



Role of Oxidative Stress in Novel Coronavirus

Sunny Chopra

Assistant Professor Department of Biochemistry
Government Medical College and Hospital, Patiala, Punjab, India

***Corresponding Author:**

Sunny Chopra

Assistant Professor Department of Biochemistry
Government Medical College and Hospital, Patiala, Punjab, India

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ABSTRACT

Worldwide, novel coronavirus 2019 (COVID-19) is a serious health problem. Emerged from Wuhan, China, it is spreading rapidly throughout globe and devastating social, health, economic and political crisis, that will leave deep scars. It is a new form of respiratory and systemic disorder sustained by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 is a viral infection, which is generally associated with inflammation, cytokine production, cell death and other patho-physiological processes, which could be link to the production of reactive oxygen species (ROS) and oxidative stress (OS). More age and underlying health conditions increases OS and ROS production in COVID-19 patients and this might be the reason of increased fatality and mortality of such patients. The aim of the present review is to understand the possible link between oxidative stress and the pathogenesis of COVID-19. It also focuses on the role of anti-oxidants to reduce the morbidity and mortality risk in patients affected by SARS-CoV-2 infection.

Keywords: COVID-19, oxidative stress, reactive oxygen species, SARS-CoV-2

INTRODUCTION

Coronavirus is a global challenge today. First case of the disease was presented with pneumonia of unknown cause on 31st December, in the town of Wuhan, China [1]. Since then, the disease outbreak was noticed and rapidly spread to the rest of the World, accounting for high morbidity and mortality. On January 30, 2020 the disease was announced as Public Health Emergency of International Concern and on 11th February 2020, new name was given to the disease as COVID-19 by World Health Organization (WHO) [2]. International virus classification commission called the novel coronavirus, as severe acute respiratory syndrome coronavirus- 2 (SARS-CoV-2) [3]. At present, the incidence of COVID-19 has been found in approximately 213 countries, around the world.

According to latest data 43,147,494 cases has been reported worldwide, till 27 October, 2020 [4].

Coronavirus (CoV): It belongs to large family coronaviridae, subfamily Orthocoronavirinae, order Nidovirales, and realm Riboviria. It is an enveloped virus with one of the largest (25-32 kilobase), positive- sense single stranded RNA genome [5]. The viral genome encodes four structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as several non-structural proteins and multiple unique accessory proteins. Whole genome is wrapped in an icosahedral protein shell [6]. Under microscope, spikes create an image reminiscent of the solar corona, from which the name derives- coronavirus (CoV) [7]. On the basis of phylogenetic relationships and genomic structure, CoV is divided into four main sub-groups known as

alpha (α), beta (β), gamma (γ) and delta (δ). The viruses belong to group α and β infect mammals while viruses belong to group γ and δ infects birds. Alpha group (α) viruses develop mild symptoms and create less fatality to humans. While viruses belong to β group, may originate from animals and transmit moderate to severe infection and can highly fatal to human beings [8].

SARS-CoV-2 is a β - group virus. It shares 79.0% nucleotide identity to SARS-CoV and 51.8% identity to MERS-CoV, indicating a high genetic homology among SARS-CoV-2, MERS-CoV and SARS-CoV [9]. Based upon the type of organ infected by SARS CoV-2, various symptoms like respiratory, gastrointestinal, hepatic, and neurologic etc will develop [10]. Based upon the amount or load of SARS-CoV-2, COVID-19 disease can be divided into three phases (Mild, Moderate and Severe) that correspond to different clinical stages of the disease.

Phase I is mild/ asymptomatic stage: This is initial 1-2 days of infection, when virus SARS-CoV-2 was being inhaled. The virus entered in nasal cavity and binds to angiotensin converting enzyme-2 (ACE2) receptors present on epithelial cells [11]. Limited innate immune response developed. Viral infection can be detected and confirmed by taking sample from nasopharynx and is confirmed by RT-PCR [12].

Phase II is moderate stage: It developed when the virus migrates down the respiratory tract along the conducting airways and a more robust immune response triggered. Virus infected epithelial cells induce cytokine response. Levels of various cytokines will increase and COVID-19 disease manifest clinically [13].

According to various case studies, about 80% of the infected patients have mild to moderate symptoms and disease is mostly restricted to the upper and conducting airways. These individuals may be monitored at home with conservative symptomatic therapy [14].

Phase 3 or severe stage: About 20% of the infected patients will progress to stage 3 and will develop pulmonary infiltrates. Out of these 20%, around 2% will develop very serious symptoms and require ventilation. Fatality and mortality rate varies with various factors like age, co-morbid conditions, immunity etc [15].

Stage 3 will develop when excessive and continuous load of SARS-CoV-2 reaches the gas exchange units of the lung. Virus propagates within cell and multiplies. Pathologically, affected tissue showed alveolar damage with fibrin rich hyaline membranes and few multinucleated giant cells. Significant high levels of cytokines and chemokines, will trigger “cytokine storm” which is associated with apoptosis and severe tissue damage [16] that contribute to increase fatality and morbidity of COVID-19 patients [17]. The cytokine production, inflammation, cell death, and other patho-physiological processes, could be link with a redox imbalance or oxidative stress (OS). It is known that an overproduction of reactive oxygen species (ROS) and deprivation of antioxidant mechanisms are crucial for viral replication and the subsequent virus-associated disease [18].

Several studies have been published on COVID-19 reported clinical features, laboratory findings and diagnostic evaluation of individuals suffering from this disease. Currently, vaccine or antiviral drugs specific for this deadly virus are under trial, so in the absence of specific treatment, there is an urgent need to find alternative mode of treatment for this disease. The current review focus on to understand the possible link between oxidative stress and COVID-19 and to review the role of some associated anti-oxidants to reduce the morbidity and mortality risk in patients affected by SARS-CoV-2 infection.

Oxidative stress (OS) and CoV: Under normal physiological mechanism, when virus or any foreign body enter in the body, inflammatory cells like neutrophils, eosinophils, monocytes and macrophages clear them by phagocytosis [19]. These inflammatory cells have NADPH oxidase enzyme, which produce certain level of superoxide ions by the process of respiratory burst. The superoxide ions are converted to various reactive oxygen species (ROS) like H₂O₂, hypochlorous acid etc. Excessive productions of ROS are controlled by antioxidants present in the body. The body maintains the balance between pro-oxidants and anti-oxidants, but under various patho-physiological conditions this delicate balance can be altered in favors of the former, thus leading to a condition known as oxidative stress (OS) [20].

Lungs are the most oxygenated organs in the human body. They are preferred target for COVID-19

because of their large area exposed to viruses [21]. CoV has long incubation period (5-14days) that elicit intensive immunological reactions, cytokine storm and immune-cell infiltration [22]. Sudden increased levels of immunocytes can produce excessive ROS. Increased oxidative stress is a major insult in pulmonary injury including acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). They will cause acute respiratory failure and substantially increase morbidity and mortality rate [23]. OS will oxidize cellular proteins and membrane lipids and quickly destroy not only virus-infected cells but also normal cells in various organs like lung and even heart and resulting in multiple organ failure. Vitamin C (ascorbic acid) and Vitamin E (α -tocopherol) are potential and inexpensive exogenous anti-oxidants. Vitamin C scavenges hydroxyl and superoxide radical anions and Vitamin E protects cell membrane from lipid peroxidation. They could alleviate cardiac and pulmonary casualties caused by COVID-19 [24].

Oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body [25]. Virus also disrupts antioxidant mechanisms, this leads to unbalanced oxidative-antioxidant status, produces OS and subsequent oxidative cell/organ damage. The link between inflammation and OS was well established [26]. It is known that an overproduction of ROS and antioxidant mechanisms deprivation are crucial for viral replication and the subsequent virus-associated disease like SARS-CoV-2 [27].

Pulmonary ischemia decreases the levels of adenosine triphosphate (ATP). Decrease levels of ATP, promotes breakdown of ATP and increase production of hypoxanthine [28]. Xanthine oxidase converts hypoxanthine into xanthine. This reaction produces various super-oxide radicals. When oxygen is reintroduced into the environment by ventilation, the activity of the enzyme xanthine oxidase enhanced [29]. In the absence of blood circulation in the lungs there is lipid peroxidation and oxidative damage due to the presence of oxygen [30].

Meta-analysis done by Hemila and colleagues observed antioxidant effect of high doses of Vit C in ICU patients. High dose of Intravenous Vitamin C infusion (e.g 200 mg/kg body weight/day) was divided into 4 doses and given to the intensive care

unit (ICU) patients. They observed that high dose of Vitamin C will shorten the ICU stay of patients by 7.8% [31]. Study done in 2014, compared pathogenesis of SARS and influenza, on non-human primates, ferrets and cats. Almost similar pathology and pathogenesis has been observed in both diseases. Study reported enhanced ROS levels and disturbance of antioxidant defense during SARS-CoV infection in experimental animal models. Both viruses affect mainly the respiratory tract and cause inflammation and necrosis centered on the pulmonary alveoli and bronchioles [32].

NADPH oxidase (Nox 2): Angiotensin converting enzyme 2 (ACE2) is a membrane bound aminopeptidase and is present in various organs like heart, vascular system and lungs. It serves as functional receptor for SARSCoV-2. SARSCoV-2 interacts with ACE2 receptors through spike protein and lead to down regulation of receptors. This down regulation may be a potential trigger for oxidative stress as impaired angiotensin II degradation could lead to activation of NADPH Oxidase 2 (Nox 2). Nox 2 is an important enzyme which generates reactive oxidant species (ROS). These ROS will activate more Nox2, so vicious circle of Nox2 activation –ROS-Nox2 has developed, which perpetuates oxidative stress [33].

Ascorbic acid is an antioxidant, given in loading dose of 1 g infusion I/V, down-regulate Nox2 activation without eliciting any side effect [34]. Tocilizumab, commonly used drug for arthritis, is now under investigation in COVID-19 patients as it behaves also as an antioxidant and may interrupt the vicious circle Nox2-ROS-Nox2 [35].

OS with age: Study done in the epicenter of the outbreak, the city of Wuhan in Hubei province, China reported a detailed clinical and epidemiologic description of the first 425 cases. Study reported that the median age of the patients was 59 years, with higher morbidity and mortality among the elderly and among those with coexisting conditions (similar to the situation with influenza) [36]. The severity and mortality risk of COVID-19 disease have been associated with the age [37]. With age, immune response diminished and ability to repair the damaged epithelium has reduced. The elderly also have reduced mucociliary clearance, and this may allow the virus to spread to the gas exchange units of

the lung more readily [38]. There is progressive loss of tissue and organ function with age and over time [39]. Harman was the first to propose that the damaging effects of ROS may play a key role in the mechanism of aging [40]. The exact mechanism of oxidative stress induced aging is still not very clear but according to Free radical theory of aging (or oxidative stress theory of aging) age-associated functional losses are due to the accumulation of reactive oxygen and nitrogen species (RONS). These RONS will lead to oxidative damage of organic macromolecules like lipids, DNA, proteins etc [41].

The mean case fatality rate for adults aged less than 60 year, is estimated to be less than 0.2%, compared with 9.3% in those aged over 80. Furthermore, co-morbidities such as diabetes, obesity, and hypertension increased mortality risk by five times, however the risk seems to be lower for younger patients than older subjects [42]. A retrospective study done on COVID-19 patients, who were hospitalized in Hainan Provincial People's Hospital (2020), showed that elderly patients (32.14%) were more likely to progress to severe disease than younger patients [43]. Elderly people suffering of diabetes, hypertension and cardiovascular diseases had a state of oxidative stress [44] and viral infection will increase this stress, giving us one possible explanation of the severity of COVID-19 in these categories of patients [45].

Rodriguez-Morales et al done meta-analysis included 19 different studies and enrolled 656 COVID-19 patients. Study reported fever, cough and dyspnoea were the most common manifestations among these patients. Around 32.8% developed ARDS, 20.3% patients required critical care, and 6.2% developed shock. Fatal outcomes were observed in 13.9% patients and old age was significantly associated with the severity of the disease [46]. Meta-analysis done by Yang et al included eight studies with 46,248 infected COVID-19 patients. They observed that hypertension, respiratory and cardiovascular system disorders may be significant risk factors for severity of COVID-19 [47]. Aging is associated with chronic oxidative stress. Theory of aging, proposed a close relationship between oxidative stress, inflammation, and aging that affects especially the regulatory immune systems of the body. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in

which chronic oxidative stress and inflammation feed each other, and consequently, increases the age-related morbidity and mortality [48].

Most diseases exhibit large geographical variations which remain unexplained despite abundant research and COVID-19 will not be an exception [49]. Some European countries e.g. Austria, Baltic States, Czech Republic, Finland, Norway, Poland, Slovakia, have low death rate than others. It was observed that some foods largely used in these countries may reduce angiotensin-converting enzyme activity or are anti-oxidants. [50]. In Korea and Taiwan, uncooked or fermented cabbage is largely consumed with potent antioxidant or anti ACE activity that might be the reason of low COVID-19 death rate [51].

Study done by Patel V et al in 2020 observed that dietary antioxidants, ascorbic acid and sulforaphane, were shown to decrease oxidative stress induced acute inflammatory lung injury in patients receiving mechanical ventilation [52]. It is suggested that age related accumulated oxidative damage and a weakened antioxidant defense system cause a disturbance in the redox balance, resulting in increased ROS.

Resveratrol is a natural stilbene derivative (trans-3, 5, 4'-trihydroxystilbene). It is widely present in different plants like grape (*Vitis vinifera*), Huzhang (*Polygonum cuspidatum*) and cranberry (*Vaccinium macrocarpon*). It decreases the production of nitric oxide in tissue and thereby reduce inflammation [53]. Shih-Chao Lin et al performed MTT and neural red uptake assays to assess survival rates of MERS-infected Vero E6 cells. They concluded that resveratrol was a potent anti viral that inhibit MERS infection and prolonged cell survival [54]. However, dietary patterns have a strong effect on ACE levels. A high-saturated fat diet increases ACE. Antioxidant activities and ACE inhibition have been largely found in many foods [55].

Viral infections and their replication constantly generate oxidized products. Melatonin (N-acetyl-5-methoxytryptamine) is a bioactive molecule. It is not viricidal, but has indirect anti-viral actions [56] due to its anti-inflammation, anti-oxidation and immune enhancing features [57]. It up-regulate anti-oxidative enzymes (e.g. superoxide dismutase) while down-regulate pro-oxidative enzymes (e.g. nitric oxide synthase), and it may also interact directly with free

radicals, functioning as free radical scavenger [58]. Study done by Gitto et al done an extensive study on newborn infants suffered with respiratory distress. They used melatonin to treat newborn infants. They observed an anti-oxidant and anti-inflammatory action of melatonin in the lungs of infants. Thus, it is likely that the application of melatonin would be beneficial in controlling the inflammation and oxidation in coronavirus infected subjects [59]. In another trial of patients suffered with severe multiple sclerosis, orally 25 mg/d of melatonin was given for 6 months. They observed significant reduction in serum concentrations of TNF- α , IL-6, IL-1 β and lipoperoxides [60].

CONCLUSION: Present review concluded that SARS-CoV-2 can trigger an OS. It could be a major factor which increases the severity of COVID-19 especially in elders and people suffering from diabetes mellitus, hypertension and coronary artery disease. Current review recommends the evaluation of oxidative stress markers and antioxidants status of each patient so that antioxidants supplementation can also be used as a novel approach to manage SARS-CoV-2.

REFERENCES

1. Pneumonia of unknown cause — China: disease outbreak news. Geneva: World Health Organization, January 5, 2020 (<https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/>).
2. Bousquet J, Akdis C, Jutel M, Bachert C, Klimek L, Agache I, et al. Intranasal corticosteroids in allergic rhinitis in COVID-19 infected patients: an ARIA/EAAACI statement. Allergy 2020. <https://doi.org/10.1111/all.14302>.
3. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report—74. <https://www.who.int/emergencies/diseases/new-coronavirus-2019/situation-reports/>. Accessed 4 Apr 2020.
4. World Health Organization (COVID-19) Dashboard. Covid19.who.int.
5. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. 1st ed. Advances in Virus Research. Elsevier Inc 2011; 81:85-164.
6. Woo PC, Huang Y, Lau SK, Yuen KY. Coronavirus genomics and bioinformatic analysis. Viruses 2(8): 1804-1820. doi:10.3390/v2081803.
7. Woo PC, Lau SK, Huang Y, Yuen KY. Coronavirus diversity, phylogeny and interspecies jumping. Exp Biol Med (Maywood) 2009; 234:1117–1127.
8. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA et al. The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020; 5: 536-544.
9. L.-L. Ren, Y.-M. Wang, Z.-Q. Wu, Z.-C. Xiang, L. Guo, T. Xu, et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chinese Medical Journal 2020; 133 (9): 1015-24.
10. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. Advances in Virus Research 2011;81: 85-164.
11. Wan Y, Shang J, Graham R, Baric SR, Li F. 2020 Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol 94: e00127-20.
12. Reyfman PA, Walter JM, Joshi N, Anekalla RK, McQuattie Pimental CA et al. Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. Am J Respir Crit Care Med 2019; 199(12):1517–1536.
13. Hancock AS, Stairiker CJ, Boesteanu AC, Monzon-Casanova E, Lukasiak S, Mueller MY et al. Transcriptome analysis of infected and bystander type 2 alveolar epithelial cells during influenza A virus infection reveals in vivo Wnt pathway down regulation. J Virol 2018; 92(21) : e01325-18.
14. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China:

- summary of a report of 72314 cases from the Chinese Center for Disease Control and Prevention. *JAMA* 2020; 323(13):1239-42.
15. Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. *Eur Respir J* 2020; 55: 2000607 [https://doi.org/10.1183/13993003.00607-2020].
16. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8(4) : 420–422.
17. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; 395(10223): 497-506.
18. Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. *Viruses* 2018; 10(8) : 392.
19. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals : Properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 2015; 30: 11-26.
20. Livan Delgado-Roche L, Mesta F. Oxidative Stress as Key Player in Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection. *Arch Med Res* 2020; 51(5):384-387.
21. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Napoli RD (2020) Features, evaluation and treatment coronavirus (COVID-19). In: StatPearls [internet].
22. L. Loffredo, F. Martino, A.M. Zicari, R. Carnevale, S. Battaglia, E. Martino, et al., Enhanced NOX-2 derived oxidative stress in offspring of patients with early myocardial infarction, *Int. J. Cardiol* 2019; 293: 56–59.
23. Hecker L. Mechanisms and consequences of oxidative stress in lung disease: therapeutic implications for an aging populace. *Am J Physiol Lung Cell Mol Physiol* 2018; 314(4): L 642–53.
24. N. Erol, L. Saglam, Y.S. Saglam, H.S. Erol, S. Altun, M.S. Aktas, et al., The protection potential of antioxidant vitamins against acute respiratory distress syndrome: a rat trial, *Inflammation* 2019; 42:1585–1594.
25. Samir D, Dalal D, Noura A. Effect of routine iron supplementation on copper level and oxidative stress status in pregnant women. *Asian Pac J Reprod* 2020; 9: 64-69.
26. Sies H. Oxidative stress: A concept in redox biology and medicine. *Redox Biol* 2015; 4 : e180-183
27. Zhang Z, Rong L, Li YP. Flaviviridae Viruses and Oxidative Stress: Implications for Viral Pathogenesis. *Oxid Med Cell Longev.* 2019 :1409582.
28. Todd A Johnson, HA Jinnah, Naoyuki Kamatani. Shortage of cellular ATP as a cause of diseases and strategies to enhance ATP. *Front Pharmacol* 2019; 10: 98.
29. Derouiche S, Djouadi A. An evaluation of stress oxidative and serum electrolytes in female hypothyroid patients. *Int J Biol Med Res* 2017; 8: 5861-5865.
30. Andrade J DR, Souza RB, Santos SA, Dahir Ramos de, Andrade DR. Oxygen free radicals and pulmonary disease. *Jornal Brasileiro de Pneumologia* 2005; 31: 60-68.
31. Hemilä H, Chalker E. Vitamin C can shorten the length of stay in the ICU: a metaanalysis. *Nutrients.* 2019;11:708.
32. Van den Brand JMA, Haagmans BL, van Riel D, Osterhaus ADME, Kuiken T. The pathology and pathogenesis of experimental severe acute respiratory syndrome and influenza in animal models. *J Comp Pathol* 2014; 151(1):83-112.
33. Khan Z, Shen XZ, Bernstein EA, Giani FJ, Eriguchi M, Zhao VT et al. Angiotensin-converting enzyme enhances the oxidative response and bactericidal activity of neutrophils. *Blood* 2017; 130(3) :328–339.
34. Basili S, Tanzilli G, Mangieri E, Raparelli V, Di Santo S, Pignatelli P et al. Intravenous ascorbic acid infusion improves myocardial perfusion grade during elective percutaneous coronary intervention: relationship with

- oxidative stress markers. *JACC Cardiovasc Interv* 2010; 3:221–229
35. Violi F, Pastori D, Pignatelli P, Cangemi R. SARS-CoV-2 and myocardial injury: a role for Nox2? *Intern Emerg Med* 2020;15:755-758.
 36. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020; 382: 1199-1207.
 37. Fauci AS, Lane HC, Redfield RR. COVID-19-Navigating the Uncharted. *N Engl J Med* 2020;382:e1268-1269.
 38. Ho JC, Chan KN, Hu WH, Lam WK, Zheng L, Tipoe GL et al. The effect of aging on nasal mucociliary clearance, beat frequency, and ultrastructure of respiratory cilia. *Am J Respir Crit Care Med* 2001; 163 (4): 983–988.
 39. Flatt T. A new definition of aging? *Front Genet.* 2012; 3:148.
 40. Harman, D. Aging: a theory based on free radical and radiation chemistry. *J.Gerontol* 1957; 2: 298-300.
 41. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998; 78 (2):547–581.
 42. Jordan RE, Adab P, Cheng KK. COVID-19: risk factors for severe disease and death. *Bmj* 2020; 368 :m1198.
 43. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect* 2020;80(6):e14-18.
 44. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al. Oxidative stress, aging, and diseases. *Clinical Interventions in Aging.* 2018;13:757-77.
 45. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z et al. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020; 395(10229):1054-1062.
 46. Rodriguez-Morales A, Cardona-Ospina J, Gutierrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana J et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis.* 2020. <https://doi.org/10.1016/j.tmaid.2020.101623>
 47. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *Int J Infect Dis.* 2020; 94:91-95.
 48. L Chen, H G Liu, W Liu, J Liu, K Liu, J Shang et al. Analysis of Clinical Features of 29 Patients with 2019 Novel Coronavirus Pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi* 2020; 43(3): 203-208.
 49. Sunyer J, Jarvis D, Pekkanen J, Chinn S, Janson C, Leynaert B, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol.* 2004; 114 (5):1033–9.
 50. Stafford N. CoVid-19: Why Germany's case fatality rate seems so low. *MJ.* 2020;369: m1395.
 51. Dang Y, Zhou T, Hao L, Cao J, Sun Y, Pan D. In vitro and in vivo studies on the angiotensin-converting enzyme inhibitory activity peptides isolated from broccoli protein hydrolysate. *J Agric Food Chem.* 2019; 67(24):6757–64.
 52. Patel V, Dial K, Wu J, Gauthier AG, WuW, LinM, et al. Dietary antioxidants significantly attenuate hyperoxia-induced acute inflammatory lung injury by enhancing macrophage function via reducing the accumulation of airway HMGB1. *Int J Mol Sci.* 2020; 21(3):977.
 53. Wang LL, Shi DL, Gu HY, Zheng MZ, Hu J, Song XH et al. Resveratrol attenuates inflammatory hyperalgesia by inhibiting glial Activation in mice spinal cords. *Mol Med Rep.* 2016; 13(5):4051–57.
 54. Shih-Chao Lin, Chi-Tang Ho, Wen-Ho Chuo, Shiming Li, Tony T. Wang, Chi-Chen Lin.

- Effective inhibition of MERS-CoV infection by resveratrol. *BMC Infectious Diseases* 2017; 17:144.
55. Huang AF, Li H, Ke L, Yang C, Liu XY, Yang ZC, et al. Association of angiotensin-converting enzyme insertion/deletion polymorphism with susceptibility to systemic lupus erythematosus: a meta-analysis. *Int J Rheum Dis.* 2018; 21(2):447–57.
 56. R.J. Reiter, Q. Ma, R. Sharma, Treatment of Ebola and other infectious diseases: melatonin “goes viral”, *Melatonin Res* 3 (2020) 43–57, <https://doi.org/10.32794/mr11250047>.
 57. Junaid, H. Tang, A. van Reeuwijk, Y. Abouleila, P. Wuelfroth, V. van Duinen, W. Stam et al. Ebola hemorrhagic shock syndrome-on-a-chip. *IScience* 2020; 23: 100765, <https://doi.org/10.1016/j.isci.2019.10.0765>.
 58. X. Wu, H. Ji, Y. Wang, C. Gu, W. Gu, L. Hu, L. Zhu, Melatonin alleviates radiation induced lung injury via regulation of miR-30e/NLRP3 axis. *Oxidative Med. Cell. Longev.* 2019 4087298, <https://doi.org/10.1155/2019/4087298>.
 59. E. Gitto, R.J. Reiter, S.P. Cordaro, R.M. La, P. Chiurazzi, G. Trimarchi et al. Oxidative and inflammatory parameters in respiratory distress syndrome of preterm newborns: beneficial effects of melatonin, *Am. J. Perinatol.* 21 (2004) 209–216, <https://doi.org/10.1055/s-2004-828610>.
 60. A.L. Sanchez-Lopez, G.G. Ortiz, F.P. Pacheco-Moises, M.A. Mireles-Ramirez, O.K. Bitzer-Quintero, D.L.C. Delgado-Lara et al. Efficacy of melatonin on serum pro-inflammatory cytokines and oxidative stress markers in relapsing remitting multiple sclerosis, *Arch. Med. Res.* 49 (2018) 391–398, <https://doi.org/10.1016/j.arcmed.2018.12.004>.