Deficient synthesis of melatonin in COVID-19 can impair the resistance of coronavirus patients to mucormycosis

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Abstract

Though it is thought that uncontrolled diabetes and the excessive use of corticosteroids are responsible for COVID-19 associated mucormycosis (CAM), researchers are on the lookout for additional reasons to explain the recent spurt of CAM in India. In the present paper it is argued that melatonin deficiency in COVID-19 plays a major role in CAM. Incidentally, melatonin is synthesized from tryptophan via the serotonin pathway and melatonin deficiency in COVID-19 arises from the faulty absorption of tryptophan from the food because SARS-COV-2 downregulates angiotensin-converting enzyme-2, which is the chaperone of the transporter of tryptophan, a key component in the process of uptake of tryptophan. The melatonin deficiency enhances the fungal virulence by facilitating iron acquisition and by promoting morphological transition of the mucor species from the yeast to the virulent hyphal form. Additionally, melatonin deficiency aggravates the suppression of T-cell immunity in the patients receiving steroids. Hence, the restoration of melatonin level should resolve the issues and help in defeating CAM, given the fact that melatonin is an iron chelator, inhibitor of myeloperoxidase, inhibitor of ferroptosis and pyroptosis and calmodulin blocker. Also, by lowering the expression of glucose-regulated protein-78, melatonin can further increase the resistance of diabetic patients to mucormycosis. Hence, clinical trials should be carried out to ascertain how tryptophan supplementation, administration of selective serotonin reuptake inhibitors (to increase serotonin, the precursor of melatonin), and exogenous melatonin help in correcting the melatonin deficiency and eliminating or reducing the propensity of the patients to CAM.

Keywords: Coronavirus, COVID-19, Mucormycosis, Melatonin, Tryptophan, ‘Black fungus’

Introduction

Mucormycosis (earlier called Zygomycosis) is a potentially fatal opportunistic infection caused by the fungi of the order Mucorales, which are commonly found in the soil and decomposing organic matters. The major risk factors of mucormycosis include uncontrolled diabetes mellitus (ketoacidosis and, in general, acidosis of any aetiology), immunosuppression, trauma and burn, use of corticosteroids, neutropenia, organ transplantation, multiple blood transfusion, chemotherapy and deferoxamine therapy in hemodialysis [1-3]. The higher incidence of mucormycosis in India is probably because of the fact that around one in six people with diabetes in the world is from India and around 82% of mucormycosis patients are diabetic [4-6].

Incidentally, a spike in Covid-19 associated mucormycosis (CAM) has recently been observed in India [7] and approximately 71% of the global cases of CAM are from India [8]. The higher incidence of CAM in India has been primarily attributed to uncontrolled diabetes and the suppression of immunity from the excessive use of steroids [5]. Other COVID-19 associated fungal infections like aspergillosis and candidiasis have also been reported, though in relatively small numbers [9-10].

Interestingly, mucor species are dimorphic and exhibit either yeast (unicellular) or virulent hyphal (multicellular) growth depending upon the conditions. Normally, aerobic condition favours the growth in the hyphal form [11]. In the invasive form, the hyphae are attached to the endothelium through coupling of glucose-regulated protein (GRP78, an endothelial cell receptor) on the host and spore coat homolog (CotH) protein on the fungus and eventually, the disease may manifest as rhino-orbital, rhino-orbital-cerebral, pulmonary or disseminated mucormycosis [8, 12, 13]. Mucormycosis infection is characterized by extensive angioinvasion leading to vessel thrombosis and tissue necrosis and is often presented with black necrotic lesions (that is why mucormycosis is popularly known as ‘black fungus’), and the spread and severity of the infection are controlled by the virulence factors of the fungi, the factors which increase their virulence in the host. One of the major virulence factors [1, 3, 12, 14] of the mucor species is the iron acquisition system, the system of ‘stealing’ iron from the host, given the fact that iron is an essential element for the survival of most of the microbes. Indeed, it has been found that fungi undergo apoptosis in iron-deprived conditions [3,14]. Accordingly, the strategy of limiting iron availability to the mucor species is an important host defense mechanism (nutritional immunity), and serum and other biological fluids are normally

fungistatic but iron addition abolishes this benefit [15]. In humans the predominant forms of iron present are sequestered/complexed with proteins such as transferrin in the circulating fluid (extracellular iron) and heme and ferritin in the cells (intracellular iron). A small amount of free, redox-active and ligand-exchangeable iron (labile iron pool) also exists as a transitory pool to regulate iron metabolism and homeostasis, though any excess free iron is rapidly sequestered [1, 3, 12]. Interestingly, fungi have developed three mechanisms [1, 3, 14-16] of iron acquisition from the host for their survival: a) reductive iron uptake, b) siderophore mediated uptake and c) uptake from heme by heme-oxygenase (by degrading heme) or by using hemophores. Reductive iron uptake from ferric chelates has three steps - reduction of ferric iron to soluble ferrous form by ferric reductase, re-oxidation to the ferric form by ferroxidase and transport into the cell by permease. On the other hand, siderophores/hemophores are low molecular weight proteins which can chelate iron by grabbing it from the protein-bound iron of the host as the iron affinity of some siderophores/hemophores can be higher than that of the sequestered iron in the host.

Given the fact that diabetes is a perennial problem in India and the long-term use of steroid is not uncommon as a large number of people suffer from arthritis and other chronic inflammatory diseases, it has been suggested by some researchers that in addition to uncontrolled diabetes and excess use of steroid, other factors like the use of zinc supplements, contaminated water in humidifiers, impure oxygen etc. may also be responsible for the spike in CAM [5, 17], though the jury is still out.

In the present paper, it is proposed that other than uncontrolled diabetes and compromised immunity from steroid use, melatonin deficiency arising per se in COVID-19, makes the coronavirus patients susceptible to mucormycosis. It is argued that impaired synthesis of melatonin in SARS-COV-2 patients significantly influences the virulence factors of the fungi in favour of them.

How does melatonin synthesis dip in COVID-19?

The formation of melatonin begins with the absorption of tryptophan (an essential amino acid) from the food. After absorption, tryptophan is first converted to 5-hydroxytryptophan (enzyme: tryptophan hydroxylase), then to serotonin (enzyme: decarboxylase) followed by N-acetylserotonin (enzyme: aralkylamine N-acetyltransferase, AANAT) and finally to melatonin

(enzyme: acetylserotonin O-methyltransferase, ASMT) [18]. Two enzymes (AANAT and ASMT), found primarily in the pineal gland, transform serotonin to melatonin (mostly in the darkness) and the synthesized melatonin is released from the pineal gland into the bloodstream mainly during nighttime. Incidentally, many other extrapineal sites like cerebellum, retina, skin, gastrointestinal tract, immune cells, thymus, thyroid, bone marrow, liver, spleen, kidney, lungs, pancreas, heart and airway epithelia also generate melatonin, some even in larger quantities than the pineal gland, though most of the extrapineal melatonin is not integrated into the circulation [19, 20].

Interestingly, in the synthesis of melatonin, the very first step of absorption of tryptophan from the food is not so straightforward. The uptake of tryptophan needs a transporter (B^0AT1, broad neutral (0) amino acid transporter 1), where ACE2 (Angiotensin-Converting Enzyme 2) performs a key role as a chaperone (aids in protein folding) for B^0AT1 [21]. As SARS-COV-2 is known to downregulate ACE2 by docking on it, the absorption of tryptophan should be significantly reduced in coronavirus patients. Hence, tryptophan deficiency in COVID-19 is expected to give rise to serotonin deficiency [22], and consequently, to melatonin deficiency.

How is the iron acquisition system of mucor species modified in COVID-19?

As iron is an essential material for the survival of fungi, there is a competition between the pathogens and the host for the same, and as discussed earlier, the host iron is almost completely sequestered and a little free iron (labile iron pool) is available for the pathogens, which is normally insufficient for their growth and virulence. However, if the disease per se increases the free iron level in blood, it becomes easier for the pathogens to gain entry into the host and grow. Indeed, COVID-19 creates such favourable conditions for the growth and invasion of the mucor species.

Incidentally, high serum ferritin can play an important role in increasing the plasma free iron. Though ferritin is known to store iron intracellularly, a small amount of ferritin is also available in the serum, which increases significantly (hyperferritinemia) in case of iron overload and, more importantly, in inflammation of any etiology as an acute phase reactant [23]. The serum ferritin is of light-chain variety (molecular weight 19 kilodalton, in contrast to the intracellular heavy-chain variety of molecular weight 21 kilodalton) and during inflammation, serum ferritin may be produced from hepatocytes, Kupffer cells, proximal tubular renal cells and

macrophages. Also, exaggerated synthesis or secretion of ferritin has been reported to occur from various stimuli like pro-inflammatory cytokines, prostaglandins, reactive oxygen species (ROS), oxidants, growth factors and hypoxia [23]. As COVID-19 is an inflammatory disease, high serum ferritin is quite common in coronavirus patients [24]. In such inflammatory states, mainly three sources operate to elevate the plasma free iron (labile plasma iron, LPI). First, during inflammation when low molecular weight serum ferritin is formed from high molecular weight intracellular ferritin, the latter releases a substantial portion of its inner core iron, which increases the free iron level in the blood [23, 24]. Second, inflammation reduces RBC half-life, which leads to macrophage-mediated RBC phagocytosis and eventually, an increase in the free iron in the plasma [25, 26]. Third, free iron is also generated from myeloperoxidase (MPO) induced degradation of heme [25]. MPO is an enzyme stored in the neutrophils and macrophages and is released into the extracellular fluid during inflammation. Interestingly, the excess free iron thus produced can assist in further raising the plasma free iron by generating highly reactive hydroxyl radical ($HO^{\bullet}$) via Fenton reaction from H_2O_2 and Fe^{2+} and/or via Haber-Weiss reaction from H_2O_2 and superoxide anion ($O_2^{\bullet -}$) in presence of Fe^{2+}/Fe^{3+}, resulting in the $HO^{\bullet}$ induced damage of cellular proteins, lipids (lipid peroxidation) and nucleic acids [26, 27]. The process can lead to ferroptosis, a type of inflammatory programmed cell death with accumulation of lipid peroxides arising from degradation of lipids in presence of free iron and ROS. Ferroptosis results in aggravation of the inflammation [28, 29] and, in effect, further generation of inflammation induced free iron in the plasma. Hence, in coronavirus patients the labile plasma iron increases by various means and the mucor species take advantage of this easily available free iron for their growth and virulence.

How can fixing melatonin deficiency restrict iron uptake by the mucor species?

Melatonin, a multifunctional neuroendocrine hormone, is celebrated for its anti-inflammatory (with some useful pro-inflammatory characteristics), antioxidant, anti-aging, analgesic, free radical scavenging and oncostatic effects [19, 20]. Melatonin modifies circadian rhythm, mood, sleep, appetite, reproduction, immune responses, cardiac functions etc. in receptor independent/receptor dependent pathways [19, 20, 30]. In the present context, melatonin has multiple roles in containing the plasma free iron. First, melatonin helps in reducing plasma free iron in SARS-COV-2 patients by chelating excess iron (both Fe^{2+} and Fe^{3+}) as melatonin is an effective metal chelator [31, 32]. Second, melatonin, being a potent anti-inflammatory agent, can help in shrinking the labile iron pool by i) limiting free iron generation from destruction of

heme by blocking [25] the enzyme myeloperoxidase (MPO), ii) restricting free iron production from inflammation induced RBC degradation [25, 26] and iii) lowering serum ferritin [33, 34], which is involved in elevating plasma free iron. Melatonin ameliorates inflammation by blocking pro-inflammatory cytokines such as interleukin IL-1β, IL-2, IL-6, tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [18, 19, 30, 35]. Melatonin also restrains inflammation by inhibiting prostaglandin synthesis, production of adhesion molecules, generation of pro-inflammatory cytokines from lipopolysaccharides (LPS) and by downregulating cyclooxygenase and NLRP3 inflammasome [18, 19, 30, 35]. Inflammasomes, which are intracellular multiprotein complexes, act as pattern-recognition receptors (PRRs) to sense pathogens and are triggered by various stimuli like pathogens, endogenous cytokines and damaged cells [35, 36]. NLRP3 mediated activation of nuclear transcription factor-kappa B (NF-κB) leads to pyroptosis, a type of programmed cell death arising from infection induced activation of inflammasomes and release of pro-inflammatory cytokines [35, 36]. Pyroptosis gives rise to exaggerated inflammation in COVID-19, and melatonin can block pyroptosis by antagonizing NF-κB and NLRP3 signaling [35, 36]. In addition to lowering proinflammatory cytokines and scavenging ROS and H_2O_2 [31], melatonin, being an inhibitor of ferroptosis [37], can lessen ferroptosis induced inflammation in coronavirus patients [29]. Hence, melatonin by chelating excess free iron and by ameliorating inflammation in multiple ways can reduce labile plasma iron and can force the mucor species to starve and, as expected, melatonin deficiency in COVID-19 deprives the coronavirus patients of the aforesaid benefits and makes them susceptible to mucormycosis.

In this context, it may be noted that zinc is also an essential nutrient for the fungi as zinc is the cofactor of several enzymes, however, in CAM, any role of excess zinc in the virulence of the mucor species may possibly be ruled out as melatonin deficiency per se leads to the lowering of zinc level in blood [38] and a significant proportion of coronavirus patients is zinc deficient and zinc deficiency leads to increased severity of the disease [39].

How can restoration of melatonin level reduce the likelihood of morphological transition of the mucor species from the yeast to the virulent hyphal form?

Another critical factor in the virulence of mucor species is the switching of their mode of growth from the yeast (unicellular) to the virulent filamentous hyphal (multicellular) form [3, 14]. Calcineurin, an important fungal regulator of calcium homeostasis and signaling, is

responsible for the transition of the mucor species from the yeast to the tissue invasive hyphal form [11, 14, 40]. Calcineurin is a Ca^{2+} and calmodulin-dependent serine/threonine protein phosphatase, where calmodulin (calcium-modulated protein) is a sensor protein that transduces calcium signals into appropriate outputs through calmodulin-binding proteins (like calcineurin), calmodulin-dependent protein kinases, and histone deacetylases [11, 40]. When calcineurin function is inhibited, growth shifts to the less virulent yeast form [11, 40]. Any material that blocks calmodulin is also expected to show antifungal activity [40, 41]. Incidentally, melatonin is a calmodulin inhibitor [41] and hence, can diminish the fungal virulence by restraining the transition of mucor species from the yeast to the virulent hyphal form.

How can melatonin further reduce the susceptibility of diabetic patients and patients receiving steroids to mucormycosis?

It is known that acidic environment helps in the growth of the mucor species as acidosis temporarily disrupts the capacity of transferrin to bind iron owing to proton mediated dissociation of iron from transferrin [13, 42] resulting in the generation of free iron. In uncontrolled diabetes, ketoacidosis generates excess free iron and produces the right environment for the proliferation of mucor species, where melatonin has a prominent role in chelating the free iron and making it unavailable to the mucor species.

Also, the expressions of GRP78 (endothelial cell receptor on the host) and CotH (receptor on the fungal surface) are increased in the presence of high glucose and iron, further increasing the propensity of diabetic patients to mucormycosis [13, 43]. Melatonin provides an added advantage to the diabetic patients by lowering the GRP78 expression [43] and, hence, attenuating the host-fungus coupling. Melatonin also lowers pyroptosis induced endothelial cell damage [35] and hence, restricts easy invasion of the mucor species through the damaged regions of the endothelial cells. In addition, melatonin deficiency is more harmful to the diabetic patients as they have lower blood melatonin than healthy people [44].

Glucocorticoid like dexamethasone is known to suppress immunity mainly by inhibiting IL-2 mediated T-cell proliferation. Incidentally, melatonin has been found to enhance T-cell associated immune responses and reduce dexamethasone-induced immunosuppression [45]. It has been found that T-cells have a major role in developing immunity against fungi [46] and

hence, the restoration of melatonin level in coronavirus patients is expected to lessen the steroid induced immunosuppression as well as susceptibility to mucormycosis.

How to correct melatonin deficiency in SARS-COV-2 patients

The preferred way to treat the melatonin deficiency is to restore the tryptophan level to normal by supplementation so that the serotonin level and consequently, the melatonin level go up [22]. In case of severe COVID-19, when there is hardly any absorption of tryptophan, parenteral supplementation of tryptophan may be considered. It should be noted that in SARS-COV-2 patients selective serotonin reuptake inhibitors (SSRIs) may not significantly raise the level of serotonin, the precursor of melatonin, as SSRIs do not act well when there is tryptophan deficiency in the system [22]. On the other hand, exogenous melatonin should help in correcting the melatonin deficiency, though keeping the serotonin deficiency unchanged. As exogenous melatonin has a short half-life (1-2 hours), different formulations like extended-release and combined immediate and extended-release may be better options for fixing the melatonin deficiency [47]. It may be noted that exogenous melatonin is safe and some mild side effects like daytime sleepiness, headache, nausea and drowsiness have been reported in case of higher doses and extended-release formulations [47]. Hence, clinical trials (prophylactic as well as therapeutic) of tryptophan supplementation, SSRIs (excluding any combination of tryptophan and SSRI to guard against serotonin syndrome [22]) and exogenous melatonin should be conducted on coronavirus patients to find out how they perform in saving the patients from the deadly consequences of mucormycosis.

In this context, it is worthy to note that exogenous melatonin has been touted by many as the silver bullet for COVID-19 and using network medicine methodologies along with clinical and multi-omics (genomics, proteomics etc.) observations, it has been found that supplementation with melatonin is associated with 28% reduced likelihood (52% for black Americans) of being infected with SARS-COV-2 [48]. Given the importance of melatonin in reducing the severity of COVID-19, at least nine clinical trials (therapeutic and prophylactic) of exogenous melatonin on coronavirus patients are under progress [49]. Hence, melatonin is expected to play a pivotal role not only in reducing the severity of COVID-19, but also in lessening or eliminating the susceptibility of the coronavirus patients to mucormycosis.

Conclusion

It is proposed that melatonin deficiency in SARS-COV-2 patients plays a major role in CAM. The deficiency of melatonin in COVID-19 arises from impaired absorption of tryptophan (the precursor of serotonin and melatonin) from the food as ACE2, the chaperone of tryptophan transporter B^0AT1, is downregulated in coronavirus patients. The melatonin deficiency in SARS-COV-2 patients provides a significant advantage to the mucor species in their growth and virulence primarily due to the creation of a milieu favourable for their smooth access to iron, an essential nutrient, as well as for their facile transition from the yeast to the virulent hyphal form. The iron acquisition of the mucor species can be restricted by correcting the melatonin deficiency, as melatonin is a good iron chelator. Also, melatonin, a potent anti-inflammatory agent, blocks myeloperoxidase and abates pyroptosis and ferroptosis induced inflammation resulting in the lowering of serum ferritin and heme degradation products and, in turn, available plasma free iron for the mucor species. Furthermore, the morphological transition of the mucor species from the yeast to the invasive hyphal form is inhibited by melatonin, which blocks calmodulin, an important sensor protein involved in the transition. In addition, melatonin can further help the diabetic patients by lowering the expression of GRP78 receptors (increased in diabetic patients), which are exploited by the mucor species to invade the host cells. Also, the T-cell suppression owing to steroid use is blunted by melatonin. Hence, with a view to fixing the melatonin deficiency and consequently, eliminating or reducing the susceptibility of the patients to CAM, clinical trials (both prophylactic and therapeutic) of tryptophan supplementation, SSRIs, and exogenous melatonin should be conducted on SARS-COV-2 patients to find out the best regimen effective against CAM.

Declaration of Competing Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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