



Vitamin B₁₂ Deficiency And Increased Susceptibility And Severity Of Covid-19: A Review

Shehwaz Anwar^{1*}, Ravindra Raut², Binish Kanwal³, Ehsan A. Yahia⁴

¹Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, Saudi Arabia.

²National Institute of Technology Durgapur, Durgapur, India.

³Department of Pharmacy, Goel Institute of Pharmaceutical Sciences, AK T University, Lucknow, India.

⁴Faculty of Nursing, Cairo University, Egypt.

Abstract

SARS-CoV-2 virus has been found to affect millions of people worldwide. It is presenting increased health and economic challenges for all the countries as a pandemic by infecting and killing a large population. Despite the considerable morbidity and mortality linked with SARS-CoV-2, no proven treatments or totally preventive strategies are available. As a result, it is very necessary to understand the pathobiological processes associated with the increased risk for and severity of SARS-CoV-2 infection. The pathogenesis of COVID-19 has been linked with three common factors including excessive inflammation, impaired immune system, and a set of pro-inflammatory cytokines. The nutritional status of COVID-19 patients has been found to be responsible for their health status, and various nutrients including vitamin A, D, C, E, B₆, B₁₂, Folate, iron, copper, zinc, and selenium, are known to boost up the immune system. These nutrients are essential for better immune system. Thus, we can assume that the supplementation of these specific nutrient might be beneficial in protection and speed up treatment of COVID-19 patients by improving the immune response against COVID-19 infection. Several damaging effects on immunological, microbiological, hematological system are noticed in COVID-19 patients that are also found in peoples having B₁₂ deficiency. B₁₂ deficiency might be responsible for the chance of death from COVID-19 via inducing impaired immune system, and causing hyperhomocysteinemia. In this review, we explore the current knowledge of COVID-19, pathogenesis of

COVID-19, and highlight the general cause of B₁₂ deficiency, and shall explore the possible role of B₁₂ deficiency in COVID-19 susceptibility and severity.

Key words: COVID-19; Pathogenesis; Micronutrients; B₁₂ deficiency; Treatment.

Introduction

Coronavirus disease-2019 (COVID-19) is a life threatening viral disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is a RNA virus that contains an enveloped and positive single strand of RNA (between 26-32 kb length) (Levy et al., 2020). There is a gene at 5' end and is called as orf1ab. All the polyproteins bearing all the non-structural proteins are encoded by this gene. The proteolytic processing of the polyproteins results in the formation of 16 proteins named as nsPs 1-16. Adenosine diphosphate-ribose 1"-phosphatase activity has been documented to be carried by nsp3 proteins. Another protein named as nsp5 protein possess the protease activity that contribute to cleavage of the polyprotein. RNA-dependent RNA polymerases are present in nsp12 protein. This is the enzyme that is responsible for the replication of the genome. However, the nsp13 protein has RNA helicase activity that is essential for genome duplication. The nsp14 protein attributes to exoribonuclease (exoN) and N7-methyltransferase activities. A nidoviral ribonuclease specific for U has been noticed to be present in the nsp15 protein. A SAM-dependent O-methyltransferase activity is possessed in the nsp16 protein (Narayanan and Nair, 2020).

This virus belongs to the sub-family of beta-corona virus belonging to family *Coronaviridae*. The identification of specific crown like surface projections by electron microscope is a specific feature of this virus (Ugwueze et al., 2020). COVID-19 crisis has been found to hit many countries by infecting and killing thousands of people. Although, many public health, and economic programs have been launched by various governments, thousands of new cases are being reported every day. It has been documented that COVID-19 is more pronounced in peoples having non-communicable disease. Individuals above the age of 60 years, as well as those with chronic diseases as asthma, diabetes, and coronary or respiratory diseases, are the most vulnerable populations to the severe-critical complications of COVID-19. Diabetes can be a causative factor for severity of COVID-19 and the resultant of SARS-CoV-2 infection (Abramczyk and Kuzan, 2021).

Transmission of this virus has been supposed to occur via respiratory droplets like cough, sneeze, or exhale, and direct contact with a COVID patient. There are some reports indicating the presence of SARS-COVID in stool and urine of confirmed patients. Hence, it may lead to risk of fecal-oral transmission. However, it is still unclear the transmission and infection of COVID-19 via consumption of food (Jin et al., 2020). COVID-19 patients are still the chief source of infection. Further, the patients who are severely affected by COVID-19 are considered to be more infectious than moderate ones (Hoehl et al., 2020).

Binding of coronavirus receptor is accomplished by a spike protein that is encoded by S gene with two subunits. S2 subunit which is trimeric, facilitate its attachment to infected cell. However, S1 subunit mediates the binding. There two distinct domains in S1 subunit called as N-terminal domain and C-terminal domain. Both of these domains brings about the binding to various cellular receptors having carbohydrate and protein at their binding site. The replication of its RNA is mediated by RNA-dependent RNA polymerase enzyme. The replication includes the discrete transcription of subgenomic mRNA that encode six major open reading frames. This common feature is shared by several accessory proteins as well as all coronaviruses (Alomari et al., 2020).

Peripheral neuropathy is a broad term that encompasses a variety of disorders caused by injury to the peripheral nervous system, which transmits sensory information to and from the brain. In general, the disorder might result in the loss of normal nerve impulses, as well as the transmission of incorrect or distorted signals. Some people may have numbness and tingling as a result of COVID-19. It is impossible to determine who would get paresthesia after COVID. The range of neurologic problems associated with the new coronavirus 2 (SARS-CoV-2) infection is growing. Peripheral neuropathy induced by SARS-CoV-2 infection has only been studied in a few trials (Bureau et al. 2020). Previous study had described a case of mixed sensorimotor neuropathy caused by SARS-CoV-2 infection that was nearly completely resolved with immune-modulation, symptomatic medication, and intense rehab. Rhabdomyolysis, myopathy, myositis, myasthenia, myasthenic syndrome, polyradiculitis with or without cranial nerve involvement, and peripheral neuropathy have all been linked to SARS-CoV-2 infection (Finsterer et al., 2021).

There is a significant morbidity and mortality linked with SARS-CoV-2. However, there are no proven treatments or totally preventive strategies against this disease. As a result, there is an urgent need to recognize the pathobiological mechanisms underlying the increased risk for and the severity of SARS-CoV-2 infection. Therefore, knowing the mechanisms that are involved in these manifestations may help in the development of therapeutic strategies. The damaged antioxidant defence system and elevated increased oxidative stress have been suggested to be implicated in the severity and mortality risk of patients having SARS-CoV infection (Anwar et al., 2022).

Pathogenesis of COVID-19

There are several symptoms associated with this disease including fever, cough, breath shortness, sore throat, and fatigue. Headache, dyspnea, hemolysis, muscle soreness, diarrhea, pneumonia, acute respiratory distress syndrome, acute cardiac syndrome, and multi-organ failure. The loss of smell and taste has been often reported (Ugwueze et al., 2020). COVID-19 infection primarily attacks the respiratory system through mucosal epithelium of the upper respiratory tract (nasal cavity and pharynx), with additional proliferation in the lower respiratory tract and gastrointestinal mucosa. Non-respiratory symptoms like acute liver and heart attack, diarrhea, and kidney failure have been further reported to occur in COVID patients. Besides, multiple

organs like nasal mucosa, heart, lung, kidney, esophagus, bronchus, stomach, ileum, and bladder, are all susceptible to it (Jin et al., 2020). Infection spread to central nervous system leading to inflammation, demyelination, with related complications. In addition, numerous hospitalized patients show other complications including cardiovascular diseases, diabetes mellitus, hypertension, and obesity.

COVID-19 pathogenesis is a multifactorial process that contributes to systemic hyper-inflammatory reaction, related thromboembolic complications, reduction in antioxidant defense, invasion of neutrophils, and release of reactive oxygen species (ROS), subsequent lysosomal membrane permeabilization. Repair mechanisms and immune system are largely affected by oxidative stress and oxidative stress is one of the key factor linked with severity of COVID-19. A great association between the pro-inflammatory elements and the reactive oxygen species (ROS) in the different lung disease including Coronavirus infection which is associated with inflammation and oxidative stress. The activation of the NLRP3 inflammasome becomes triggered by the release of ROS into cytosol. NLRP3 inflammasome has been known to be involved in many inflammatory processes by activating caspase-1 and release of IL-1 β (Pérez de la Lastra et al., 2021).

It has been proposed that neurological symptoms are more prevalent in individuals with severe illness, but not in those over the age of 65. Peripheral neuropathies are common in COVID-19 patients, and they are caused mostly by immunological processes and neurotoxic side effects of medications used to treat COVID-19, as well as, to a lesser extent, by peripheral nerve compression caused by extended ICU bedding (Bureau et al., 2020). The precise method of SARS-Cov-2 entrance into the central nervous system is unknown, but currently considered routes include retrograde neuronal transport across infected neurons, olfactory nerve invasion, infection of the vascular endothelium, and white blood cell migration across the blood-brain barrier. Although SARS-Cov-2 may have direct access to the CNS, only two instances of SARS-Cov-2 in cerebrospinal fluid have been recorded. Angiotensin-converting enzyme-2 is one proposed route for direct nervous system entrance (ACE2) (Reza-Zaldivar et al., 2021). Peripheral nerve neuropathy, including polyradiculitis, has been reported to be very common among COVID-19 patients. GBS, medicines used to treat COVID-19 symptoms, pre-existing diabetes, and compression neuropathies owing to prone bedding in the ICU are the most prevalent causes of SARS-CoV-2-associated peripheral neuropathy (Finsterer et al., 2021).

The serious progression of COVID-19 causes a cytokine outbreak, with an increase in the synthesis of pro-inflammatory cytokines (Shakoor et al., 2021). Immune-mediated inflammation, is important in the pathogenesis of COVID-19. The emergence of COVID-19 was associated with a steady decrease in lymphocyte count and a significant rise in neutrophils. In the meanwhile, inflammatory indicators including MIP1A, TNF, C-reactive protein, interleukin (IL)-6, ferritin, and MCP1, IP-10, have been documented to be substantially elevated. Furthermore, lymphopenia, and a decrease in both CD8+ and CD4+ T lymphocytes have been identified as key features of SARS. The substantial decline in lymphocyte populations in SARS might be due to two mechanisms including lymphocyte depletion via apoptosis or pyroptosis, or redistribution

of circulating lymphocytes (Cao et al., 2020). Reactive oxygen species have a critical role in receptor activation, signaling, and gene expression. Excessive generation and insufficient clearance of reactive oxygen species (ROS) leads to oxidative stress, which is a significant risk factor in the development of a variety of diseases (AlMatroodi et al., 2020) such as liver diseases (Yahya and Anwar, 2020). Repair mechanisms and immune control system are affected by oxidative stress (AlSahli et al., 2021). Damage to numerous biomolecules, membranes, and tissue injuries are caused by oxidative stress, which is mediated by increased generation and accumulation of reactive oxygen species (ROS) (Anwar et al., 2021). Oxidative stress has been known to be significant factor of the inflammatory response. There is a significant relation between oxidative stress, reactive oxygen species, and the pro-inflammatory elements various lung disease including COVID-19 infection. Therefore, oxidative stress can be a significant cause that leads to the severity of COVID-19, in those patients having chronic illnesses as well as impaired antioxidant system (Derouiche, 2020).

To avoid SARS-CoV-2-associated neuropathy, neurotoxic medications such as daptomycin, linezolid, lopinavir, ritonavir, hydro-chloroquine, cisatracurium, clindamycine, and glucocorticoids should be used with caution, and patients in the ICU should be suitably bedded (Finsterer et al., 2021). Safety precautions to avoid COVID-19 infections include frequent washing of the hands with alcohol, soap, and water, social distancing, use of non-medical fabric mask, avoiding touching eyes, mouth, and nose, and practicing respiratory hygiene.

Hyperglycemia and COVID-19

Hyperglycemia has been suggested to have a great impact on the risk of mortality in patients with COVID-19 (Bode et al., 2020). Several mechanisms have been proposed for hyperglycemia-caused worse prognosis for COVID-19. Angiotensin-converting enzyme-2 (ACE-2) receptor is a plasma membrane protein, and is expressed mainly in lungs. ACE-2 receptor is responsible for infection of COVID-19 to host cells that leads to COVID-19-related ARDS and interstitial pneumonitis. Glycation of ACE2 receptor, encourages the binding of the SARS-CoV-2 virus to ACE2 receptor and thus the severity of COVID-19 disease increases in hyperglycemic conditions (Brufsky, 2020). Further, pulmonary dysfunction and structural changes in lung tissue like collapse of portions of the lung and augmented permeability of the blood vessels are induced by hyperglycemia. As a result, the lung of diabetic peoples are more potential target for severity of COVID-19. The replication of SARS-Cov-2 in monocytes is promoted by hyperglycemic conditions. Hence, T-cell response is supposed to be inhibited in the presence of elevated level of glucose. Besides, the dysfunctional immune system including both innate and adaptive immunity in diabetic patients lead to vulnerability and severity of COVID-19. Hyperglycemia induced abnormal inflammatory response and immune-over response, as well as elevated lactate levels in modulating the inflammatory immune response are another potential mechanisms that facilitate SARS-CoV-2 infection (Wang and Meng, 2020).

Role of specific dietary nutrients in the management of COVID-19 disease

A poor diet is a significant risk factor for a variety of chronic illnesses, including cardiovascular disease, diabetes, and obesity. A deficient diet will result in food deficiencies, which will almost certainly contribute to chronic disease. Diets rich in sodium and low in whole grains, berries, vegetables, nuts, and seeds have been linked to an increase in mortality rates. Malnutrition is one of the root cause for the poor health as well as disease conditions because it leads to poor immune system and increased risk of infection. It is well known that nutrients and bioactive molecules significantly affect the activity of immune system (McAuliffe et al., 2020). Similarly, the nutritional status of the COVID-19 patients have the great influence on their health status. An adequate nutritional status and appropriate intake of specific nutrients have been reported to be essential in COVID-19. It has been suggested that proteins of high biological values (present in fish, eggs, dairy, and lean meat) with all essential amino acids may attribute the anti-inflammatory effect. Further, certain lipids like omega-3-fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may have the ability to inactivate enveloped viruses via modulation of the optimal host lipid conditions that are required for viral replication. Therefore, the reduction of the severity and/or improve the recovery of patients with COVID-19 can be gained by supplementation with DHA and EPA. Additionally, anti-inflammatory property of polar lipids, including sphingolipids, phospholipids, or glycolipids is supposed to be beneficial in COVID-19. Besides, these polar lipids are very important in COVID-19 because of their potential to block platelet-activating factor (PAF) as well as its receptor. Food -rich in carbohydrate like glucose are not advised to COVID-19 patients (Fernández-Quintela et al., 2020). Antioxidant supplementation in therapeutic regimens has been recommended to COVID-19 patients ((Derouiche, 2020).

Micronutrient deficiency and susceptibility to COVID-19

There are several reports regarding the potential interactions of immune system and nutritional status. Various micronutrients are gifted by nature with amazing immunomodulatory effects. The health of patients suffering from various infectious diseases is reported to be improved by supplementing the micronutrients to patients. Similarly, micronutrients are supposed to boost the immune response against COVID-19 (McAuliffe et al., 2020). Micronutrient deficiencies are documented to impair immune system. Hence, deficiency in any micronutrient can attribute to increased susceptibility to various infectious diseases including COVID-19. In short, micronutrients play important role in the development and maintenance of physical barrier, mediation of inflammatory processes, production of antimicrobial proteins, and activity of immune cells. Thus, micronutrients may alter risk of serious infection and survival, susceptibility to COVID-19 infection, progression to various symptoms. Vitamins and minerals are important in counteracting the toxic effects of ROS that have a strong potential to kill cells. The lack of these nutrients can lead to suppression of immune system, resulting in the increased susceptibility to infection. A group of organic compounds that are known to have an essential role in normal physiologic functions. However, these substances are not endogenously synthesized in human body and are obtained from diet. These compounds are called as vitamins. There are

fat soluble (A, D, E, and K), and water soluble (C, and B complex) vitamins. Vitamin B complexes comprises thiamine (B₁), riboflavin (B₂), niacin (B₃), pantothenic acid (B₅), vitamin B₆, folate (B₉), and vitamin B₁₂. The nutrients for better immune system include vitamin A, D, C, E, B₆, B₁₂, Folate, iron, copper, zinc, and selenium. Thus, we can assume that the interaction of these nutrients with immune system might be beneficial in protection and speed up treatment of COVID-19 patients by supporting the immune response against COVID-19 infection (Guha and Chakraborty, 2021).

Vitamin B₁₂

Vitamin B₁₂ (B₁₂) or “cobolamin” is one of the essential vitamins, and cannot be synthesized by human beings through their own metabolic reactions (Al-Musharaf et al., 2021). It is a water soluble, largest and most complex, cobalt containing, vitamin molecule, and is exclusively produced by certain microorganisms (Andres et al., 2016). It is very crucial for DNA synthesis, methylation, folate metabolism, erythropoiesis, neurodevelopment and nervous system functions, as well as regulation and in the synthesis of fatty acids. Besides, it involves in the metabolism of proteins, phospholipids and neural transmitters and energy production (Van Sande et al., 2013). B₁₂ functions as a cofactor in the methionine synthase reaction, which converts homocysteine into methionine. Therefore, the deficiency of vitamin B₁₂ could increase the level of plasma homocysteine which raises the risk for cardiovascular diseases. Further, the formation of S-adenosylmethionine (a universal methyl donor) requires methionine. S-adenosylmethionine is a universal methyl donor which is required for methylation of phospholipids, neurotransmitters, amines, DNA, RNA, and myelin basic protein. It is well known that impaired methylation of DNA caused by reduction of S-adenosylmethionine may lead to altered fetal metabolic programming, and increased risk for chronic diseases later in life. In the mitochondria, methylmalonyl-CoA mutase catalyze the conversion of methylmalonyl-CoA to succinyl-CoA, and vitamin B₁₂ works as a cofactor for this enzyme. Hence, the deficiency of B₁₂ causes the elevated concentration of methylmalonyl CoA, and the formation of a by-product methylmalonic acid (MMA) starts. Thus, carbohydrate and lipid metabolic pathways might be affected by insufficiency of vitamin B₁₂ (Siddiqua et al., 2014). In the human body, B₁₂ occurs in three forms including natural form, hydroxycobalamin and in its two active forms i.e., methylcobalamin (Me-Cbl) and adenosylcobalamin (Ado-Cbl) (Smith and Coman, 2014).

B₁₂ is not synthesized by human body so it is naturally obtained by ingestion of animal proteins such as beef, poultry, fish, eggs, dairy products and fortified plant based foods (Vashi et al., 2016; Vanderjagt et al., 2011). B₁₂ metabolism is a complex and multistep process and B₁₂ deficiency can occur by defect in any one of step. B₁₂ from dietary intake occurs as bound to protein and which is released by the hydrochloric acid in stomach. This free form of B₁₂ is protected from chemical denaturation in the stomach by binding with haptochorrins. Haptochorrins are glycoproteins that are secreted by salivary and stomach glands. Now in the stomach, intrinsic factor secreted by stomach binds to B₁₂, and it helps in the active absorption in ileum (Van Sande et al., 2013) where it is released ultimately as vitamin B₁₂ (Sadasivan and Friedman, 2012). Main

storage site for B₁₂ is the liver that converts B₁₂ into its active forms for utilization. Liver is one of the most important organs with multiple functions. It has an active role in metabolism (Al Matroodi et al., 2020; Rahmani et al., 2020). A synthetic form of B₁₂ is used for commercial and pharmacological purpose and is popularly known as cyanocobalamin.

B₁₂ is extremely crucial for two cellular reactions. Me-Cbl works as cofactor for methionine synthase (MS; EC 2.1.1.13), and is needed for the synthesis of methionine from homocysteine in cytosol (Van Sande et al. 2013) and methyltetrahydrofolate to tetrahydrofolate (Langan and Zawistoksi, 2011; McCracken et al., 2006). In the second reaction, Ado-Cbl is required as cofactor for methylmalonyl-CoA mutase (MCM; EC 5.4.99.2) that catalyzes the conversion of methylmalonyl CoA to succinyl CoA in mitochondrion (Van Sande et al., 2013). The deficiency of B₁₂ results in accumulation of homocysteine and/or methylmalonate in serum (Smith and Coman, 2014; Van Sande et al., 2013). Methylmalonyl CoA is a normal product of catabolism of branched chain amino acids, cholesterol and odd chain fatty acids. The decreased activity of methylmalonyl CoA mutase results in increased concentration of Methylmalonyl CoA and its upstream metabolite, propionyl CoA. Under normal circumstances, citrate synthase catalyzes the condensation of acetyl CoA, with oxaloacetic acid to form citrate. The enhanced concentration of propionyl CoA makes its incorrect uptake into the Krebs cycle, and propionyl CoA joins with oxaloacetate to produce MCA. Thus, instead of making citrate required for normal citric acid cycle, MCA is produced.

B₁₂ deficiency

Since the most common source of B₁₂ is animal source, vegans and vegetarians are at the risk of its deficiency. However, the type of food and amount of B₁₂ intake determine its bioavailability. B₁₂ deficiency is a major public health problem (Hvas and Nexø, 2006) and this condition is multifactorial (Hannibal et al., 2016), and can be caused by nutritional deficiency, malabsorption syndromes, drug nutrient interactions, genetic defects such as inherited metabolic disorders, autoimmune diseases and other gastrointestinal problems (O'Leary and Samman, 2010; Smith, 2008). Several medicines including proton pump inhibitors (PPIs) such as lansoprazole (Prevacid), omeprazole (Prilosec OTC), esomeprazole (Nexium), rabeprazole (Aciphex), and pantoprazole (Protonix), H₂ Blockers such as famotidine (Pepcid AC) and cimetidine (Tagamet); and certain diabetes medicines such as metformin (Glucophage) cause the B₁₂ deficiency. The peoples, who are on strict vegan diet, develop B₁₂ deficiency. The bioavailability of B₁₂ is an important factor that determines the status of B₁₂ in human body and is dependent on an individual's gastrointestinal absorption. Pernicious anemia is considered as most frequent underlying cause of B₁₂ deficiency because it is associated with lack of intrinsic factor to bind with ingested B₁₂. Abdominal surgery, pancreatic insufficiency, fish tapeworm infections and severe Crohn's disease are other causes of B₁₂ deficiency because they results in reduced ileal absorption of B₁₂. Elderly peoples, vegans, pregnant and/or lactating woman are particularly at increased risk of B₁₂ deficiency. Due to predominant vegetarian dietary pattern, poverty, overcrowding general neglect towards nutrition and lack of public services, the pregnant women are more vulnerable to

develop B₁₂ deficiency (Ramirez-Velez et al., 2016). This micronutrient is actively transported from placenta to foetus. The fetus is unable to synthesize the B₁₂ so it uses B₁₂ supplied by placenta for its biochemical reactions (Van Sande et al., 2013). Therefore, B₁₂ deficiency occurs frequently during pregnancy (Chandyo et al., 2017). Due to hemodilution, hormonal changes, alterations in the concentration of B₁₂ binding proteins and placental transport of B₁₂ to the fetus, a gradual decline occurs in the serum B₁₂ concentration starts first through the third trimester of pregnancy and the status of B₁₂ reaches its lowest value at 32 weeks of pregnancy (Van Sande et al., 2013). Long term B₁₂ deficiency has been proved to have adverse effects on pregnancy (O'Leary and Samman, 2010).

B₁₂ has been proved to be important in preventing recurrent pregnancy loss. Scientific studies have proved that overall 12-15% of clinically recognizable pregnancies end in spontaneous miscarriages and the prevalence of recurrent pregnancy loss is between 15% in general population. Low level of B₁₂ deficiency and high concentration of homocysteine have been confirmed to be the underlying cause of prior pregnancy loss in 33% of women by Votre and coworkers. It was found that loss of pregnancy was caused by low level of B₁₂ and its prevalence was around 38.4% (Chandyo et al., 2017).

Sufficient amount of B₁₂ is extremely critical for pregnancy, healthy cell division and normal placental function. Maternal B₁₂ deficiency enhances the risk of many pregnancy complications (Garima et al., 2016; Chandyo et al., 2017). The higher incidences of infertility, spontaneous abortion, preterm and low weight newborns are the key symptoms for B₁₂ deficiency that ensure the B₁₂ supplementation for women (Scolamiero et al., 2014).

Insufficient concentration of B₁₂ or deficiency of B₁₂ induces faulty oocyte production, state of homocysteinemia and defective placentation which lead to recurrent pregnancy loss (Sawant, 2015). Besides, cell growth and replication of fetus and placenta depends on maternal B₁₂ status therefore its deficiency may cause neural tube defects, intrauterine growth retardation, preeclampsia and early miscarriage (Vanderjagt et al., 2011) and pre-term birth. Both folate and B₁₂ are strongly correlated with initial stages of embryogenesis (Garima et al., 2016). Furthermore, low B₁₂ concentration during pregnancy has a strong connection with obesity and insulin resistance in adulthood (Ramirez-Velez et al., 2016).

Malnutrition has the potential to impair every part of the neurological system. A lack of vitamin B₁₂ results in a spinal cord lesion or subacute combined degeneration, in which a demyelination process causes the damage to myelin sheath in the dorsal and lateral columns. Hypomethylation, phospholipid metabolism, and the neurotoxic effects of homocysteine might all be involved in the pathways of B₁₂ induced neuropathy. However, distinguishing vitamin B₁₂ deficiency-related polyneuropathy from cryptogenic sensory polyneuropathy (CSPN) might be challenging clinically. Histopathological investigations have revealed that central nervous system myelin is broken down and vacuolized in B₁₂ deficient situations. In vitamin B₁₂ deficiency induced polyneuropathy, axonal neuropathy is observed in nerve biopsies and nerve conduction

investigations, in contrast to the demyelinating features present throughout the spinal cord. Various risk factors for B₁₂ deficiency are shown in figure 1.

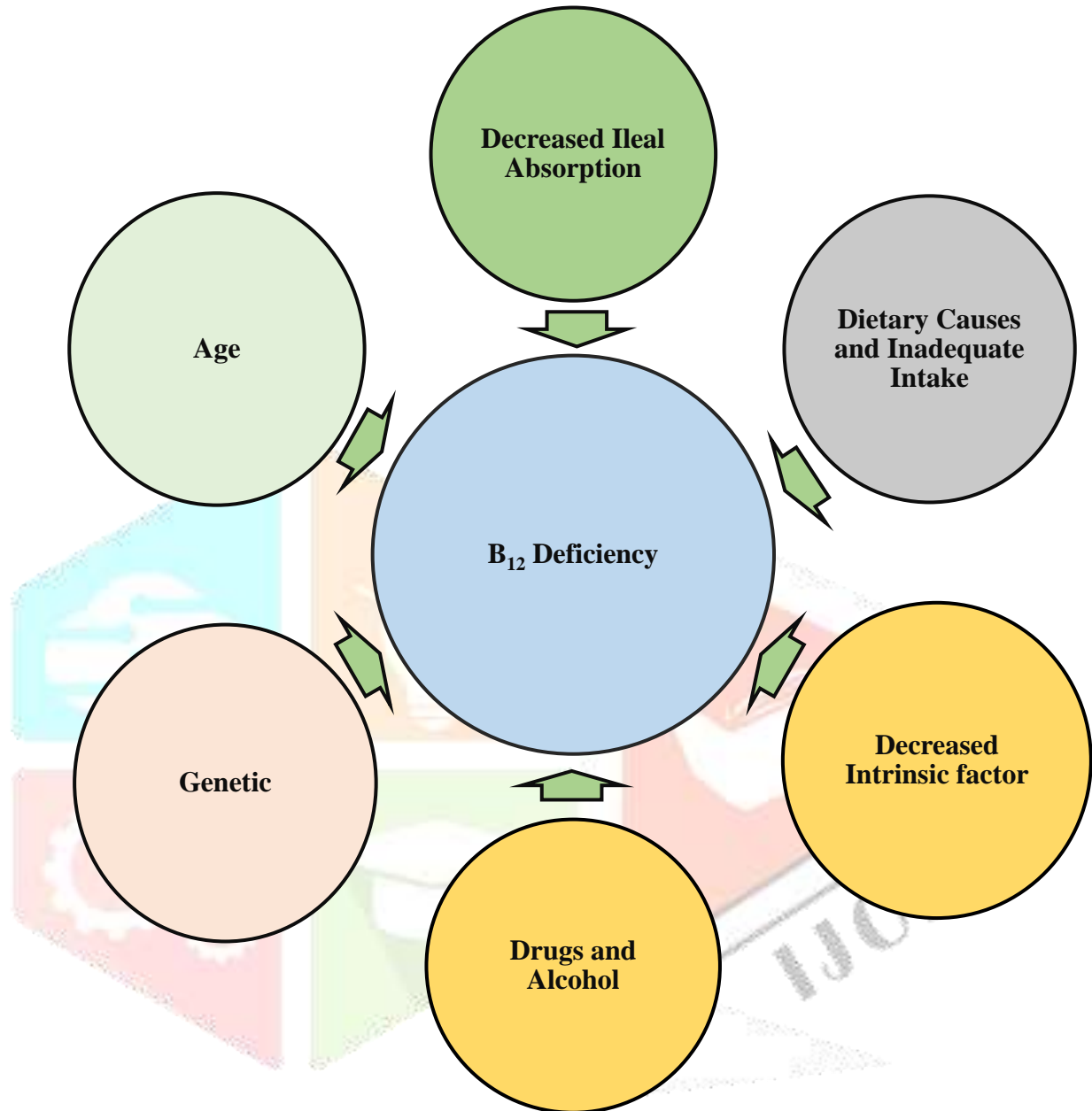


Figure 1- Various risk factors for B₁₂ deficiency. There are several risk factors that can lead to B₁₂ deficiency in human body.

B₁₂ is indispensable for metabolism of all cells in human body. In B₁₂ deficiency, multi organ systems can be affected, and hence, B₁₂ deficiency is associated with wide spectrum of clinical manifestations. Clinical manifestations of B₁₂ are nonspecific and highly variable and a single and unique clinical feature cannot be applicable for B₁₂ deficiency in all patients (Wong, 2015). Hematological and neuropsychiatric manifestations are considered to be most significant symptoms of B₁₂ deficiency. Peripheral neuropathy, demyelination and nerve damage, paraesthesia, numbness, memory loss, irritability, psychosis, dementia, megaloblastic anaemia, pancytopenia, glossitis, stomatitis and mild jaundice are very common symptoms associated with

B₁₂ deficiency (Langan and Zawistoski, 2011). B₁₂ deficiency induces severe oxidative stress which induces the oxidative damage of various biomolecules and cellular components (Bito et al., 2017). Protein glycation and oxidative stress leads to severe health complications in different diseases (Anwar et al., 2020). Superoxide dismutase (SOD) is an important antioxidant enzyme which counters the oxidative stress induced by reactive oxygen species (Anwar and Younus, 2017a, b; Khan et al., 2014; Anwar et al., 2014). B₁₂ deficiency has been reported to significantly reduce the activity of SOD (Bito et al., 2017). The interaction between B₁₂ and folate contributes to megaloblastic anemia that is noticed in the deficiency of both vitamins (Stabler, 2013). In a clinical study on type 2 diabetic patients, the low intake of folate and B₁₂ have been found to be linked with low serum levels of these two nutrients and hyperhomocysteinemia (Al-Maskari et al., 2012). General symptoms of B₁₂ deficiency are included in figure 2.

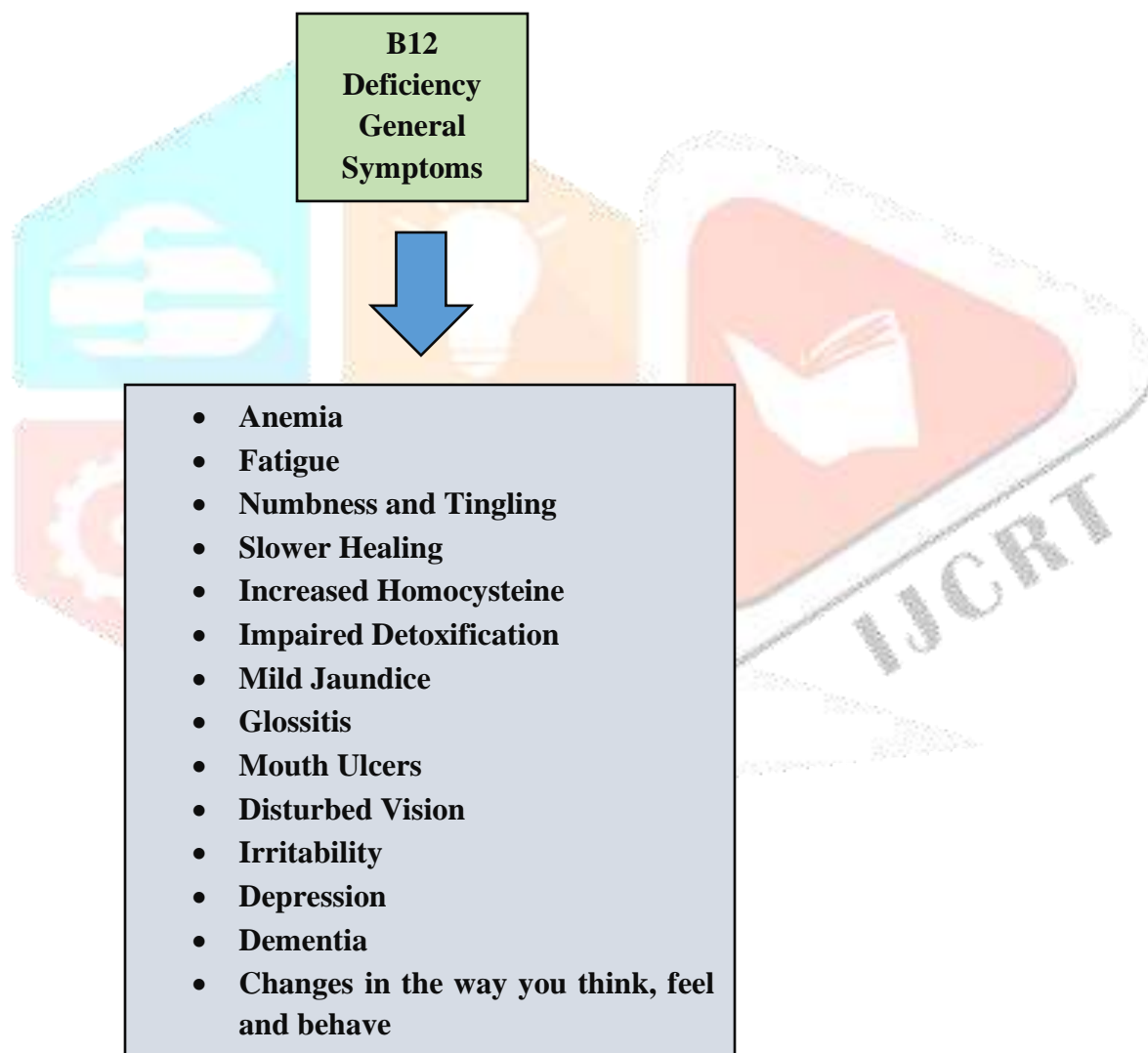


Figure 2- General symptoms of B₁₂ deficiency. After getting the B₁₂ deficiency, many symptoms are shown by human body.

Prevention and treatment of B₁₂ deficiency

Liver is one of the most important organs with multiple functions. It has an active role in metabolism (Al Matroodi et al. 2020; Rahmani et al. 2020). B₁₂ deficiency is not only limited to poor pregnancy outcomes, impaired physical and cognitive development and increased risk of morbidity in children but also affect national productivity and economics (Ramirez-Velez et al., 2016). There are several studies that proved the long term outcomes are positively related to duration and severity. Treatment regimes are always based on the cause. The treatment with supplementation should be started as soon as possible to overcome the adverse effects of B₁₂ deficiency because long term outcome is always linked with duration and severity of deficiency. It is a big challenge to provide or not provide the treatment to patients who have conflicting laboratory test results and clinical symptoms (Hvas and Naxo, 2006). A study done by Solomon suggests that treatments to patients with severe signs of B₁₂ deficiency, should be necessary as well as independent of clinical test results (Solomon, 2005).

According to WHO report, Estimated Average Requirement (EAR) is 0.2 µg/day for a normal adult woman. For a pregnant woman, EAR is suggested to be 2.2 µg/day and a Recommended Nutrient intake (RNI) is 2.6 µg/day in pregnancy because nutrients are never 100% absorbed. For a lactating woman, EAR results in 2.4 µg/day and RNI of 2.8 µg/day because they secrete 0.4 µg/day in their milk (Van Sande et al., 2013).

Diverse recommendations are suggested for initial and maintenance therapy of B₁₂ deficiency. To plan a successful strategy for treatment of B₁₂, decision on dose, route and form of B₁₂ to be used and determination of the need for continuous follow up are needed. Several form of B₁₂ including cyano, hydroxyl and methylcobalamin are available that can be employed. (Hvas and Naxo, 2006). To date, management of B₁₂ deficiency with intramuscular B₁₂ injections is very popular, but new routes of vitamin B₁₂ administration that include oral and nasal are being researched (Lane and Rojas-Fernandez, 2002).

Current clinical practice in UK recommends the treatment of B₁₂ deficiency with intramuscular hydroxocobalamin. Hydroxycobalamin is preferred over cyanocobalamin in correcting defects in B₁₂ metabolism because it has improved retention, greater availability to cells and no need of decyanation. For patients without neurological disorders, the standard initial therapy is set to be 1000 µg hydroxycobalamin by intramuscular injection thrice a week for two weeks while maintenance treatment for such patients is with hydroxycobalamin 1000 µg by intramuscular injections every 3 months. B₁₂ deficiency with neurological symptoms should be treated with 1000 µg hydroxycobalamin intramuscularly on alternate days until there is no further improvement (Devalia et al., 2014). The degree of recovery depends on the severity and duration of the neurologic abnormalities. It is very crucial to monitor the effect of treatment and the dose of treatment should be adjusted according to change in the concentration of urinary MMA, plasma homocysteine and red cell indices (Smith and Coman, 2014).

High dose oral B₁₂ therapy shows equal efficacy equal to that of intramuscular injections in the treatment of B₁₂ deficiency and has increased its popularity. A study with radioactively labeled oral B₁₂ has shown the evidence that 0.5 % to 4% of oral B₁₂ can be absorbed by passive diffusion in both normal controls and patients with pernicious anemia. Daily oral treatment with 2000 µg with parenteral therapy showed significant reduction in levels of methylmalonic acid and significantly increased levels of B₁₂ as well as maintained normal B₁₂ status (Hvas and Naxo, 2006). Another study comparing oral with intramuscular B₁₂ (1000 µg doses, daily for 10 days, then weekly for 4 weeks, and monthly thereafter) showed that two groups had similar improvements in B₁₂ status after 90 days. It is to remember that mild dietary deficiency can be treated with oral B₁₂ but introduction of animal foods alone is not sufficient.

B₁₂ deficiency and COVID-19 susceptibility

It has been shown that symptoms of post-COVID-19 patients are very similar to that of persons having B₁₂ deficiency. Hence, B₁₂ can be a possible factor in the poor health of these peoples. Weak immunity and poor socioeconomic status can be an important factors determining the vulnerability to COVID-19. Further, elder peoples, pregnant ladies, infants, those with underlying diseases, and those living in long-term care units are more vulnerable to Coronavirus. Decreased immunity is a significant factor for the infection in these peoples. B₁₂ had been accounted for to have a significant role in cellular immunity, particularly with natural killer cells as well as CD8⁺ cells. Further, B₁₂ goes about as immunomodulator for cellular immunity. B₁₂ has been known to assist immune system to work properly (Dehghani-Samani et al., 2020). As a result, the deficiency of B₁₂ may lead to inappropriate immune response. Therefore, it is possible that B₁₂ deficiency is a potential modifiable risk factor for the susceptibility of COVID-19 infection (Wee, 2020).

Undiagnosed vitamin B₁₂ deficiency can increase the risk of death from COVID-19. B₁₂ deficiency not only suppresses the immune system, making it difficult to fight infection and develop antibodies, but it also causes hyperhomocysteinemia (HHcy), which can lead to harmful blood clot (i.e. myocardial infarction, pulmonary embolism, stroke, and deep vein thrombosis). Hyperhomocysteinemia is an independent and important contributing risk factor for vascular disease. The studies have concluded that extreme COVID-19 attributes to vascular disease that affects the endothelium's cells and lining. In addition, hyperhomocysteinemia leads to blood vessel inflammation, thrombosis, as well as vascular and endothelial damage. A person having both COVID-19 and B₁₂ deficiency is at the greater chances of death (Sally Pacholok, 2020).

Moreover, B₁₂ metabolism becomes impaired due to SARS-CoV-2. Additionally, B₁₂ deficiency and COVID-19 infection share several symptoms together including coagulation cascade activation, elevated oxidative stress, and lactate dehydrogenase, hyperhomocysteinemia, renal and pulmonary vasculopathy, and vasoconstriction (Grange et al., 2015; Sabry et al., 2020). Likewise, B₁₂ deficiency can bring about disorders of the central nervous systems, respiratory, and gastrointestinal systems (Shakoor et al., 2021).

As discussed previously, B₁₂ is very crucial for the synthesis of red blood cells, healthy nervous system, formation of myelin, cellular growth, and the quick synthesis of DNA (Lopes, 2020). Further, B₁₂ works as a modulator of gut microbiota. The low concentration of B₁₂ are involved in increased inflammation, ROS generation, and oxidative stress. Further, B₁₂ is indirectly involved in activation of platelet and coagulation cascades, endothelial dysfunction, disruption of myelin sheath integrity, megaloblastic anemia, and decreased immune responses. However, B₁₂ treatment is reported to lessen oxidative stress. B₁₂ further improves circulation, and it works as analgesic, and anti-inflammatory agent. As a result, it may likely diminish the damage of COVID-19 patients (Shakoor et al., 2021). Besides, the study based on early computer modelling and laboratory-based research has indicated that vitamin B₁₂ may have the potential to bind with at least one of the viral proteins, and thereby B₁₂ is supposed to slow down viral replication. In a recent study, B₁₂ supplements was found to possibly diminish organ damage associated with COVID-19 and related side effects (dos Santos, 2020). A cohort study was performed in Singapur to evaluate the effect of vitamin B₁₂ along with magnesium and vitamin D on progression to severe outcome in older COVID-19 patients. This study showed that supplementation of B₁₂ (500 µg), vitamin D (1000 IU), and magnesium to patients having COVID-19 had diminished severity of COVID-19 in older patients. Further, these supplements were also found to decrease the requirement for oxygen and support of intensive care (Shakoor et al., 2021).

Pregnant women or recently pregnant women having obesity, hypertension, and diabetes are supposed to be at higher risk of developing severe COVID-19. Pregnancy-related physiological alterations have a direct effect on the immune system, nervous system, cardiovascular function, and coagulation. These can have an effect on the development of COVID-19 disease in either a positive or negative way (Wastnedge et al., 2021). There is a chance of women contracting respiratory problems, heart rhythm abnormalities, or acid-base imbalance (Lopes et al., 2020). Therefore, it is very important that pregnant must have some additional protections against COVID-19. It is advised that the occurrence of possible symptoms (like fever, cough or difficulty in breathing) must lead to contact to their healthcare provider. Some researchers have suggested that the third trimester might be the most vulnerable time for infection. Therefore, additional investigation are required in order to trigger monitoring programs at the end of the second trimester. During this pandemic, it is critical to control pregnant women before and after birth, as well as their children (Salem et al., 2020).

Narayanan and Nair have suggested that methylcobolamin may inhibit the association between RNA and incoming NTP. Thus, methylcobolamin has a possible potential to inhibit RNA-dependent-RNA polymerase activity of COVID-19-nsp12 enzyme. Therefore, lower viral titres and reduced disease morbidity can be obtained due to inhibition of this enzyme. It is suggested that the ability of methylcobalamin to inhibit nsp12 protein, and hence, viral replication can be a possible an effective approach in COVID-19 treatment (Narayanan and Nair, 2020). A case report has suggested that recovered COVID-19 patients are more vulnerable to B₁₂ deficiency. Therefore, a regular test of B₁₂ deficiency in such cases is highly recommended and subsequent treatment for its deficiency is very necessary to avoid further worsening. Further, it was

concluded in this case report that B₁₂ therapy may be very beneficial in the recovery of COVID-19 patients (Alshammari, 2021).

Role of vitamin B complex in COVID-19 treatment

There are several evidences that indicate the link of vitamin B with immune system. Thiamine has been documented to improve immune system and have been reported to decrease the risk of cardiovascular disease, diabetes, kidney disease, aging-related disorder, cancer, neurodegenerative disorders, and mental disorders. It has well known that thiamine deficiency can lead to insufficient antibody responses. Since, antibodies, and importantly T-cells, are needed to fight and eradicate the SARS-CoV-2 virus. As a result, sufficient levels of thiamine likely to help with proper immune responses against SARS-CoV-2 infection (Shakoor et al., 2021).

Riboflavin and UV light have the potential to cause irreversible damage to nucleic acids like DNA and RNA. Infectious titer of SARS-CoV-2 in human blood, plasma, and platelets has been reported to be decreased by the use of riboflavin-UV (Shakoor et al., 2021; Keil et al., 2020). Recently, it has been suggested that inflammatory storm in COVID-19 patients can be controlled by targeting IL-6. Niacin as a building block of NAD, has a significant role in decreasing the levels of pro-inflammatory cytokines including IL-1, IL-6, and TNF. Furthermore, niacin has been shown to have anti-inflammatory effect, and to decrease neutrophil infiltration in patients having lung damage induced by ventilator, niacin decreases and has an anti-inflammatory effect. Considering lung-protective, and immune-boosting abilities of niacin, it is suggested to be beneficial as adjunct treatment for COVID-19 patients (Shakoor et al., 2021).

Deficiency of vitamin B₆ (pyridoxine) results in immune dysregulation. It has been suggested that COVID-19 patients with high inflammation might have deficiency of this vitamin. The peoples having diabetes and cardiovascular disease or elder peoples have been found to be deficient with pyridoxine, and are included in high risk group for COVID-19 (Merigliano et al., 2018; Shakoor et al., 2018). Besides, COVID-19 patients have been noticed with immune dysregulation and at increased risk of coagulopathy (Desbarats et al., 2020). In a recent study, it was found that supplementation of pyridoxal 5'-phosphate (PLP) controlled immune responses, decreased pro-inflammatory cytokines, preserved endothelial integrity, and avoided hypercoagulability in COVID-19 patient. Moreover, vitamin B₆ (along with B₂ and B₉) was shown to increase the production of IL-10 that is a potent immunosuppressive and anti-inflammatory cytokine. IL-10 has the ability to deactivate macrophages and monocytes while inhibiting antigen-presenting cells and T cells (Mikkelsen et al., 2019). COVID-19 patients have been reported to often mount an aggressive T cell response and secretion of pro-inflammatory cytokines in response to the virus. Therefore, it is possible that PLP can help to reduce the cytokine storm as well as inflammation as experienced by some COVID-19 patients (Shakoor et al., 2021).

Folate is a vitamin that is needed for DNA and protein synthesis as well as the adaptive immune response. Further, folate has been found to inhibit an enzyme known as furin. Furin is an enzyme that has been linked to viral and bacterial infections. Hence, most of treatment therapies for infection control are based on inhibition of furin. Recently, folic acid has been shown to inhibit furin, blocking the binding of SARS-CoV-2 spike protein, and thereby restricting cell entry, and virus turnover (Sheybani et al., 2020). On the basis of structure-based molecular docking, researchers have discovered that folic acid and its derivatives, tetrahydrofolic acid and 5-methyl tetrahydrofolic acid have strong and stable binding affinities against the SARS-CoV-2. As a result, folic acid can be suggested to be used as a preventive solution for COVID-19 treatment (Shakoor et al., 2021).

Discussion

So far, the general trend of this pandemic has involved an increase in COVID-19 cases, with a jump in the summer and a greater one in the autumn. Even so, a second wave of elevated infections has been observed in areas that suffered a surge shortly after the outbreak began, accompanied by a decline in the number of cases. Human activity is the most important aspect for elevated number of infections. Further, a large proportion of peoples is less stringent in taking COVID-19 precautions, including physical separation, hand-washing, and mask use. The cases are increasing in places, where less people wear masks, and more people assemble indoors for feed, drink, religious rites, celebrate, and socialize, including with families (Maragakis, 2020).

Two mRNA vaccines, BNT162b2 (Pfizer-BioNTech), and mRNA-1273 (Moderna) have been approved by Food and Drug Administration (FDA) in December 2020 for emergency use and are reported to be 95% and 94% efficacious, respectively after completion of two doses. In February 2021, the FDA granted an EUA for Ad26.COV2.S (Johnson & Johnson/Janssen) which is a human adenovirus type 26 (Ad26) vectored vaccine and it is 66% effective after a single vaccine dose. BNT162b2 can be given to people over the age of 16, while mRNA-1273 and Ad26. The lack of effective antiviral agents and the shortage of vaccine for COVID-19, challenges to health system due to severity of COVID-19 is forcing urgency to search new valuable therapeutic strategies to boost the immune system, antioxidant defense and antiviral system of the body. For this purpose, several nutritional interventions for viral infections are recommended that include multivitamins including C, D, A, B complex, zinc, selenium, omega-3-fatty acids, and plant polyphenols. Besides, frequent hydration is suggested to be very important. COV2.S can be given to people over the age of 18 (WHO, 2021).

There are many preliminary reports indicating the role of robust immune response in recovery of COVID-19 patients. Several studies have concluded that pregnant women are more vulnerable to respiratory infections. As a result, they could be more prone to COVID-19 infection as compared to general population. Furthermore, the normal immune responses that arise during pregnancy, as well as the potential complications

from the cytokine storm triggered by COVID-19 infection, placed COVID-19 infected pregnant women at risk of severe morbidity and even death (Liu et al., 2020). The alterations in hormone levels, and decreased lung volumes due to a gravid uterus, and slightly immunocompromised state may increase susceptibility towards more rapidly worsening clinical path and a higher threat of injury to both mother and fetus. During pregnancy and lactation, the requirement for B₁₂ increases and B₁₂ needs during pregnancy are very high that it is not fulfilled through diet alone (Siddiqua et al., 2014). It is very necessary to protect women of reproductive age from B₁₂ deficiency for maternal and fetal wellbeing. Poor maternal B₁₂ status is related with many problems in offspring (Jeruszka-Bielak et al., 2017).

Hyperglycemia has been reported to be an important contributor to severity of SARS-CoV-2. Although DM may not raise the likelihood of COVID-19 infection, it can affect its outcome and severity. According to epidemiological research, because to COVID-19, DM increases the risk of hospitalization, critical care utilization, and death rate (Mahluji et al., 2021). Metformin has been demonstrated to affect calcium-dependent intestinal absorption of vitamin B₁₂, which may be reversed with calcium supplementation. Thus, metformin-related vitamin B₁₂ deficiency should be carefully observed in COVID patients and B₁₂ deficiency must be investigated as a probable confounder in COVID-19's severe effects on the elderly and people with diabetes (Wee et al., 2021).

T cells, B cells, the complement system, and phagocytes are the four members of the immune system. The immune system is divided into two parts: innate immunity and adaptive immunity. These help to keep the body well by warding off infections. A well-balanced diet and nutrition strengthen the immune system. Vitamin B₁₂ as a cofactor for methionine synthase plays a significant role for the synthesis of purines and pyrimidines in all cells, including fast-dividing immune cells. Most specialists intuitively consider the possibility of B₁₂ deficiency causing immune system dysfunction and thus a putative cause of COVID-19 susceptibility. Unfortunately, there are not too much studies supporting the use of cobolamin in reducing the risk of complications associated with COVID-19 infection.

In a recent study, post-COVID syndrome was evaluated among the survivors of COVID-19. Among the symptoms were nausea, cough, arthralgia, dyspnea, myalgia, and cognitive disturbances. Furthermore, severity of COVID-19 was reported to be associated with lung fibrosis, hypercoagulability leading to venous thrombosis and embolism, and kidney damage. Anemia is characterized by fatigue, post-exercise malaise, dyspnea, and cognitive dysfunction. Iron supplementation improves subjective fatigue and quality of life (Garg et al., 2021). There is, however, a connection between vitamin B₁₂ deficiency and elevated levels of fatigue and depression (Huijts et al., 2012). A healthy diet has been found to have a positive considerable effect on COVID-19 recovery rates. Inflammatory symptoms towards homeostasis can be counteracted by specific medication therapies and/or healthy lifestyles like proper diet and regular exercise. Further supplementation of micronutrients including multivitamins, minerals like iron, zinc, and selenium, and antioxidants is highly recommended in treatment therapies of COVID-19. Further, we recommend to

determine the response of COVID-19 patients to specific diets having all nutrients like vitamins, antioxidants and mineral elements in future studies. Further, their long term effect on lung function abnormality, pshycological impairment, depression, and post-traumatic stress disorders are also suggested to be studied in peoples recovered from COVID-19.

5. Conclusion

A proper nutritious diet is considered to be very crucial for an optimal immune response. A diet that is poor in nutrients or is deficient in these nutrients may lead to disease burden. There are evidences suggesting the role of nutrients deficiency in the development of COVID-19. As a result, we believe that that nutritional deficiencies might be involved in the onset of COVID-19, and worsen its severity. Vitamin B not just to supports throughout the development and maintenance of a healthy immune system, but it could also help to prevent or suppress COVID-19 symptoms and cure SARS-CoV-2 infection. B₁₂ is known to be beneficial for health system because it downregulate pro-inflammatory cytokines and inflammation, helps in the reduction of breathing difficulties and gastrointestinal complications, protects from hypercoagulability, and and shortens the hospital stay for COVID-19 patients. B₁₂ (cobalamin) deficiency is linked with several health complications. Therefore, we suggest that supplementation of cobolamin along with other nutrients like multivitamins, zinc, folate, selenium etc., to COVID-19 patients to reduce the symptoms and to treat the disease. Further, we suggest to do extensive clinical research in this regards as well as long term effect of B₁₂ on post-COVID symptoms.

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All authors declare that they have no conflicts of interest.

Ethical approval

In the writing of this review, we did not conduct any experiment. We just used the data from different publication. We provided the references. Hence, we did not any ethical approval.

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