Review

Does the melatonin supplementation decrease the severity of the outcomes in COVID-19 patients? A mini review of observational data in the *in vivo* and *in vitro* studies

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ABSTRACT

The Coronavirus Disease 2019 (COVID-19) is a global pandemic and there is no specific treatment for reducing the severity of this disease up to date. The majority of the treatments remain supportive and empirical. The aim of present study is to assess the relationship between melatonin supplementation and its effect on the severity of the outcomes in covid-19 patients. All published studies up to April 4 of 2021 were searched by using the databases of PubMed, ISI Web of Science, SCOPUS and Google Scholar. Finally, 201 studies have been acquired.

After screening titles, abstracts and justifying the inclusion criteria, eight studies were finally selected in our study. Four studies were observational and case series with total 216,792 participants. Three studies performed on laboratory in the molecular level and one was carried out in mice. The results have suggested that melatonin decreases the severity of the outcomes of COVID-19 patients in their early stage or even in their critical conditions. Furthermore, the melatonin decreases pneumonia and reduces the ground glass lung damage observed in the image findings. Also, it plays an important role as anti-inflammatory, anti-viral and antioxidant activities. Melatonin inhibits the main protease of sares-cov-2 virus and decreases the viral load in molecular level. Regarding the *in vivo* studies, melatonin is more effective for reducing acute lung injury than other treatments. Although, further clinical studies are required.

Key words: melatonin, coronavirus, COVID-19, anti-inflammation, antioxidant

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1. INTRODUCTION

The recent outbreak of coronavirus disease 2019 (COVID-19) has led to 131,654,872 million infected patients and 2,862,891 deaths in worldwide as of April, 4, 2021. The most important causes of mortality in COVID-19 are due to the comorbidities of the patients, including diabetes *mellitus*, asthma, cardiovascular disease, cerebrovascular disease, malignancy and hypertension (1-5). Previous studies indicated that the level of some pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, interferon gamma increased in COVID-19 patients (6, 7). Some studies reported cytokine storm in the intensive care unit (ICU) patients. Furthermore, the number of neutrophils, C-reactive protein (CRP) level are also increased in ICU patients (8). However, there is no specific treatment for COVID-19 individuals yet. The majority of treatments remain supportive and empirical (9). These include antiviral, immunosuppressant's and supportive therapeutic options which are used to reduce inflammatory condition and cytokine storm. As well as, some studies reported beneficial effect of melatonin in COVID-19 individuals (10-13).

Melatonin, a methoxyindole, is a bioactive molecule mainly produced by the pineal gland during night (14, 15). Melatonin widely exists in food stuff including nuts, medical herbs, fish, eggs, fruits including grapes, cherries, strawberries and also vegetable such as tomatoes and peppers, mushroom and milk (15-21) (Figure 1).

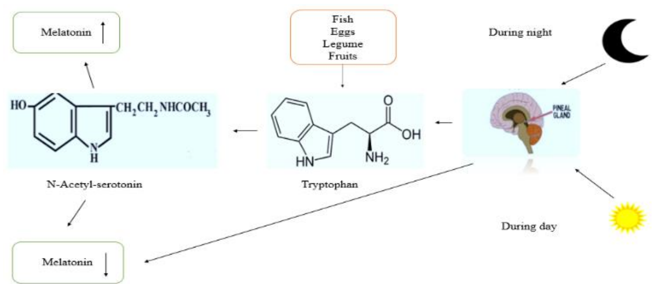

Fig. 1. The circadian rhythm of melatonin production in pineal gland.

Darkness increases melatonin synthesis and sunlight suppressed melatonin production. In addition, food intake also increases melatonin level.

Melatonin is used as a medicine or as dietary supplement globally. Its plasma levels exhibit circadian rhythm with peak at night and baseline during the day (22). Thus, it has been used as a sleep-aid supplement (23). However, many studies have reported its antioxidant, anti-inflammatory and immune promoting activities (23-29). For example, melatonin reduces tumor necrosis factor (TNF-α), interleukin-6 (IL-6), IL-1β, IL-8, elevates level of anti-inflammatory cytokine such as IL-10 (30-34) and lowers oxidative stress, muscle atrophy, apoptosis with the increase in immune system function (35-37) (Figure 2).

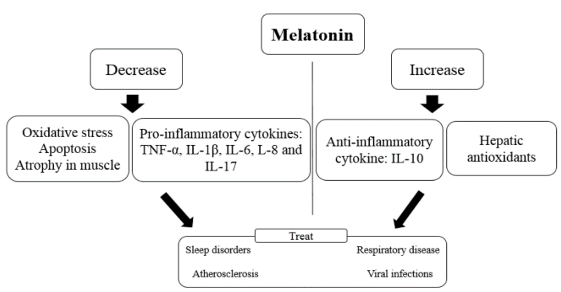

Fig. 2. The regulatory effects of melatonin on the productions of cytokines

Melatonin downregulates the pro-inflammatory cytokines while upregulates the anti-inflammatory cytokines, thus, suppresses the cytokine storms in SARS-CoV-2 infection. It modulates immune function to decrease oxidative stress and inflammatory reaction.

As to its antioxidant activity, melatonin has an aromatic indole ring that makes it being an electron donor (36-38) and it also upregulates expression of superoxide dismutase (SOD) and glutathione peroxidase (GR) (38). All these make it a more potent antioxidant than that of vitamin C and vitamin E (38). Melatonin is safe for humans, even the doses are more than 100 mg/day (10-13, 29, 39-42). Melatonin is produced by mitochondria and thus, many tissues and organs can produce melatonin including skin, retina, gut and lymphocytes (43-48).

It has been reported that the synthesis and secretion of the melatonin are controlled by the immune-pineal axis which might alter the melatonin source from the pineal gland to immune-competent cells in the different phases of inflammatory response (49-51). Previous studies have shown that the switching melatonin source from pineal gland to immune cells is mainly controlled by the Nuclear factor-kappa B (NF-κB) pathway. NF-κB binds to κB responsive elements, leading to deactivation of the noradrenalin stimulated melatonin production and inducing melatonin production in activated macrophages (52, 53).

The anti-inflammatory effect of melatonin, at least, partially through the NF-κB pathway and this has been documented in the asthma animal model (54, 55). The promising effect of melatonin on cytokines is mediated by adjusting the immune system and oxidative stress (56, 57). Several mechanisms by which melatonin acts in immune responses have been suggested. 1. An association between the lower levels of pineal melatonin and the severity of viral sickness is observed. This mechanism may be partly ascribed to regulating the mitochondrial metabolism and phenotype of immune cells (58). The interaction of pineal melatonin with mitochondrial metabolism and the melatonergic pathway afford essential facts to explain the viral infections. 2. Aryl hydrocarbon receptor (AhR) mediates the effect of coronavirus on the host's immune response. The AhR may be responsible for the early “cytokine storm” and alteration in mitochondrial and immune cell functions and these are associated with alterations in the melatonergic pathway (59). 3. A main indicator of the response to viral infection is the disrupted host melatonin circadian rhythm which impacts the viral replication. A strong inhibitor of virus replication is the circadian gene *Bmal1*, emphasizing the function of circadian factors in regulating viral infections (60). 4. Melatonin is a major inhibitor of NLRP3 inflammasome, the inhibition of melatonin production by virus increases NLRP3 inflammasome, and therefore, to promote viral replication and spreading (61-63). 5. The gut dysbiosis and increased gut permeability is linked with reduced melatonin production. For example, butyrate’s function to conserve the gut barrier is mediated by to increase melatonin production in the mitochondria of intestinal epithelial cells (63-65). In addition, the synthesis of melatonin by the lungs is due to inflammatory response to the lung injury (66).

The pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) activate the synthesis of melatonin in immune cells. Alterations in melatonin source cause the migration of cells to the location of the injury, where, regulatory roles are played by the locally synthesized melatonin. With regards to an immune-pineal axis, it should be contemplated that melatonin synthesized by lungs defends versus PAMPs/DAMPs stimulation which changes the macrophage phenotype (57, 67). Therefore, an essential element of the advancement and resolution of the innate immune response is the autocrine activity of melatonin. In this respect, by inhibiting the NF-κB pathway, melatonin blocks its synthesis consequently on local inflammatory responses (68).

The positive relationship between melatonin levels and decreasing severity of the outcomes of COVID-19 patients has been documented (14, 69). However, there is no systematic review for assessing this relationship. Thus, the aim of the current study is for this purpose.

2. METHODS

This review was performed according to the preferred items for systematic review and meta-analysis (PRISMA) guideline.

2.1. Search strategy.

A systematic search was carried out to find all relevant studies on databases such as PUBMED, SCOPUS, ISI Web of Science and google scholar until April 4th, 2021 by using title/abstract. The search results were imported to ENDNote X9.3. the duplicates articles were removed. The search terms included "Corona-virus" OR "COVID-19" OR "Coronavirus" OR "SARS-CoV-2" OR "severe respiratory syndrome coronavirus 2" and melatonin.

In Pubmed, keywords were searched through [tiab] and [MeSH] tags. No limitation was applied during the search. The reference lists of retrieved papers were also examined to avoid missing any published data

2.2. Inclusion criteria.

Two investigators independently selected the articles through mentioned search strategy. Publications that fulfilled the following criteria were eligible for inclusion: 1) all studies that have observational design (cross-sectional, case-control or cohort); 2) studies that examined the relationship between melatonin supplement and coronavirus conditions were include.

2.3. Exclusion criteria.

The exclusion criteria were editorials, letters, secondary studies, narrative reviews, commentary studies, or the studies which were not bases on our PICOS. The data of all included papers were extracted on Excel 2016. The studies were included without any country or language limitation.

2.4. Data extraction.

From each eligible study, we extracted the following information: year of publication, first author, and study design, sample size, exposure outcome. The extraction checked independently by two researchers.

3. RESULT

A total 201 articles were found on the database, after removing duplicate articles, 190 articles remained. Then the articles screened by title and abstract and after screening eight articles remained (Figure 3). The characteristics of 8 studies in this systematic review are presented in Table 1.

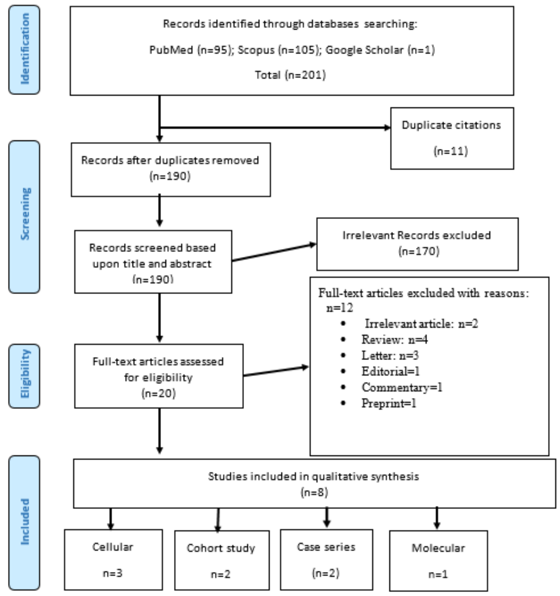

Fig. 3. Literature search and filtering of studies according to the PRISMA flow diagrams.

Table 1. characteristics of the selected studies of melatonin on COVID-19 infection.

Author (year)	Population	Country	Study type	Treatment	Main finding
Zhou Y. *et al.* (70)	26779	USA	Observation	Melatonin	Melatonin supplementation reduces 28% PCR positive and 52% in African Americans after adjusting for age, sex, race, smoking history, and various disease
Castillo RR. *et al.*(71)	10	Philippines	Case series	Melatonin	Melatonin adjuvant therapy improves the recovery of pneumonia in COVID19 patients.
Feitosa EL. *et al.* (72)	-	Brazil	Cellular level	Melatonin	The inhibitory effect over the SARS-CoV2 main protease is characterized as a big step towards the introduction of melatonin in the front line for the treatment.
Jehi L. *et al.* (73)	16	USA	Case series	Melatonin	Melatonin is effective to treat PCR positive participants (0.001>)
Al-Zaqri N. *et al.* (74)	-	Saudi Arabia	Molecular level	Melatonin	Melatonin is an antagonist to COV-2 protein and decreases viral load
Zhang Y. *et al.* (75)	-	China	*In vivo*	Melatonin	Melatonin is an effective therapeutic agent for acute lung injury
Fernandes PA. *et al.* (76)	288	Brazil	Cellular level	Melatonin	The negative correlation between the ability of human lung synthesizing melatonin (MEL-Index) and the expression of genes relevant for the entrance (SARS-CoV-o2), the trafficking and the virus proliferation.
Ramlall V. *et al.* (77)	189,987	Columbia	Retrospective study	Melatonin	Melatonin supplementation has positive outcomes in COVID-19 and non-COVID-19 intubation participants.

3.1. Description of studies

One study has been carried out in the laboratory on macro molecular proteins, four articles performed in human (observational and case series) as well as two studies carried out at molecular base under laboratory condition. Also, one study subjects are mice.

The sample size of review literature that carried out in human were 216,792 (cohort study: n= 216,766 & case series study n=26).

Zhou *et al*. (70) conducted an observational study to determine whether administration of melatonin reduces the chances for library positive reverse transcription–polymerase chain reaction (RT-PCR) as acquiring for coronavirus disease 2019 (COVID-19) in black Americans. Their analysis revealed that chances of reporting a positive PCR test were significantly lower in people taking melatonin compared with those who did not take melatonin after adjusting sex, age, smoking, race, and various disease comorbidities. In contrast, melatonin could not reduce library positive reverse transcription–polymerase chain reaction (RT-PCR) in white Americans. Although there are a few excellent sources of melatonin and tryptophan, which are indirect sources of melatonin, this study did not take into account dietary intake as co-founder. Furthermore, some vitamins and minerals are required as triggers in the melatonin production pathway and could implicate different levels of melatonin.

Castillo *et al*. (71) performed a case series study for assessing the association between using high dose of melatonin and coronavirus pneumonia in 5 female and male. They illustrated that the using high dose of melatonin was well tolerated as well as they indicated that the high dose of melatonin administration had a beneficial effect in pneumonia of COVID-19 patients. However, they reported that the melatonin has beneficial effect in shorter time to improve clinical outcomes.

Also, the melatonin has fewer side effects except sleepiness and melatonin supplementation decreased the ground glass pattern in image finding. Besides, average hospital stays of patients who given high dose of melatonin was decreased significantly.

Feitosa1 *et al*. (72) carried out a study on SARS-CoV-2 main protease for assessing the inhibitory effect of melatonin on this protease. in this study, the interaction between the main protease and melatonin was assessed. They found that an interaction between main protease amino-acid residues of SARS-CoV-2 and melatonin would increase binding energy of main protease and give a new context for potential therapeutic effect in severe cases. So, melatonin inhibits COVID-19 protease, they emphasise that this can be the main step for treatment SARS-CoV-2 conditions by melatonin.

Al-Zaqri *et al*. (74) have investigated the structure and chemical properties of melatonin using electronic structure methods and molecular-mechanics tools. They performed a detailed quantum-mechanical investigation of the melatonin. Natural bonding orbital research showed the strength of different intramolecular interactions. The various frontier molecular orbital data explain the nature and physical parameters of melatonin, and the non-linear optical properties are compared with urea which is a standard material. They have revealed a significant interaction between melatonin and coronavirus protein, that cause to inhibition of the virus proteins leading to its destruction. They have shown that the melatonin can be defending against COV-2 protein. So, it decreases viral load.

Zhang *et al*. (75) performed a study on mice. They have investigated the effect of melatonin by using acute lung injury animals, they revealed injection of melatonin significantly decreased the pulmonary injury and reduced the infiltration of macrophages and neutrophils into lung. Melatonin prevents the activation of the NLRP3 inflammasome by suppressing the release of histones. They showed that the administration of intratracheal melatonin is effective therapeutic for reducing acute lung injury.

Fernandes et *al*. (76) carried out a study on 455 genes of 288 human lunges. They represented that the immune competent cells synthesized melatonin in lung and constitutively by resident alveolar macrophages. Furthermore, they presented that the virus is less detected in lunges that synthesis melatonin as well as higher level of melatonin index inversely associated with the expression of genes that promote virus mobility inside the cells. The high melatonin index subjects prevent the entrance virus in AT2 epithelial cells. therefore, nasal rout treating participants with melatonin may decrease the rate of infection in participants.

Furthermore, Ramlall *et al*. (77) performed a study among 189,987 participants with a mean age of 65 years old, found that the exposure with melatonin associated with positive outcomes in intubation COVID-19 and non-COVID-19 individual with history of respiratory disease. In summary, the study results have demonstrated that melatonin may decrease the severity outcome in COVID-19 patients. Furthermore, the melatonin decreases pneumonia and reduces lung ground glass damage. All these may be mediated by its anti-inflammatory, anti-viral and antioxidant activities. (Figure 4).

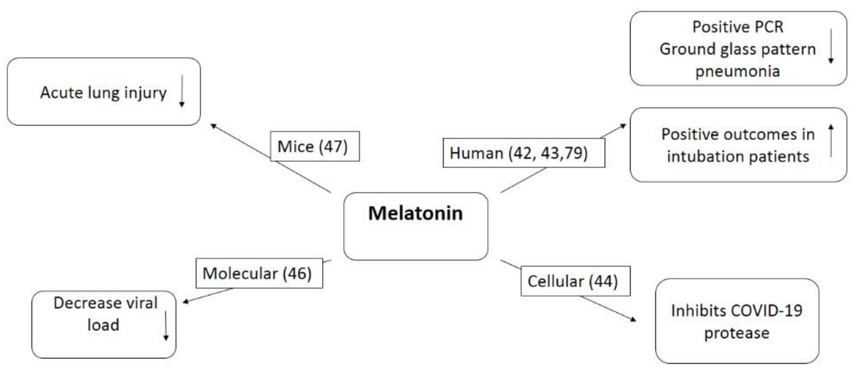

Fig. 4. Effects of melatonin on coronavirus in clinical trials and cell and molecular level.

4. DISCUSSION

Coronavirus has rapidly emerged as a global pandemic and it can cause severe outcomes in individuals who are afflicted with this virus. In this systematic review, we suggest that supplementation of melatonin in COVID-19 patients has a beneficial effect to limit the pneumonia and reduce severity of outcomes.

The cytokine storm takes place in COVID-19 patients by increasing IL-1, IL-6, TNF-α. The cytokine storm can be subsided by melatonin since melatonin improves immune functions (42-45). It is reported that coronavirus was involved in the expression of the gene in tryptophan metabolism (37). This will result in the reduced melatonin production in patients who are infected by the virus (78). Since the tryptophan is the precursor of melatonin. The association of low level of melatonin and severe outcomes in elderly COVID-19 patients is biomarker of this disease since elderly patients have dramatically reduced melatonin levels. Study suggests that to increase tryptophan resource in the COVID-19 patient is a promising remedy (39) to increase melatonin production and achieves therapeutic effect in bacterial and viral infections (46). Melatonin plays this role by decreasing IL-6 as an important biomarker in COVID-19 patient (47, 48).

Animal studies have also showed melatonin's beneficial effects in protecting viral, bacterial and parasite infections. These beneficial effects are attributed to the immunomodulation, enhancing antioxidant and anti-inflammatory activities of melatonin (49). Moreover, melatonin can decrease sepsis conditions by reducing cytokine storm and suppressing the expression of nitric oxide (NO), tumor necrosis factor (TNF-α), interferon- y and interleukin-12.

The small scale clinical trial carried out by Castillo *et al*. has provided the first hand evidence to show that high dose of melatonin is effective to treat moderate to severe COVID-19 patients. Even the patients have the comorbidities of hypertensive, diabetic, chronic kidney disease, chronic gout with moderate acute respiratory distress syndrome, melatonin treatment reduced ground glass pattern of lung damage and promoted them to symptom free (53). Huang *et al* carried out an *in vitro* study; they also showed the melatonin reduces pneumonia by its anti-viral effect in influenza (39). Also, Feitosa *et al* (74) reported that the melatonin inhibits the main protease of SARS-CoV-2

This is the first systematic review for assessing melatonin supplement in COVID-19.
The limitation of this study was: 1) there is lack of enough studies for assessing association melatonin and COVID-19 in humans and lack of RCT studies for evaluating this correlation in the treatment group with placebo. 2) It also requires variety of studies to analyze all ages and different doses in different subgroups.

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AUTHORSHIP

MG searched on data base and screened the articles by title and abstract. Strategy of search checked by KM. AM double checked the data extraction. The MG wrote the manuscript. KM and AM edited and revised it. The final version confirmed by all authors.

CONFLICT OF INTEREST

The authors declare that there is not conflict of interest.

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