

Potential role of gut microbiota in patients with COVID-19, its relationship with lung axis, central nervous system (CNS) axis, and improvement with probiotic therapy

Nazanin Alibeik¹, Elham Pishgar², Ramin Bozorgmehr³, Farshad Aghaaliakbari¹, Neda Rahimian^{1,4*}

¹Department of Internal Medicine, School of Medicine, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran

²Gastrointestinal and Liver Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran

³Department of Surgery, School of Medicine, Shahid Madani Hospital, Alborz University of Medical Sciences, Karaj, Iran

⁴Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences (IUMS), Tehran, Iran

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) is a pandemic disease caused by a new corona virus. COVID-19 affects different people in different ways. COVID-19 could affect the gastrointestinal system via gut microbiota impairment. Gut microbiota could affect lung health through a relationship between gut and lung microbiota, which is named gut-lung axis. Gut microbiota impairment plays a role in pathogenesis of various pulmonary disease states, so GI diseases were found to be associated with respiratory diseases. Moreover, most infected people will develop mild to moderate gastrointestinal (GI) symptoms such as diarrhea, vomiting, and stomachache, which is caused by impairment in gut microbiota. Therefore, the current study aimed to review potential role of gut microbiota in patients with COVID-19, its relation with lung axis, Central Nervous System (CNS) axis and improvement with probiotic therapy. Also, this review can be a guide for potential role of gut microbiota in patients with COVID-19.

Keywords: Coronavirus disease 2019; Gut microbiota; Lung axis; Central nervous system; Probiotic

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is a pandemic caused by a new corona virus, named as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV-2). Accordingly, this disease threatens public health of communities as a critical event (1). The virus was initiated in Hubei, China, at late 2019 and then spread rapidly in many countries. There-

fore, World Health Organization (WHO) stated this disease as an endemic and international concern on January 30, 2020 (2). COVID-19 led to 6,192,698 cases of infection and more than 131,572 deaths since April 3 2020 in Iran (3).

The COVID-19 functions as it uses angiotensin converting enzyme-2 (ACE2) receptors in various tissues (4). Spike protein of the virus detects the receptor and then attacks the host via transmembrane

*Corresponding author: Neda Rahimian, MD, Department of Internal Medicine, School of Medicine, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran; Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences (IUMS), Tehran, Iran. Tel: +98-9125210321 Fax: +98-2188942622 Email: Rahimian.n@iums.ac.ir

protease serine 2 (TMPRSS2) (5). Both ACE2 and TMPRSS2 proteins must be expressed in various tissues spontaneously, especially in lung and gastrointestinal system (GI) to enter the cells (6). In addition, COVID-19 causes inappropriate immune responses, including the increased pro-inflammatory cytokines and subsequently cytokine storm induced inflammatory pathway, leading to the Acute Respiratory Distress Syndrome (ARDS) and multi-organ failure (7). A study performed in Wuhan, China reported that mortality rate in patients with COVID-19 is mostly due to severe inflammation and cytokine storm, and there was a significant difference between patients expired and discharged regarding WBC value, Lymphocyte count, and Interlukine-6 (IL-6) levels (2, 8). Immune responses and pro-inflammatory cytokines cause a change to gut microbiota as well as a subsequent change in integrity of gut barrier, which is accompanied with secondary infection and pneumonia (9).

Since SARS-COV-2 is similar to SARS-COV and causes respiratory symptoms, many studies were performed on findings of chest radiography (10). In contrast, the findings showed that SARS coronavirus viral RNA is detectable in feces of patients that a month has passed from their illness besides respiratory secretions (11). Accordingly, this fact can be considered as the reason for the presence of gastrointestinal (GI) symptoms such as diarrhea, vomiting, and stomachache in patients with COVID-19 virus, which are caused by impairment in gut microbiota (12). Therefore, the GI symptoms in patients with COVID-19 should not be ignored (13). Moreover, epidemiologic studies conducted in various regions showed that the incidence rate of GI symptoms in patients with COVID-19 is increasing over time, and Pan et al. reported this as 50.5% in 204 patients (14, 15). Therefore, the current study aimed to review the potential role of gut microbiota in patients with COVID-19, as well as its relationship with lung axis, Central Nervous System (CNS) axis, and improvement with probiotic therapy.

Gut microbiota. In human GI, there are more than 10^{14} cells and 500 to 1000 bacterial species named gut microbiota (16). Microbiota is an asset of microorganisms including bacteria, fungi, archaeobacteria, and viruses living in various body organs like the human intestine, in response to the external environment changes (17). The microbial population can be

transmitted from a generation to the other one, and this plays a key role in preserving human health (18).

Various projects on human microbiome showed that microbiota plays an important role in the development of immune system, defeating pathogenic microbes, intestinal synthesis of vitamins, defecation of toxin, basic metabolism of body, digesting the indigestible substrates, and in total, in human beings' survival (19, 20). Imbalance of gut microbiota is associated with several diseases, including cancer, diabetes, obesity, depression, and cardiovascular diseases (CVD) especially gastrointestinal (GI) disorders (21). Moreover, several studies showed that symbiotic bacteria in human play a key role in the functions of lung and brain (22, 23). According to variations in the human microbiome in each region and its direct effect on disease and treatment, studying the microbiome is currently known as an important field of research (24).

In patients with COVID-19, the structure, function, and diversity of their gut microbiota are impaired, since the expression of ACE2 decreases during a viral infection (25). Moreover, ACE2 regulates the expression of B0AT1 amino acid transporter that is responsible for control tryptophan (Trp) uptake in the gut (26). Trp regulates the expression of the antimicrobial peptides mRNA levels via the mammalian TOR (mTOR) pathway that has an effect on the microbiota (27). So, a decline in the expression of ACE2 leads to the decreased Trp absorption in intestine and antimicrobial peptides secretion, the increased pathogen invasion, and consequently to gut dysbiosis (28).

Many patients with COVID-19 who underwent treatment with antibiotic, indicated dysbiosis and GI symptoms such as diarrhea, vomiting, and stomachache, named as Antibiotic Associated Diarrhea (AAD) (29). Accordingly, AAD is a common complication of antibiotic therapy, occurring in approximately 50 to 75% of those patients who receive antibiotics (30). Additionally, in patients aged more than 65 years old infected with COVID-19, the incidence of GI symptoms is higher than those aged under 65 years old (31). Because of aging-associated alterations in composition, diversity, and functional features of intestinal microbiota and in the incidence of dysbiosis in these individuals (32), exploring some strategies to restore a normal balance in gut microbiota is essential for improving an interaction between gut microbiota and key organs such as the lung and

the brain. Correspondingly, this can increase severity of pulmonary and brain damages through gut-lung axis and gut-brain axis in patients with COVID-19.

Gut-lung axis. Gut could affect lung health through a relationship between gut and lung microbiota, which is named gut-lung axis (33). Evidences showed that there are microorganisms in lung similar to gut microbiota; however, in gut, Bacteroidetes and Firmicutes are predominant, and in lung, Bacteroidetes, Firmicutes, and Proteobacteria are predominant (34).

Dysbiosis in the gut microbiota plays a role in pathogenesis of various pulmonary disease states, so GI diseases were found to be associated with respiratory diseases (35). It was seen that inflammatory bowel disease (IBD) in about 50% of patients is accompanied with pulmonary disease (36). However, they had no history of respiratory disorders. Additionally, the excessive antibiotics' consumption was found to be related to gut microbiota impairment, which is associated with the increased risk of lung cancer in people consuming penicillin, cephalosporin, macrolide, and quinolone (37).

It was reported that the gut-lung axis might be reciprocal. Therefore, endotoxins and microbial metabolites can affect lung function, inflammation, and the gut microbiota (38). In this line, several studies reported some changes in function of the combination of gut microbiota and respiratory infection (37). In this line, Chunxi et al. (2020) reported that "increasing evidence suggests an important and complex crosstalk between the gut and lung, as well as between the gut microbiota and host immunity". Gut microbial dysbiosis is believed to be associated with the etiology or/and development of common respiratory diseases, such as asthma, COPD, CF, lung cancer, and respiratory infection. To date, the understanding of the mechanism involving the gut-lung axis is still in its infancy and remains to be further elucidated (39). Dumas et al (2018) study demonstrated that "Administration of microbes (using probiotics or faecal transfer), microbe components, or products favoring their growth (e.g., prebiotics) has been suggested to confer host protection through direct competition with the disease-causing microbes, enhancement of epithelial barrier functions, or immune modulation during respiratory diseases (37)." Also, Sencio et al. (2021) study reported that "The gut microbiota has a critical role in pulmonary immunity

and host's defense against viral respiratory infections. The gut microbiota's composition and function can be profoundly affected in many disease settings, including acute infections, and these changes can aggravate the severity of the disease" (40). The findings reported that the COVID-19 virus can be transmitted to the GI tract and gut through systemic blood circulation and lymphatic flow after impairing lung tissue (22).

In general, gut and respiratory system were observed to be related to each other by modifying immune system functions (33). The balanced gut microbiota has an important effect on lung immunity, so that germ free (GF) mice with no gut microbiota are not able to scavenge pathogens in lung (41). In response to an infectious pathogen like coronavirus, the healthy gut microbiome are considered as important components in preserving optimum immune system functions, in order to inhibit immune overreactions, which is harmful for vital organs such as lungs (42). Of note, an overreaction and under-reaction of immune system both can lead to the exacerbation of clinical complications of patients with COVID-19 such as pneumonia and ARDS (43).

ARDS is a serious clinical sign, in pathogenesis of which, the destruction of gut microbiota plays an essential role (44). It was reported that the lung microbiota is different in patients with ARDS and patients with no ARDS. Experimental studies over decades have indicated that the gut microbiome population have a prominent role in the pathogenesis of ARDS (45): antibiotic-suppressed and germ-free mice are prevented from the lung impairment and mortality of experimental sepsis and a lot of clinical trials studies have indicated that prophylactic suppression of gut microbiome population by antibiotics is preventive towards multi-organ impairment and death in patients with critical conditions (46, 47). However, the mechanism of the gut microbiome population involvement in ARDS is unclear, an assumption is that the lung microbiome is full of gut microbiome in human ARDS (48). Enrichment of the lung microbiome by gut-related microbiome lead to independent of the upper respiratory tract, related with severity of systemic inflammation states and cause to the resistance of live bacteria in the lung microbiome (49). Alveolar inflammatory cytokine such as tumour-necrosis factor- α (TNF- α) is a main mediator in ARDS patients that is highly related by changes in lung microbiome (50).

Therefore, gut and lung microbiota can be recognized as a predictive factor for ARDS as well as for laboratory results of COVID-19 patients (51). It was observed that viral infection of influenza in the respiratory system of mice could also increase Enterobacteriaceae and decrease lactobacilli and lactococci populations of the gut microbiota (52).

Gut-central nervous system (CNS) axis. It is worth noting that coronaviruses lead to neurological impairment. Several neurological complications of patients with corona, such as cranial neuropathy, encephalopathy and Guillain-Barre Syndrome, have been demonstrated (53). Also, genome sequencing indicated the presence of SARS-CoV-2 in the cerebrospinal fluid. According to various researches, current study proposed the hypothesis that COVID-19 affecting CNS by gut-CNS axis (54). Many health aspects of brain are affected by gut function and flora (55). Gut microbiota affects the CNS through the gut nervous system, named as the gut-brain axis (56). Notably, due to the similarity of the intestinal nervous system to the autonomous CNS, the intestinal nervous system is known as the second brain (57). Various studies have previously reported the reciprocal relationship between gut and brain axis, which is from the gut microbiota to brain and from brain to gut microbiota using neural, endocrine, immune, and humoral links (58).

Over one hundred million neurons exist in the gut, which can be linked to brain through the secretion of neurotransmitters (59). The neurotransmitters are secreted from three major sources, including neurons in brain, enterochromaffin cells, and gut microbiota that reach brain by blood flow (60). Many gut microbiota are able to produce neurological active compounds such as tryptophan, serotonin, and leptin that have no appropriate functions in the absence of flora (61). Gut microbiota can directly affect some neurological disorders such as Parkinson, Alzheimer's disease, Autism, Depression, and other psychiatric diseases through gut-brain axis. In contrast, stress and depression can consequently affect gut-brain axis (62, 63). Although some recent studies showed the importance of the gut-brain axis, one of the gaps in our current knowledge is the absence of a relationship between the SARS-COV-2 and nervous system, especially with the gut-brain axis (23). In addition, neuro-invasive and neurovirulence profiles were reported in many patients with types of coronaviruses

such as SARS-COV-1 and MERS-COV, belonging to genetic group of beta corona virus COVID-19 (64).

In SARS pandemic disease during 2002-2003, patients showed some neurological complications, and SARS-COV-1 was seen in the cytoplasm of neurons by autopsy of brain tissue that consequently led to tissue's inflammation (65). Experimental mice transgenic studies for ACE2 receptor showed that SARS-COV-1 virus could lead to brain attack through olfactory nerves, which is accompanied with neuronal death (66). In contrast with SARS-COV-1, MERS-COV binds to Di-Peptidyl Peptidase 4 (DPP-4) receptor from the cell surface to the cytoplasm, which is widely expressed in whole body, especially on the brain. Since SARS-COV-2 has a genetic and structural and clinical aspects similar to SARS-COV-1 and MERS-COV, many mechanisms involved in destructing gut-brain axis could also be identical (67).

Several previous studies showed that COVID-19 accompanied with GI symptoms such as diarrhea, nausea, vomiting, and abdominal pain in patients would cause neurological complications (10, 68). In addition, several animal studies reported that the brain does not grow at the same rate with the body in mice models with no microbiome (69). Moreover, it was shown that essential processes of brain such as development, myelination, neurogenesis, and microglial activation are vigorously dependent on gut microbiota combination (70). As explained earlier in previous sections, SARS-COV-2 affects gut microbiota through entering GI system, especially ACE2 receptor in intestine, which consequently causes dysbiosis in the gut (17). Thereafter, it reaches CNS through blood flow or vagus nerve pathway and then causes neurological complications (71). Neurons and glial cells in CNS also express ACE-2 receptors on their surface, leading SARS-COV-2 to enter brain cells (72). As well, SARS-COV-2 use the DPP-4 receptor from the brain cell surface to the cytoplasm. COVID-19 virus can directly reach CNS through olfactory nerve and blood pathways, and then cause neurological impairments (23).

Neurological symptoms such as headache, the impaired consciousness, and paresthesia might be mild at first, which subsequently become severe and cause the indication of some problems such as loss of consciousness, convulsion, and brain stroke (73). Therefore, it is logical to assume that when the COVID-19 virus reaches to the gut, the severe clinical symptoms occur along with neurological disorders.

Probiotic. The intestine involves the regulation of lung and CNS function in various ways, such as nervous system, hormone system, and immune system. Increasing evidence shows that the SARS-CoV-2 can cause intestinal dysfunction, microbial imbalance, and immune disorder. Through the microbe-gut-brain and lung axis, the intestine, especially the intestinal bacteria, is most likely to be the major approach for SARS-CoV-2 affecting the brain and lung function. It is therefore likely that brain and lung dysfunction would be secondary complication after SARS-CoV-2 infection.

One of the most important factors affecting the gut microbiota is dietary regimen (74). Food is a main source of energy that lead to growth, develop, immunity, tissue repair, homeostatic regulation, as well as source of energy for gut microbiota (75). Perceiving the association between the gut microbiome population and diet is necessary for improvement of next-generation therapeutic supplementation that target the microbiota in health (76). Food is able to affect the gut microbiome directly, which lead to alter biochemical reactions pattern in the gastrointestinal system (77). Intestinal microbes absorbed and transformed various nutrients into other metabolites that are ingested by the host (78). The various products of the biochemical transformation including short-chain fatty acids (SCFAs), biogenic amines (such as histamine) or other amino-acid-derived metabolites such as serotonin or gamma-aminobutyric acid (GABA) have main role in health and disease condition (79). Moreover, the production leads to induce alteration in gut microbiome composition. Non-digestible carbohydrates food may be ferment in the intestinal lumen and lead to produce several SCFAs including acetate, propionate and butyrate (80). SCFAs metabolites have main role in several biological pathways that provide energy sources for colonic epithelial cells in gastrointestinal lumen. In addition, fermentation of prebiotic carbohydrates including inulin and fructo-oligosaccharides lead to induce proliferation of important gut microbiome such as *Bifidobacterium* spp. and *Lactobacillus* spp. in the gastrointestinal lumen (81).

In this mean, the more affection of the gut microbiota with probiotic source is so prominent in human and animal disease treatment. Regular consumption of probiotics, foods or supplements containing beneficial bacteria is a critical approach to establish eubiosis condition, which is a state of gut microbiota

balance (82).

The word “probiotic” results from Greek and means “for life.” In 1954, Ferdinand Vergin understood the word “probiotic” in a paper with “Anti-und Probiotika,” title that various microorganisms were reported to make a list of beneficial bacteria and to assess the detrimental effects of antibacterial agents and antibiotics on the intestinal microbe (83). Due to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are known as live and non-pathogenic microorganisms, so their sufficient consumption can affect health status through direct and indirect mechanisms (84). Clinical studies also confirmed that probiotics are effective factors on the improvement of treatment process of spectrum GI diseases such as Ulcerative Colitis (UC), acute infectious diarrhea, Irritable Bowel Syndrome (IBS), antibiotic resistance of diarrheal pathogens, and necrotizing enteritis (85).

Nowadays, most probiotic species in dietary supplements or pharmaceutical products belong to *Lactobacillus* and *Bifidobacter*. However, other bacterial species like bacteria that produce lactic acid, are also able to be applied in producing wide spectrum of fermented foods (diary and non-diary) (84). Modulation of the gut microbiota improves a several health disorders; probiotic consumption with a high-fat diet (HFD) demonstrated imbalanced of the gut microbiota composition with a decrease in the Gram-positive bacteria phyla Firmicutes and Actinobacteria in animal models (86). In contrast, in another animal model of lipid disorders, the probiotic consumption of *Lactobacillus* caused to prominent alters in the gut microbioma population, including an elevated amount of Bacteroidetes and Verrucomicrobia and a decreased ratio of Firmicutes. It is obvious that probiotic sources consumption has prominent roles in maintaining the gut microbiota in humans and animals gastrointestinal system (87).

Health effects of probiotics on the gut microbiome can be performed in two ways, either dependent on living bacterium and its metabolic activity, or dependent on non-living compounds derived from microbiota (88). Notably, probiotics produce the antimicrobial factors or metabolic compounds (89), which compete for receptors with other gut microbes in the gut mucosa and consequently suppress the growth of other microorganisms and compete for active site of receptors and binding with other intestinal microbes on the intestinal mucosa (90). Probiotic species of

Lactobacillus may improve gut barrier function, which leads to the modification of immune response; the modulation of gut epithelial response; the decreased bacteria translocation; endotoxemia; and various diseases such as GI infections, IBS, and IBD (89, 91). Moreover, probiotics may modify metabolic function besides directly affecting gut microbiota combination. Another mechanism involved in the effect of probiotics on gut microbiome is their ability to bind to enterocytes, which leads to the prevention of pathogen binding to the host gut epithelium and the production of antimicrobial metabolites such as bacteriocin, lantibiotics, short chain fatty acids (SCFA), and lactic acid. Moreover, active oxygen compounds like hydrogen peroxide (H₂O₂) are also among the mechanisms known in this regard (92-95).

CONCLUSION

In this review we have summarized the findings that indicate evidence for bidirectional interactions the gut-lung axis and gut-CNS axis via the host microbiome in COVID-19 with implications for post-COVID-19 GI complications as well as for gut infections and consequently for lung and brain impairment.

In summary, GI injury in COVID-19 patients can impair the structure, function, and diversity of their gut microbiota. Consequently, this can increase severity of pulmonary and brain damages through gut-lung axis and gut-brain axis in patients with COVID-19. The microbiota relation with lung is resulting from similar microbiota in gut and respiratory system were observed to be related to each other by modifying immune system function. The balanced gut microbiota has an important effect on lung immunity and the germ free mice with no gut microbiota are not able to scavenge pathogens in lung. Moreover, gut microbiota can affect the brain by using neural, endocrine, immune, and humoral pathways. In this mean, over one hundred million neurons exist in the gut, which can be linked to brain through the secretion of neurotransmitters and many gut microbiotas are able to produce neurological active compounds such as tryptophan, serotonin, and leptin that have no appropriate functions in the absence of flora. Therefore, exploring some strategies to restore a normal balance in gut microbiota is essential for improving an interaction between gut microbiota and

key organs such as the lung and the brain. Since the human microbiome is adaptable and can be re-seeded with the help of dietary changes, we reported an important nutrient in this field. Probiotics are recommended as a part of the dietary management of gut microbiome function. New types of probiotics may be used as further considerations in order to promote health, prevent disease, and treat various disorders. It is currently assumed that targeting brain and lung-gut microbiota axis through diet and dietary supplements containing probiotic can be an effective way in this regard. However, further studies are required to evaluate the association between probiotics and COVID-19 management.

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