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Review Article

ANGIOTENSIN-CONVERTING ENZYME 2 AND TMPRSS2 EXPRESSIONS IN COVID-19

ALEETA MARIA JOLLY, JAYA THOMAS*

Department of Pharmacology, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham, Ponekkara, Kerala, India.
Email: jayamarythomas@gmail.com

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters into host cells through the angiotensin-converting enzyme 2 (ACE2) receptor and transmembrane serine protease 2 (TMPRSS2) helps in propagation. Expression of ACE2 gene was found to be higher in kidney, testis, heart, thyroid, and adipose tissue whereas expression of TMPRSS2 was found to be highest in the kidney, small intestine, and prostate. ACE2 and TMPRSS2 expression increases as age increases. Estrogen has a protective role against SARS-CoV-2. Thus mortality rate of SARS-CoV-2 is more in men compared to women. ACE2 is also expressed in adipose tissue therefore as body mass index increases the disease severity increases. Smoking upregulates the lung airway expression of ACE2 and TMPRSS2. Alcoholics have more chances to get a COVID-19 as it elevates the expression of the angiotensin II type 1 receptor. Administer an antioxidant-rich diet to fight against COVID-19. Patients with hypertension, diabetes, cardiovascular disease, chronic lung disease, chronic kidney disease, and cancer cause more severity to COVID-19. ACE2 expression increased while administering drugs such as ACE inhibitors, angiotensin II type I receptor blocker, thiazolidinediones, and NSAIDs thus elevates the severity of COVID-19 infection. Anti-histo-blood natural antibodies act as a protective role in blood group O. Blacks and Histapanic community have a higher risk of infection and mortality compared white people. Temperature and humidity are negatively correlated with COVID-19. ACE2 expression is elevated in the alveolar cells of the population exposed to air pollution thus elevates the exposure to the SARS-CoV infection. In this article, we will compare the modifiable and non-modifiable risk factors of COVID-19 that affect both first and second waves.

Keywords: COVID-19, Angiotensin-converting enzyme 2, Transmembrane serine protease 2, Obesity, Smoking, Alcohol, Antioxidant, Comorbidity, Climate, Pollution.

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INTRODUCTION

Coronaviruses have single-stranded RNA and a diameter of 80–120 nm. It is pleomorphic or spherical and covered with glycoprotein. The different coronaviruses include alpha, beta, gamma, and delta coronavirus. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is from the beta coronavirus family. SARS-CoV and Middle East Respiratory Syndrome coronavirus are other beta coronaviruses that cause disease in humans [1]. Angiotensin-converting enzyme 2 (ACE2) is an exopeptidase receptor in which the SARS-CoV-2 enters into the host target cells [2]. The S protein that is a transmembrane spike glycoprotein of SAR-CoV-2 binds to the ACE2 receptor. ACE2 is a member-bound receptor that belongs to the carboxypeptidase family. Transmembrane protease serine 2 [TMPRSS2] allows the virus and cellular membrane to fuse thus SARS-CoV-2 enters the cells and undergoes replication [3]. TMPRSS2 has a key role in cleaving the glycoprotein of the virus thus helps propagation [4] as shown in Fig. 1. In this review article, we will discuss various modifiable and non-modifiable risk factors of SARS-CoV-2 infection in wave 1 and wave 2.

RATE OF EXPOSURE

The expression of ACE2 and TMPRSS2 is predominantly high in nasal epithelium compared to saliva or blood [5]. An *in vivo* study was conducted and found significantly higher expression of ACE2 and TMPRSS2 in conjunctiva whereas a clinical study was conducted to analyze the expression of ACE2 and TMPRSS2 in conjunctival samples in humans and no significant expression was found in conjunctiva but there might be other unidentified receptors [6,7]. An *in vivo* study was conducted and found expression of ACE2, TMPRSS2, and Furin in eustachian tube and cochlea so it is important to take necessary precautions against SARS-CoV-2 during mastoidectomy [8]. The expression of the ACE2 gene was estimated and found higher in kidney, testis, heart, thyroid, adipose tissue, and small intestine whereas lungs, liver, bladder, and adrenal gland showed only medium expression. Blood, spleen, bone marrow, muscles, and blood vessels had the lowest expression [9]. The expression of TMPRSS2 was highest in kidney, small intestine, and prostate. Lung expression was found in bronchi, bronchioles and no expression in alveoli. The expression is also seen in the stomach, colon, pancreas, and salivary gland [10] as shown in Tables 1 and 2. Thus, the expression of ACE2 and TMPRSS2 in other regions reveals the symptoms and complications of COVID-19 will not be confined to pulmonology but also affect other regions of the body. Thus serious precautions have to be taken to avoid complications among patients who are affected with COVID-19 along with other diseases. In the first wave, a single mask was used by the population whereas in the second wave the population started to use double mask to prevent exposure through the mouth and nose.

AGE

ACE2 and TMPRSS2 expression increases as age increases thus the pediatric population have mild symptoms. A study was conducted on the expression of ACE2 and TMPRSS2 in the pediatric population to that of adults and found that the expression of genes was more in the adult population which indicates mild symptoms in SARS-CoV-2 pediatric population [11]. A study was conducted on the expression of ACE2 and found that it elevates the expression as age increases but the population excluded neonates and geriatrics in the study [12]. The various factors involved in the vascular ageing process are oxidative stress and impairment in antioxidant defense mechanisms. The thoracic aorta thickness elevates on ageing and thus increases RAS components [13]. Hypoxia conditions also regulate the expression of ACE2 [11]. The ACE2 expression and immune signature were analyzed and found immunopathological damage in elderly patients rather than younger patients. The rate of exposure of SARS-CoV is the same in all age groups but severity and mortality elevate on age in the first

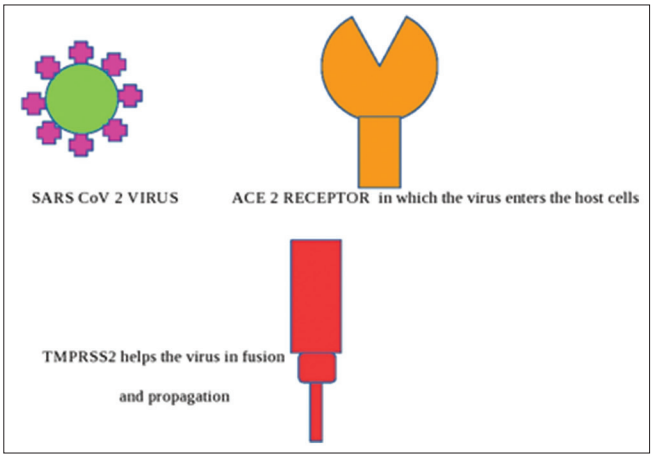

Fig.1: Severe acute respiratory syndrome coronavirus 2 receptors

Table 1: Expression of ACE2 in various organs

ACE2 Expression	
Higher expression	Kidney Small intestine Adipose tissue Thyroid Heart Testis
Medium expression	Lungs Adrenal gland Bladder Liver
Lowest expression	Blood Blood vessels Muscles Bone marrow Spleen

ACE2: Angiotensin-converting enzyme 2

Table 2: Expression of TMPRSS2 in various organs

TMPRSS2 expression	Kidney
	Prostrate Small intestine Bronchi Bronchioles Stomach Salivary gland Pancreas Colon

TMPRSS2: Transmembrane Serine Protease 2

wave [14]. In the second wave, children are more affected compared to the elderly population [15].

GENDER

The mortality rate of SARS-CoV-2 is more in men compared to women. Estrogen has a protective role against SARS-CoV-2 infection. It activates the immune response and suppresses the replication of the virus. A study conducted on ACE2 expression and immune signature found that immunopathological damage is more in men compared to women [14]. Androgens upregulate the TMPRSS2 gene expression thus helps in SARS-CoV-2 virus replication and propagation [16]. ACE2 expression alone cannot elevate the mortality, TMPRSS2 expression should also be considered to assess the mortality of the SARS-CoV-2 infected patients. In the second wave women especially pregnant and lactating are more affected compared to men [15]. This might be because of lack of vaccination among pregnant and lactating women thus less immunity compared to vaccinate.

OBESITY

Obesity is one of the etiology of oxidative stress and stimulates the RAS pathway. A study was conducted and found that obese mouse upregulates RAS components and ROS levels. When angiotensin receptor blocker (ARB) was given it reduced the RAS components and ROS levels. In obese patients angiotensin is mainly produced in adipose tissue. ARBs inhibit adipocyte hypertrophy, reduces interleukins, and oxidative stress [17]. Obesity is one of the major risk factors for severe SAR-CoV-2 virus infection. ACE2 is also expressed in adipose tissue. Moreover, adipose tissue acts as a major source of inflammatory molecules and may aggravate SARS-CoV-2 virus infection. A large number of obese patients are admitted to intensive care unit (ICU) with SARS-CoV-2 virus infection. As body mass index (BMI) increases the disease severity increases. A report from New York City suggests that among 5700 COVID-19 patients 41.7% were obese patients. A study reported that 88.2% of non-survivors of COVID-19 infection have BMI >25kg/m^2. In the human body adipose tissue act as a reservoir for Adenovirus, influenza A virus, *Mycobacterium tuberculosis*, *Trypanosoma gondii*, megalovirus, HIV, cyto influenza A [18,19]. Due to lockdown for many months the majority of the population has gained weight due to overeating and lack of physical activity thus it might have elevated the severity of COVID-19 in the second wave [20]. Thus reduction of excess fat might help to prevent COVID-19 severity and mortality.

SMOKING

The WHO reported that smoking elevates the mortality rate in COVID-19 patients. Smoking also elevates the severity of COVID-19 infection. Guan *et al*. reported that 25% of people admitted to ICU due to COVID-19 are smokers and 7.6% were former smokers. A clinical study reported that adverse outcomes were nine-fold more prevalent in the smoking group compared to the non-smoking group and less improvement and stabilization of the disease in the smoking group [21]. Smoking upregulates the lung airway expression of ACE2 and TMPRSS2 [5]. Smoking causes air pollution and elevates ACE activity a hundred-fold. Smokers elevate ACE2 expression in type2 pneumocytes and alveolar macrophage mainly at the apical end of small airway epithelium when compared to non-smokers [21]. Due to lockdown smoking frequency was elevated among the smokers [22]. This might be one of the risk factors for the severity in the second wave. Thus, it is important to quit smoking to prevent the severity and mortality of COVID-19 infection.

ALCOHOL

There is a relationship between alcohol consumption and viral infection. A clinical study reported that 70% of patients with Hepatitis C virus and HIV virus have a history of alcohol consumption [23]. Alcoholics have more chances to get a community-acquired infection as it affects immunity. It acts both natural as well as acquired immunity by interfering with the natural killer cells and target cells, altered cytolytic activity, and signal transduction thus affects the neuroendocrine system. It also stimulates inflammatory reactions and CD8 responses. Interleukins-1,6 and tumor necrosis factors play a key role in alcohol-induced lung injury. Alcohol inhibits the synthesis of anti-inflammatory cytokines. Both moderate and severe drinkers cause pulmonary injury. Alcohol also causes severe oxidative stress and antioxidant deficiencies. Moreover, alcohol elevates the expression of the angiotensin II type 1 receptor [21]. During lockdown day's alcohol consumption has elevated among the alcoholic population thus it may be another risk factor for the severity in the second wave [24]. It is important to stop or reduce alcohol consumption during the period of COVID-19 to prevent severity and mortality due to the SARS-CoV-2 infection.

FOOD

Dr Linus Pauling, a Nobel prize winner, suggested that ascorbic acid (Vitamin C) plays a key role in preventing the common cold. So during the period of COVID-19, it is important to administer Vitamin C-rich foods such as broccoli, lime, lemon, orange, tangerine, grapefruit, tomato, cauliflower, sweet potato, kiwi, papaya, and strawberry. It is important to administer an antioxidant-rich diet to fight against the oxidative stress which is produced by the viral infection. A study was conducted in Thailand that concluded Plasmodium falciparum malaria was prevented in certain populations as the population was administered with fava beans. Fava beans contain quinine-like chemicals which have antimalarial activity; moreover, hydroxychloroquine is used to treat COVID-19 patients. COVID-19 patients worsen their health when they have protein energy-wasting, hypokalemia, and hypophosphatemia. Acute kidney injury was reported in some COVID-19 patients so it is important to give adequate amounts of protein in the diet [25]. A study was conducted which reported that a low protein diet elevates angiotensin II synthesis in the lungs of pregnant rats [26]. Another study conducted revealed a high prevalence of reduced potassium in COVID-19 patients. There is a positive correlation between low potassium levels and the severity of COVID-19. More potassium is lost through the urine due to the destruction of ACE2 by the SARS-CoV-2 virus. Hypokalemia leads to ECG variations, myocardial injury, and alkalosis. In severe hypokalemia conditions, more days are necessary to give negative PCR results in COVID-19 patients [27]. Unani medicine which is a plant-based medicine suggests that several plants such as liquorice, onion, garlic, tulsi or holy basil, black pepper, cinnamon, turmeric, lemon, and honey are found to have antiviral property [28] as shown in Table 3. Bennett *et al*. reported that due to lock down most of the population took to unhealthy food habits such as chips, sweets, and processed food [29]. Thus it also might be another factor for the severity of the second wave. Dietary habits play a key role during the period of COVID-19 thus administering antioxidant and Vitamin C-rich food along with food that has antiviral actions.

Table 3: Foods with antiviral property

Food with antiviral property	Licorice
	Onion
	Garlic
	Tulsi
	Black pepper
	Cinnamon
	Turmeric
	Lemon
	Honey

COMORBIDITIES

The patients with various diseases have more chances to cause COVID-19 as well as higher severity than the patients without other comorbidities [30]. As the number of comorbidities increases, the severity of infection also increases [31]. Patients with hypertension, diabetes, cardiovascular disease, chronic lung disease, chronic kidney disease, and cancer cause more severity to COVID-19 [30]. Yang *et al*. reported that 32 non-survivors of the ICU with COVID-19 had cerebrovascular disease of 22% and diabetes 22%. Guan *et al*. reported that among COVID-19 patients 23.7% had hypertension, 16.2% had diabetes, 5.8% had coronary heart disease, and 2.3% had cerebral vascular disease. Zhang *et al*. reported that 30% had hypertension and 12% had diabetes [32]. Sanyaolu *et al*. conducted a meta-analysis on comorbidities on COVID-19 and found that 15.08% of people had hypertension, 11.70% with cardiovascular disease, 9.40% with diabetes, 1.50% with HIV or Hepatitis B, 1.50% with malignancy, 1.40% with respiratory illness, 0.80% with renal disorders, and 0.01% with immunodeficiency [30]. Hence, it is important to maintain adequate blood pressure, blood glucose level, and cholesterol levels in patients during the period of COVID-19 to reduce the severity and mortality of the disease.

DRUGS

ACE2 expression increased while administering drugs such as ACE inhibitors, angiotensin II type I receptor blocker, thiazolidinediones, and NSAIDs [32,33]. Further studies are needed to evaluate if patients with COVID-19 that have a medication history of the above drugs will elevate the severity of infection. COVID-19 elevates the coagulation activity thus in severe COVID-19 infection treatment with anticoagulants improves the condition of the patients. Alterations of diabetes medication are necessary for COVID-19 patients. Metformin should be avoided in patients with the risk of lactic acidosis, respiratory distress, renal impairment, and heart failure. Metformin also has interactions with lopinavir which is a COVID-19 medication. DPP-4 inhibitors have anti-inflammatory actions and have interactions with lopinavir, ritonavir, and atazanavir. SGLT2 inhibitors should be avoided in patients with the risk of hypovolemia, electrolyte imbalances, and ketoacidosis. GLP-1 receptor agonists have anti-inflammatory actions and should be avoided in patients with gastrointestinal side effects and aspiration. Sulfonylureas have interactions with lopinavir, ritonavir, and hydroxychloroquine. Pioglitazone has anti-inflammatory action and should be avoided in patients with fluid retention and heart failure. It has interactions with favipiravir. Insulin is recommended for critical patients and has interactions with hydroxychloroquine [34]. More studies have to be conducted on COVID-19 medication interactions with other drugs thus giving rational prescriptions for the COVID-19 patients.

BLOOD TYPE

A study was conducted and concluded that people with blood groups B and AB with Rh-positive have more chances to test positive for COVID-19 whereas people with blood group O have less chance to be positive [35]. A study in Hong Kong reported that O blood groups have a low infection of SARS-CoV. The main site for the expression of the SARS-CoV virus is in the histo-blood group. Natural anti-A and B antibodies bind to S protein and inhibit the binding with ACE2. SAR-CoV replication is at ABH antigen which is controlled by the FUT2 gene. Anti-histo-blood natural antibodies act as a protective role in the blood group O [36]. Blood group O also shows protective effects from malaria [37]. A clinical study reported that populations with blood group A showed more severity in ICU than other blood groups [38]. Blood grouping is a non-modifiable risk factor of COVID-19. A, B blood group people are more vulnerable to COVID-19.

RACE

Blacks and Hispanics have a higher risk of infection and mortality compared to the white people due to the presence of diabetes, hypertension, asthma, obesity, and smoking. Black and Hispanics need more ventilatory support compared to white [39]. Devan Hawkins reported that black people are employed with occupations that have more proximity with others. Blacks, Hispanics, and Asians are more employed in health-care sectors, hospitals, social assistance industries, animal slaughtering, respiratory therapists, nurses, physical therapists, medical assistants, bus drivers, flight attendants, etc. [40]. Lower usage of RAAS inhibitors is seen among blacks which might be the reason for COVID-19 infection [41]. Further studies are needed to make sure if COVID-19 has more correlation with occupation, race, disease, and drugs.

CLIMATE

The ambient environment for SARS-CoV-2 to survive is at a temperature of 5–15°C and with humidity between 3 and 10 g/m^3. Humidity is negatively correlated with COVID-19. Huang *et al*. conducted a study and reported that 60% of COVID-19 cases are from countries with temperatures between 5 and 15°C. About 73.8% of COVID-19 cases were at regions with 3–10 g/m^3 humidity [42]. Viruses thus survive in the droplets at high humidity >70% under the physiological contents of salt. At moderate humidity 40–60% evaporation takes place thus salts

get concentrated and deactivates the viruses. At low humidity <30% salts crystallize from the solution and may activate the virus. During winter the humidity is low thus viral aerosols exist longer in the air due to their smaller size; therefore, elevating the spread of the virus. During the rainy season, low temperature and saturation humidity facilitate the transmission of the virus through large aerosol particles. In contrast to the high humid countries such as India, Iran, Iraq, and the Midwestern United States, the temperature at day time is >20°C but the night temperature is <10°C, thus facilitating the spread of the virus. SARS-like viruses can thus live in cool and arid weather for several days. A recent report suggests that the arid climate also reduces the immunity of the host by causing impairment in mucociliary clearance [43]. Thus temperature and humidity in your region need to be considered during the period of COVID-19.

POLLUTION

COVID-19 patients with chronic exposure to air pollutants have a more severe form of disease and mortality is higher among those populations. ACE2 expression is elevated in the alveolar cells of the population exposed to air pollution thus elevates the exposure to the SARS-CoV infection [44]. Air pollutants act as free radicals causing oxidative stress and inflammatory cells. Exposure to pollution reduces the ability of macrophages to undergo phagocytosis, therefore unable to inactivate the virus [45]. A study reported that an increase of 1% PM2.5 causes an 8% increase in mortality of COVID-19 [44]. Regions with high air pollution index have double mortality than those with low air pollution index [45]. A 2003 study from China on SARS reported that regions with moderate pollution have higher mortality than regions with low pollution [46]. Air quality index, PM2.5, NO_2 play a key role in the transmission of SARS-CoV-2 infection [14]. Hence, it is important to control air pollution during the period of COVID-19.

CONCLUSION

COVID-19 pandemic is a challenging situation all over the world. Complete immunization against COVID-19 among every age group of both sexes is required. Exercise regularly to reduce complications of COVID-19. Avoid smoking and alcoholism during the COVID-19 pandemic to reduce the severity of the disease. Populations with other comorbidities have maintained their blood pressure, glucose levels, cholesterol levels, etc., for reducing mortality among the population with COVID-19. Avoid unnecessary consumption of OTC medications without the instructions of the medical practitioner. Maintain cleanliness among yourselves as well as the environment and surroundings. It is important to assess more risk factors that elevate ACE2 and TMPRSS2 expression. Thus, the population more vulnerable to COVID-19 and its complications can be analyzed. Precautions should be taken among the vulnerable groups of COVID-19. Thus, SARS-CoV-2 virus can be wiped out from the community.

REFERENCES

1. Wang L, Wang, Y, Ye D, Liu Q. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. Int J Antimicrob Agents 2020;55:105948.
2. Kumar D, Malviya R, Sharma PK. Corona virus: A review of COVID-19. EJMO 2020;4:8-25.
3. Kai H, Kai M. Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors lessons from available evidence and insights into COVID-19. Hypertens Res 2020;43:648-54.
4. Meyer D, Sielaff F, Hammami M, Böttcher-Friebertshäuser E, Garten W, Steinmetzer T. Identification of the first synthetic inhibitors of the Type II transmembrane serine protease TMPRSS2 suitable for inhibition of influenza virus activation. Biochem J 2013;452:331-43.
5. Sharif-Askari NS, Sharif-Askari FS, Alabed M, Temsah MH, Al Heialy S, Hamid Q, *et al*. Airways expression of SARS-CoV-2 receptor, ACE2, and TMPRSS2 is lower in children than adults and increases with smoking and COPD. Mol Ther Methods Clin Dev 2020;18:1-6.
6. Zhang BN, Wang Q, Liu T, Dou SQ, Qi X, Jiang H, *et al*. Expression analysis of 2019-nCoV related ACE2 and TMPRSS2 in eye tissues. Zhonghua Yan Ke Za Zhi 2020;56:438-46.
7. Lange C, Wolf J, Auw-Haedrich C, Schlecht A, Boneva S, Lapp T, *et al*. Expression of the COVID-19 receptor ACE2 in the human conjunctiva. J Med Virol 2020;92:2081-6.
8. Uranaka T, Kashio A, Ueha R, Sato T, Bing H, Ying G, *et al*. Expression of ACE2, TMPRSS2, and furin in mouse ear tissue, and the implications for SARS-CoV-2 infection. Laryngoscope 2021;131:E2013-7.
9. Li M.Y, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty 2020;9:1-7.
10. Vaarala MH, Porvari KS, Kellokumpu S, Kyllönen AP, Vihko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 2001;193:134-40.
11. Naqvi SS, Phulpoto AH, Memon AQ, Channo MA, Mangi MR, Maher A. Association of mortality and raised inflammatory markers such as serum LDH, serum ferritin and CRP in hospitalized patient with Covid-19 infection. Age (years) 2021;42:17-84.
12. Bunyavanich S, Do A, Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. JAMA 2020;323:2427-9.
13. Yoon HE, Kim EN, Kim M, Lim JH, Jang I, Ban TH, *et al*. Age-associated changes in the vascular renin-angiotensin system in mice. Oxid Med Cell Longev 2016;2016:6731093.
14. Li H, Xu XL, Dai DW, Huang ZY, Ma Z, Guan YJ. Air pollution and temperature are associated with increased COVID-19 incidence: A time series study. Int J Infect Dis 2020;97:278-82.
15. Iftimie S, López-Azcona AF, Vallverdú I, Hernández-Flix S, De Febrer G, Parra S, *et al*. First and second waves of coronavirus disease-19: A comparative study in hospitalized patients in Reus, Spain. PLoS One 2021;16:e0248029.
16. Cianfarani S. Pediatric endocrinology in the time of the COVID-19 pandemic. In: Hormone Research in Paediatrics. Basel, Switzerland: Karger Publishers; 2020. p. 1.
17. Ramalingam L, Menikdiwela K, LeMieux M, Dufour JM, Kaur G, Kalupahana N, *et al*. The renin angiotensin system, oxidative stress and mitochondrial function in obesity and insulin resistance. Biochim Biophys Acta 2017;1863:1106-4.
18. Kassir R. Risk of COVID-19 for patients with obesity. Obes Rev 2020;21:e13034.
19. Iannelli A, Favre G, Frey S, Esnault V, Gugenheim J, Bouam S, *et al*. Obesity and COVID-19: ACE 2, the missing tile. Obes Surg 2020;30:4615-7.
20. Krams IA, Jõers P, Luoto S, Trakimas G, Lietuvietis V, Krams R, *et al*. The obesity paradox predicts the second wave of COVID-19 to be severe in Western countries. Int J Environ Res Public Health 2021;18:1029.
21. Engin AB, Engin ED, Engin A. Two important controversial risk factors in SARS-CoV-2 infection: obesity and smoking. Environ Toxicol Pharmacol 2020;78:103411.
22. Chagué F, Boulin M, Eicher J, Bichat F, Saint-Jalmes M, Cransac A, *et al*. Alarming increased rate of smoking and associated lifestyle behaviours in patients with chronic cardiac diseases during COVID-19 pandemic related lockdown. Arch Cardiovasc Dis Suppl 2021;13:127.
23. Testino G. Are patients with alcohol use disorders at increased risk for Covid-19 infection? Alcohol Alcohol 2020;55:344-6.
24. Alpers SE, Skogen JC, Mæland S, Pallesen S, Rabben ÅK, Lunde LH, *et al*. Alcohol consumption during a pandemic lockdown period and change in alcohol consumption related to worries and pandemic measures. Int J Environ Res Public Health 2021;18:1220.
25. Kalantar-Zadeh K, Moore LW. Impact of nutrition and diet on COVID-19 infection and implications for kidney health and kidney disease management. J Renal Nutr 2020;30:179-81.
26. Gao H, Tanchico DT, Yallampalli U, Yallampalli C. A low-protein diet enhances angiotensin II production in the lung of pregnant rats but not nonpregnant rats. J Pregnancy 2016;2016:4293431.
27. Li X, Hu C, Su F, Dai J. Hypokalemia and clinical implications in patients with coronavirus disease 2019 (COVID-19). MedRxiv 2020;2020:20028530.
28. Ali I, Alharbi OM. COVID-19: Disease, management, treatment, and social impact. Sci Total Environ 2020;728:138861.
29. Bennett G, Young E, Butler I, Coe S. The impact of lockdown during the COVID-19 outbreak on dietary habits in various population groups: A scoping review. Front Nutr 2021;8:53.
30. Sanyaolu A, Okorie C, Marinkovic A, Patidar R, Younis K, Desai P, *et al*. Comorbidity and its impact on patients with COVID-19. SN Compr Clin Med 2020;2:1069-76.
31. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, *et al*. Comorbidity and its impact on 1590 patients with COVID-19 in China:

A nationwide analysis. Eur Respir J 2020;55:2000547.
32. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 2020;8:e21.
33. Giavina-Bianchi P, Aun MV, Agondi RC, Kalil J. Debate on drugs that may aggravate COVID-19. J Allergy Clin Immunol 2020;8:2452-3.
34. Apicella M, Campopiano MC, Mantuano M, Mazoni L, Coppelli A, Del Prato S. COVID-19 in people with diabetes: Understanding the reasons for worse outcomes. Lancet Diabetes Endocrinol 2020;8:782-92.
35. Latz CA, DeCarlo C, Boitano L, Png CY, Patell R, Conrad MF, *et al*. Blood type and outcomes in patients with COVID-19. Ann Hematol 2020;99:2113-8.
36. Guillon P, Clément M, Sébille V, Rivain JG, Chou CF, Ruvoën-Clouet N, Le Pendu J. Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. Glycobiology 2008;18:1085-93.
37. Terao C, Bayoumi N, McKenzie CA, Zelenika D, Muro S, Mishima M, *et al*. Quantitative variation in plasma angiotensin-I converting enzyme activity shows allelic heterogeneity in the ABO blood group locus. Ann Hum Genet 2013;77:465-71.
38. Gómez J, Albaiceta GM, García-Clemente M, García-Gala JM, Coto E. DNA genotyping of the ABO gene showed a significant association of the A-group (A1/A2 variants) with severe COVID-19. Eur J Intern Med 2021;88:129-32.
39. Golestaneh L, Neugarten J, Fisher M, Billett HH, Gil MR, Johns T. The association of race and COVID-19 mortality. EclinicalMedicine 2020;25:100455.
40. Hawkins D. Differential occupational risk for COVID-19 and other infection exposure according to race and ethnicity. Am J Ind Med 2020;63:817-20.
41. Doumas M, Patoulias D, Katsimardou A, Stavropoulos K, Imprialos K, Karagiannis A. COVID19 and increased mortality in African Americans: Socioeconomic differences or does the renin angiotensin system also contribute? J Hum Hypertens 2020;34:764-7.
42. Huang Z, Huang J, Gu Q, Du P, Liang H, Dong Q. Optimal temperature zone for the dispersal of COVID-19. Sci Total Environ 2020;736:139487.
43. Yuan S, Jiang SC, Li ZL. Do humidity and temperature impact the spread of the novel coronavirus? Front Public Health 2020;8:240.
44. Domingo JL, Marquès M, Rovira J. Influence of airborne transmission of SARS-CoV-2 on COVID-19 pandemic. A review. Environ Res 2020;188:109861.
45. Comunian S, Dongo D, Milani C, Palestini P. Air pollution and COVID-19: The role of particulate matter in the spread and increase of COVID-19's morbidity and mortality. Int J Environ Res Public Health 2020;17:4487.
46. Contini D, Costabile F. Does air pollution influence COVID-19 outbreaks? Atmosphere 2020;11:377.