendation on not to use direct oral anticoagulants with antiviral therapy in COVID-19 infected patients (Testa et al., 2020).

5.3 N-acetylcysteine (NAC)

A glutathione precursor, N-acetylcysteine (NAC) has emerged as a potential medication against COVID-19. N-acetylcysteine (NAC) is used in paracetamol overdose medication by intravenous administration (Prescott et al., 1979). There has been a lot of studies done on NAC and several other potential therapeutic activities have appeared. There have been many articles published that propose the therapeutic approach with N-acetylcysteine in COVID-19 infection (Andreou et al., 2020; Assimakopoulos & Marangos, 2020; Bauer, Kapoor, Rath, & Thomas, 2020; De Flora, Balansky, & La Maestra, 2020; Ibrahim et al., 2020; Jorge-Aarón & Rosa-Ester, 2020; Nasi et al., 2020; Poe & Corn, 2020). An article by Poe & Corn, provided a hypothesis on N-acetylecyseine (NAC) being a promising drug against COVID-19 infection. In that article, the authors provided evidences that, as being a glutathione precoursor and is able to work as an anti-oxidant, NAC can be effective against high levels of reactive oxygen species in COVID-19 infected patients. As COVID-19 infected patients having lowered level of CD4+ and CD8+ counts, and in HIV, lowered level of CD4+ and CD8+ counts were attenuated by administering oral NAC. By stating this, Poe & Corn argued that NAC can also increase the CD4+ and CD8+ counts. The author also provided in vitro evidences and argued that NAC would be a potential agent in reducing the levels of TNF-α, IL1β, IL18 as well as IL-6 in COVID-19 infected patients (Poe & Corn, 2020). A study was published in 2017, with the objective to observe the effect of N-acetylcysteine (NAC) on vonWillebrand factor multimers in arteial thrombi. Both in vitro and in vivo stidies confirmed that the anti-thrombotic activity of N-acetylcystein (NAC) is done by cleaving the von Willebrand factor multimers that helps in platelet activation as well as platelet aggregation (De Lizarrondo et al., 2017). Also, we have previously discussed that COVID-19 induce thrombosis can occur through the elevation of von Willebrand factor. These studies by De Lizarrondo et al. provides strong directoins about N-acetylcysteine (NAC) to be used against COVID-19 infection. Although, with out any clinical

trials, using any drugs in repurposing manner is not cosider as ideal. Thus, randomized clinical trials are necessary in order to establish the efficacy of N-acetylcystein against COVID-19 infection. Furthermore, there is a phase II non-randomized clinical trial ongoing to see the efficacy of N-acetylcysteine (NAC) in COVID-19 infected patients (ClinicalTrials.gov, 2020). There has not been any drug-drug interaction observed with NAC and investigational antiviral agents used against COVID-19 infection.

Chapter 6

Conclusion

Starting from the beginning of the pandemic, the COVID-19 virus affected the world and its public health as well as economics. Not only the virus is causing fever, cough, pneumonia or ARDS, but this virus is also showing thrombotic events. As the mechanism is not clearly understood, we provided a review of possible mechanisms of thrombosis formation in COVID-19 infected patients and focus on how hypercholesterolemia can be a vulnerable comorbidity in terms of getting COVID-19 infection. Furthermore, we explained the reactive oxygen species (ROS) formation in COVID-19 infection via renin-angiotensin-aldosterone system. Evidences on ROS can generate endothelial cell dysfunction was specified. Our findings also suggest the release of von Willebrand factor due to endothelial dysfunction. With that, we hypothesized the high ROS production may be the major cause of thrombosis development in COVID-19 patients. Later on, we discussed the relation of hypercholesterolemia with elevated ROS levels. Patients with hypercholesterolemia have high levels of LDL, and upon oxidation it forms into OxLDL. Our findings also provided with the possibilities that OxLDL in the presence of high neutrophils can generate more ROS as well as NETs. The high levels of ROS leads to oxidative stress which further results in thrombosis formation as we have discussed, also, organ injuries and hyperinflammatory states which are demonstrated by critically ill

COVID-19 patients. In addition, we provided potential drug agents, such as statins, antithrombotic drugs and N-acetylcysteine. However, caution should be taken on taking them as some of these medications have drug interactions with the investigational antiviral agents used against COVID-19 infection. Although our review has provided hypothesis on the basis of published evidences and data, there are some aspects that our article will direct for further investigation.

The following points require thorough investigation by related expertise:

I. This review has shown several articles that provided with data of COVID-19 infected patients having high levels of von Willebrand factor. We strongly supported that this phenomenon is caused by oxidative stress induced endothelial dysfunction. Study on ROS measurements in COVID-19 patients with thrombotic events are needed to establish our hypothesis.

II. There was only one study mentioned in this article with the mortality data of COVID-19 infected patients with hypercholesterolemia as comorbid. More retrospective study needs to be done to gather information on hypercholesterolemic patients with COVID-19.

III. Furthermore, we have also argued that hypercholesterolemia as a vulnerable comorbid because they can generate excessive ROS and NETs. We provided in vitro and in vivo studies on OxLDL and neutrophils to support our argument. With that, it is required to measure the OxLDL level in COVID-19 infected patients.

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