

The molecular story of COVID-19; NAD⁺ depletion addresses all questions in this infection

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Abstract

The emerging new Coronaviridae member, nCoV 19, outbreak announced a pandemic by WHO with an increasing morbidity and mortality rate worldwide. nCoV 19 known as the third highly pathogen coronavirus in human population after the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), the nCoV 19.

The renin–angiotensin (RAS) signaling pathway, oxidative stress and cell death, cytokines storm and endothelial dysfunction are four major pathways involved in the pathogenesis of nCoV 19. Acute respiratory distress syndrome (ARDS) generally develops with a massive oxidative/nitrosative stress following virus entry and RAS activation. The DNA damage subsequent to oxidative burst activates poly-ADP ribose polymerase-1 (PARP-1), viral macrodomain (NSP3) poly (ADP-ribose) glycohydrolase (PARG) and transient receptor potential channel, melastatin 2 (TRPM2) in a sequential manner ultimately leading to apoptosis and necrosis due to NAD and ATP depletion. Regarding the molecular mechanisms involved in nCoV 19 pathogenesis, angiotensin II receptor blockers and/or PARP, PARG and TRPM2 blockers could be engaged as therapeutic candidates for inhibition of RAS and quenching oxidative stress, respectively. In this review, the molecular aspects of nCoV 19 pathogenesis would be studied precisely and possible therapeutic targets would be proposed. It is recommended to evaluate the proposed drugs and supplements via registered clinical trials along with conventional guideline-based multi-drug regimen.

Key Words: COVID-19; nCoV 19; Oxidative stress; PARP; PARG; TRPM2

1. Introduction

The new viral pneumonia caused by the 2019 novel coronavirus (nCoV19) is a highly contagious disease, and the ongoing outbreak has been declared by WHO as a global public health threat (1, 2). The nCoV 19 known as the third highly pathogen coronavirus in human population after the severe acute respiratory syndrome coronavirus (SARS-CoV-2002) and the Middle East respiratory syndrome coronavirus (MERS-CoV-2012) in the 21st century (3). The nCoV19 categorized in Coronaviridae family along with about 40 species as single-stranded positive-sense RNA viruses. The nCoV 19 classified in β -coronaviruses cluster based on sequence analysis study together with SARS-CoV and MERS-CoV (4) with more than 82% similarity to those of SARS-CoV. Coronaviruses known as human infectious agents in the last few decades while primarily identified as a source for enzootic infections in birds and mammals (5). At present, the incidence of coronavirus disease 19 (COVID-19) have been found in more than 100 countries around the world (6). According to the latest data, the number of confirmed cases in the world reached 244919, up to the March 20, 2020, of which 10031 deaths, were reported.

Considering the lack of any registered therapeutic or preventive strategy for COVID-19, there is an urgent need to find an alternative solution for prevention and control of global distribution of virus. Therefore, the molecular mechanisms involved in nCoV 19 pathogenesis or recruited by the virus would be studied precisely in this review and possible therapeutic targets and a number of approved drugs would be introduced.

2. Pathogenesis and Therapeutic Targets

2.1 Clinical manifestations- Fever, nonproductive cough, nasal congestion and fatigue as the clinical characteristics of COVID-19 start after less than a week of infection. About 75% of patients show severe disease as seen by computed tomography scan on admission (1). On the day 10 to 20 of the symptomatic infection, pneumonia occurs which is associated with reduced oxygen saturation, blood gas deviations and sharp changes of chest X-ray as prominent signs of COVID-19. Lymphopenia and elevation of inflammatory markers including C-reactive protein and pro-inflammatory cytokines considered as diagnostic clinical laboratory manifestation (1, 7).

The exact mechanism of nCoV-19 pathogenesis is still unknown, although Lu et al revealed nCoV 19 is genetically similar to SARS-CoV-1 and MERS-CoV, 79% and 50%, respectively. In addition, homology modelling showed the structure of receptor binding domain in nCoV 19 is similar to SARS-CoV-1. This evidence suggests nCoV 19 pathogenesis resembles SARS-CoV-1 infection (8, 9), thus too much information can be gained based on this similarity for a better understanding of COVID-19.

2.2 Coronavirus life cycle- Coronaviruses are believed to enter the host cells through interaction of spike protein (S protein) and angiotensin-converting enzyme 2 (ACE2) receptor, also known as COVID-19 specific cellular receptor (10, 11). The one step proteolytic cleavage mediated by host cell protease in certain SARS-CoV S protein residue (position S2'), facilitates direct membrane fusion between viral and plasma membrane (12). Previous studies have shown SARS-CoV cell entry also occurs through ACE2 receptor as the preferred receptor (13, 14).

While MERS-CoV has developed a strange two-step furin- mediated membrane fusion (15). Upon entry into the host cells, the viral RNA genome is translated into two polyproteins and structural proteins in the cytoplasm, which helps the assembly of virus progeny (16). Ultimately, following assembly of emerging envelope glycoproteins and the genomic RNA, budding occurs in the endoplasmic reticulum-Golgi intermediate compartment (7).

2.3 The renin–angiotensin (RAS) signaling pathway- Particularly, ACE2 is an ACE homolog belongs to ACE family of dipeptidyl carboxydipeptidases which obviously counter balances the ACE physiological function. ACE is responsible for production of angiotensin II by cleavage of angiotensin I which subsequently binds to angiotensin Type-1 Receptor (AT1R) and alters blood pressure, body fluids and electrolyte homoeostasis. In contract, ACE2 inactivates angiotensin II by catalyzes the elimination of the C-terminal phenylalanine residue in order to generate angiotensin 1–7. This peptide potentially exerts vasodilatory action and negatively regulates renin–angiotensin system. Thus, in nCoV 19 pathogenesis, RAS acts as a double edge sword, as a receptor for the virus entry and a negative regulator for severe symptoms of infection and lung injury (17, 18).

Earlier studies in SARS-CoV pathogenesis proved the binding of the SARS-CoV protein S to ACE2 receptor, is associated with ACE2 downregulation, excessive production of angiotensin by ACE and less vasodilatory function of heptapeptide angiotensin 1–7. This process leads to excessive production of pro-atrophic, pro-fibrotic, pro inflammatory and

pro- oxidant agents thus exacerbates lung injury and enhances pulmonary vascular permeability (Fig. 1) (19).

The lungs provide a massive surface of about 100 m² for viral entry. On the other hand, existence of alveolar epithelial type II cells (AECII) creates an appropriate viral reservoir in human alveolar epithelial cells. Moreover, multiple viral process-associated genes in AECII, could simplify virus progeny release in these cells (19, 20).

2.4 Oxidative stress and cell death- Oxidative stress is triggered by the imbalance between the oxygen reactive species (ROS) production and clearance. ROS as metabolic by-products including hydrogen peroxide (H₂O₂), Superoxide radicals (O₂ •⁻), singlet oxygen (1 O₂), hydroxyl radicals (•OH) and peroxynitrite anion (ONOO⁻) are produced using biological systems (21). Both viral infections and RAS activation produce ROS in a reproductive manner leading to oxidative burst. Increased ROS levels lead to destructive effects on cellular macromolecules such as lipids, proteins and especially nucleic acids (22).

The exogenous (environmental) and endogenous (intracellular) sources of free radicals constantly cause DNA damage. Another source of free radical generation which is generally underestimated is the exposure to viral infections (23).

Oxidative stress-mediated DNA damage is repaired primarily via the base excision repair (BER) pathway which appears to be the simplest pathway among the three excision repair pathways (23).

Normally, poly-ADP ribose polymerase-1 (PARP-1), a DNA base repair enzyme, activated by DNA breaks and contributes to BER pathway for maintenance of genome stability.

Upon activation, PARP-1 rapidly uses the substrate NAD^+ to transfer poly ADP-ribose (PAR) to itself, nuclear acceptor proteins and damaged DNA (24).

PARP-1 has ADP-ribosyl transferase (monomer or polymer) activity and functions as an antiviral agent through ADP-ribosylation of viral genome (RNA or DNA) and inhibition of viral transcripts translation. However, several viral families, including Togaviridae, Hepeviridae and Coronaviridae encode for a macrodomain protein with poly (ADP-ribose) glycohydrolase (PARG) activity which hydrolyzes ADP-ribose units from proteins and nucleic acids to facilitate optimal replication and virulence(25).

Excessive activation of PARP occurs to compensate ADP-ribose hydrolyzation of PARG which is associated with catalytic consumption of NAD^+ followed by ATP reduction leading to depletion of energy and cell death (26).

On the other hand, PARP and subsequent PARG activation following the deep DNA damage generates large amounts of free ADP-Ribose units which bind to transient receptor potential channel, melastatin 2 (TRPM2) through a functional ADP-Ribose hydrolase domain in its C terminus (27). Intracellular calcium concentration and ADP-ribose known as activators of TRPM2 (27, 28). Lysosomal and plasma membrane TRPM2, cause Ca^{2+} influx across the plasma membrane and release of lysosomal Ca^{2+} , providing high concentration of Ca^{2+} in the cytosol. The overload of cytosolic Ca^{2+} initiates apoptosis and probably necrosis (29, 30). This might be the possible explanation of severe lung injury in COVID -19 patients. Moreover, TRPM2 ability to response to oxidative stress made it a promising target for development of new small molecule and therapeutic approach such as aptamer (31) and gene editing tools (32, 33)(Fig 1).

2.5 Cytokines storm- A well-synchronized immune response is essential for control and eradication of nCoV and other viral infections. However, maladjusted and uncontrolled immune responses to viral progeny release may cause cytopathic effect during nCoV infections. The host innate immune system detects virus and a rapid immune response initiates after virus-cell interaction (34).

The antigen-presenting cells (APCs), including macrophages present nCoV antigens to T cells subsequent to nCoV 19 entrance into the host cells. This process leads to T cell activation and production of cytokines in various T cell subsets i.e. Th17, followed by a massive release of cytokines due to positive feedback loop between cytokines and immune cells. On the other hand, during nCoV 19 replication, virus genomic dsRNA activates interferon regulatory factors (IRFs) and TLR-3-induced NF- κ B pathway which culminates in production of type I IFNs and proinflammatory cytokines in large quantities (35).

Dysregulation of immune responses following hyper-inflammation and cytokine storm, may lead to multiple organ failure, pulmonary tissue damage, and reduced lung capacity which is well-known in patients with COVID-19 infection (34, 36).

Present data in COVID-19 infected patients showed a significantly increased level of plasma pro-inflammatory cytokines including MCP1, MIP1 α , MIP1 β , IL1- β , IL1RA, IL7, IL8, IL9, IL10, IP10, PDGFB basic FGF2, GCSF, GMCSF, IFN γ , TNF α , and VEGFA (37).

In conclusion, viral infection initiates a detrimental cycle of oxidative stress-mediated functions including PARP and PARG activity, ADP ribose increase, TRPM2 activity, apoptosis and/or necrosis (parthanatos) (38) and inflammatory and vasodilator agents

release. Together, all these mechanisms result in endothelial dysfunction and extravasation of immune cells in alveolar space and finally a ground glass pattern in chest X ray. The trapped immune cells release large amounts of cytokines which leads to systemic inflammatory response syndrome (SIRS), a figure usually seen in septic shock and other cause of acute respiratory distress syndrome (ARDS) like paraquat (1,10-dimethyl-4,40-bipyridinium dichloride; PQ) poisoning (39)(Fig 1).

2.6 Endothelial dysfunction- The earliest and one of the most important indicators of endothelial dysfunction is chronic reduction of nitric oxide (NO) synthesis, release or activity and/or increased NO degradation by reactive oxygen species (ROS) (40). The reduced NO bioavailability results in proliferative, pro-oxidant, pro-inflammatory, and pro-thrombotic responses. Thus various pathological disorders affect endothelial function through changing the molecular mechanisms involved in regulation of NO bioavailability (41).

The appropriate environment for coronavirus life cycle is an oxygen-depleted condition. In the hypoxic situation, ROS generation, and HIF-1 α activation occurs sequentially, which (42), consequently induces the expression of furin enzyme (43) and ultimately viral activation.

In the hypoxic milieu, nitric oxide release enhanced by function of nitric oxide synthases (NOSs) using L-arginine amino acid for maintenance of nitroso/redox balance (41, 44). The NOSs function is dependent to adequate amounts of tetrahydrobiopterin (BH₄) in its

active reduced form (45, 46). In the oxidative stress state, extra free radicals interfere with redox homeostasis and adequate BH₄ production (47). It could be concluded the altered redox homeostasis leads to reduced levels of NO in COVID-19 patients with oxidative burst and RAS activation.

Decreased levels of NO, shows various properties attributed to inflammation and cardiovascular events concomitant with endothelial dysfunction. Low levels of NO is capable of inducing vascular smooth muscle cells (VSMCs) proliferation (48), LDL oxidation (49), and vascular cell adhesion molecule-1 (50) and Monocyte chemoattractant protein 1 (MCP1) expression (51) through inhibition of NF-kappa B signaling pathway. Moreover, decreased NO following oxidative burst, stimulates matrix metalloproteinases-2 and 9 (52, 53) as well as induction of pro-inflammatory cytokines and chemokines expression (54). Platelet aggregation (55), leukocyte adhesion (56) and thrombolysis stimulation (54) are also considered as other characteristics of reduced NO level contributing in endothelial dysfunction.

Although, decreased NO production is prominent in COVID-19 patients, a few clinical manifestation of patients described due to slightly increased level of NO. NO is considered an important factor in vascular homeostasis (49) due to the role of NO in inhibition of contractile machinery and vasodilation. In this process, NO which was produced by endothelial cells, spreads into VSMCs and generates cyclic guanosine-3,5-monophosphate (cGMP). Subsequently, cGMP-dependent protein kinase is activated and removal of cytosolic Ca²⁺ occurs which is associated with inhibition of the contractile machinery and ultimately, vasodilation (54, 57, 58). On the other hand, NO release in peripheral vessels

may worsen hemodynamic homeostasis and decrease the blood pressure and organ perfusion.

Another mechanism for NO release from endothelial cells, might be due to shear stress and ischemia (49, 54), which enhanced by acetylcholine, bradykinin and serotonin (54)(Fig 1).

3. Possible therapeutic options for COVID-19

Regarding the molecular mechanism involved in nCoV 19 pathogenesis, several proteins including ACE2, ATR1, NADPH oxidase, PARP, PARG Macrodomein or NSP3, and TRPM2 could be considered for target therapy using synthetic drug or natural compound candidates which are further discussed in following sections.

3.1 Vitamin D- Recently, variety of vitamins have been suggested for treatment or improvement of nCoV 19 infection symptoms. Vitamin D showed significant effects through binding to the ACE2 receptor which mediates acute lung injury in host cells during nCoV 19 infection (59, 60). The high expression of ACE2 receptor on the surface of human alveolar epithelial cells significantly facilitates coronavirus internalization and infection (19). Based on experimental studies, administration of vitamin D agonist, calcitriol, exerts protective effects on lung injury through modulating components of RAS such as ACE I and ACE II, renin and Ang II (59). Thus, vitamin D deficiency may directly promote hypertension through impacts on members of RAS (61).

Another protective function which has been proposed for vitamin D, is immunomodulation through suppressing pro-inflammatory cytokines. Hence, vitamin D supplements may ameliorate the cytokine storm following nCoV 19 infection. (62). In vitamin D deficiency, the ACE II receptor binding sites are exposed on the cell surface which could

further enhances the viral entry into the host cells. Thereby, it seems vitamin D is considered as a critical player in capability of nCoV 19 infection. Furthermore, although vitamin D is considered as a known PARP inhibitor (63), vitamin D provides increased amounts of extracellular calcium which functions as a potent activator of TRPM2 (97). Thus, administration of vitamin D in COVID-19 patients is still controversial.

3.2 Thalidomide- Thalidomide previously known as an effective NSAID, while the application of thalidomide was banned due to its teratogenic effects. On the other hand, various antiviral properties including inhibition of PARP, anti TNF and NADPH oxidase inhibition effects have been assigned to thalidomide (99).

3.3 Trehalose- Trehalose is a non-reducing disaccharide and generally applied as a stabilizer in drug formulation. Trehalose inhibits PARP1 and PARP2, thus could be used as a filler in vitamin B3 or NAD tablets and further prescribed for nCoV 19 infected patients through a registered clinical trial (64).

3.4 N-acetylcysteine (NAC)- NAC has been used for treatment of poisoning with toxin and some drugs due to its PARP inhibitory and antioxidant activity, thus might be a suitable candidate in treatment and control of nCoV 19 infection symptoms (65).

3.5 Tannins- Tannins are water-soluble polyphenolic compounds which have been known as health-promotor components (66). Several studies have reported the antiradical activities of tannins (66). Moreover, the anti-inflammatory effects of tannins were also reported in previous studies (67, 68). Based on the molecular mechanisms recruited by nCoV 19 during pathogenesis in human body, COVID-19 is considered as an inflammatory disorder, thus tannins as the magic antioxidants can reduce the disease morbidity and mortality due to their role in redox homeostasis maintenance. In addition, inhibitory effect of tannins on

PARG enzyme was reported previously (38). An in silico study showed the tannins also bind to ADP ribose specific binding site in TRPM2 and supposed to be the TRPM2 inhibitor but this should be more evaluated by further experiments. Gallotannin as hydrolysable tannins showed to inhibit cytokine expression (38).

3.6 Flufenamic Acid and clotrimazole- These drugs inhibit TRPM2 in a non-selective manner and are capable to inhibit or activate other ion channels as well (69). Flufenamic acid activates AMP-activated protein kinase (AMPK α -P) and suppresses NFkB expression and ultimately shows anti-inflammatory function (70).

Another small molecule against PARP such as olaparib or other paribs might be appropriate candidate for COVID19 treatment (69).

3.7 NAD⁺ and niacin- In the depicted molecular pathology pathway of COVID-19, almost all procedures lead to or originate from NAD⁺ depletion. NAD⁺ depletion mediated by uncontrolled PARP activity leads to decreased sirtuin 1 (SIRT1) activity indirectly. SIRT1 deacetylates nuclear proteins using NAD⁺ to regulate the expression of genes including tumor suppressors, cytokines and proto-oncogenes and ultimately modulate inflammation, cell survival and apoptosis mechanisms (71). NAD and ATP are prerequisite for each other and consumption of NAD in large amounts decreases ATP levels leading to impairment of all activities and integrity of the cell. In COVID-19-mediated ARDS, aldosterone level is decreased and patients are hypovolemic in spite of RAS activation. It seems that aldosterone synthesis is silenced somewhere in CNS or adrenal gland. The logical interpretation is serotonin shortage which is an important molecule and has several roles in biology including stimulation of aldosterone secretion (72). In COVID-19 patients, the resources of tryptophan-as the raw material for serotonin and NAD synthesis- spend and

in ARDS course of the disease serotonin is decreased and hypoaldosteronism causes hyponatremia and hypovolemia. Fatigue and various degrees of mood disorders are the consequences of NAD, ATP and serotonin reduction, which could be addressed by concomitant prescription of NAD, Niacin (Vitamin B3) and/or its precursor L tryptophan with a PARP or PARG inhibitor. It is possible that administration of NAD alone, along with high activity of PARP and PARG, worsen the clinical manifestation.

3.8 Losartan- Based on previous experimental studies, losartan, the angiotensin II receptor antagonist, reduces the synthesis of TGF-1 β and PARP. Hence, losartan seems to be able to prevent or control chronic fibrotic diseases such as cardiac hypertrophy and asthma in addition to hypertension (73-76). In an animal study, losartan therapy improved paraquat-induced pulmonary fibrosis. It seems losartan acts through inhibition of TGF-1 β mRNA expression and synthesis of collagen. This could provide enough evidences for application of losartan for the prevention of pulmonary fibrosis (39).

Moreover, Apart from direct anti-hypertensive effects of losartan, it provides significant reduction of platelet aggregation by ristocetin and reduced hematocrit with hemoglobin, following administration in newly diagnosed hypertensive patients which is suggestive for applying losartan for thrombosis and atherosclerosis as well (77).

Another mechanism of action for losartan considered to be the immunomodulatory function and significantly regulating IFN- γ , IL-6, IL-17F and IL-22 cytokines in PBMCs of rheumatoid arthritis patients (78).

Another animal study provided evidence of losartan significant effects on severe acute lung injury (ALI) and ARDS (ALI/ARDS), which might be due to inhibition of NF-kappaB and

MAPK signaling pathways (79). Activation of RAS system ignites a strong oxidative stress as the key pathogenic mechanism in COVID19.

An earlier hypothesis illustrated that losartan as an angiotensin receptor 1 (AT1R) blocker in RAS pathway could be useful for patients infected by COVID-19 who experience pneumonia (80). Losartan and olmesartan, AT1R antagonists, commonly applied for treatment of hypertensive patients which have overexpression of ACE2, major receptor for virus entry in both rat and human (81, 82).

Although, half of COVID-19 patients experience hypotension during their hospitalization but based on premature estimation, percentage of SARS patients of the currently ongoing epidemic can be properly cured with AT1R blockers without risking exacerbated hypotension (20). These candidates of SARS-CoV-2 therapeutics suggested for treating patients prior to the development of acute respiratory syndrome but this hypothesis certainly should be approved with experimental and clinical investigation (20). COVID-19 infection induces some degree of fibrosis in lung which correlates with the severity of the disease, thus, losartan may protect the involved organs from fibrosis and decrease morbidity of disease.

Based on the existing evidences, TRPM2 could be an appropriate therapeutic target in nCoV 19 life cycle. There are several small molecules inhibiting TRPM2, including Flufenamic acid (FFA) (83, 84), 2-(3-methylphenyl) aminobenzoic acid (3-MFA) (83), N-(pamylcinnamoyl) anthranilic acid (ACA) (85), econazole, clotrimazole (86) and 2-aminoethoxydiphenyl borate (2-APB) (87, 88), while, none of them are TRPM2 channel specific (89). Furthermore, some nucleoside analogues like adenosine monophosphate (AMP) and 8-bromoadenosine 5'-diphosphoribose (8-Br-ADPR) have also been mentioned

as TRPM2 inhibitors (27, 90). Two novel TRPM2 channel inhibitors have been synthesized (69) by modification of ADPR analogues including 8-phenyl-2'-deoxy-ADPR which specifically inhibits TRPM2 without interfering Ca^{2+} release induced by cADPR, NAADP or IP3 (91).

3.9 Monoclonal Antibodies and recombinant proteins

Neutralizing antibodies against nCoV 19 surface antigens prevent virus entry of which, S protein considered as a good candidate. These monoclonal neutralizing antibodies provide a passive immunity in the time of exposure like palvizumab as a putative example that has been applied for prevention of RSV infection (92). Anti-inflammatory antibodies like anti IL6 receptor (Tocilizumab) and anti ITGA4 (Natalizumab) may inhibit inflammation and cellular extravasion (101, 102). Another proposed strategy is administration of soluble ACE2 receptor to scavenge the virus. The similar procedure has been applied in HIV infection using soluble CD4 in HIV infection.

4. Conclusion

As a clinical manifestation, ARDS occurs during viral infection like nCoV 19, septic shock, poisoning and chemical warfare agents. The prognosis of ARDS is poor and there is no specific treatment for it. ARDS generally begins with a massive oxidative/nitrosative stress and the subsequent DNA damage activates PARP, endogenous PARG and TRMP2 activity which ends in apoptosis, necrosis and parthanatosis. nCoV 19 expresses NSP3 as a potent extraneous PARG and likely activates RAS that provides fuel for oxidative stress in this circuit. This detrimental cycle consumes NAD and decreases antioxidant capacity thus enhances

inflammation and cytokin release. NAD is consumed in large scale by PARP and its depletion inhibits the activity of other protective protein like SIRT1 and CD38. Expression of NFkB and cytokines and blood and immune cell defects are the consequences of SIRT1 and CD38 inhibition respectively. L tryptophan is the common raw material for NAD and serotonin synthesis and NAD depletion leads to reduction of serotonin indirectly. Since serotonin is important for aldosterone secretion and has several positive cardiovascular effects, COVID-19 patients suffering hypovolemia and hypoaldosteronism due to serotonin depletion in spite of RAS overactivation that usually ends to hypervolemia and hypertension. According to above vision of nCoV 19 molecular pathology, there are several therapeutic candidates engaged for COVID-19 treatment. Inhibition of RAS activation by Angiotensin II receptor blockers like losartan with known PARP inhibitory effect could quench oxidative stress and interrupt this circuit. Moreover, renin inhibitors like aliskiren which suppresses RAS could also be recommended for COVID-19 treatment. PARP inhibitors like trehalose, olaparib, losartan, vitamin D and NAC are another therapeutic options in nCoV 19 infection. Apocynin is an NADPH oxidase inhibitor and could cut the detrimental circuit. PARG and TRPM2 antagonist including tannins, fluenamic acid and clotrimazole proposed for TRPM2 inhibition as well. In addition to suggested drugs, taking NAD or Vitamin B3 plus L-tryptophan could replenish NAD and serotonin and recover the body toward hemostasis. It should be considered that using NAD alone could worsen the COVID-19 symptoms, thus PARP, PARG and TRPM2 inhibitors might be prescribed in the first step. Vitamin D and losartan appeared to overexpress ACE2 and might increase viral entrance. Vitamin D also increases extracellular calcium and could activate TRPM2. However, the level of AgII in COVID-19 is high and upregulation of ACE2 following losartan intake runs the protective brunch of RAS pathway. In conclusion, it seems

interruption of the explained lethal circles may convert COVID-19 to a simple common cold.

The above drugs and supplements could be examined via registered clinical trials along with conventional multi drug regimen and anti-viral therapeutic guidelines.

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Legend

Figure 1. Four major pathways involved in the pathogenesis of nCoV 19. 1-The renin-angiotensin (RAS) signaling pathway, 2- oxidative stress and cell death, 3- cytokines storm and 4- Endothelial dysfunction.



