RESEARCH ARTICLE

The Effect of Melatonin on C-reactive Protein, Serum Ferritin and D-Dimer in COVID-19 Patients

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ABSTRACT

Aim: To evaluate the effect of melatonin on C-reactive protein, serum ferritin and D–dimer level in adult patients with severe coronavirus disease 2019 (COVID-19).

Method: This single center, prospective, randomized clinical trial conducted in Al-Shifaa hospital at Mosul, Iraq from 1st December 2020 to 1st June 2021 on 158 patients divided into two groups 82 patients in melatonin group (given 10 mg melatonin) and 76 patients in control group. Then C-reactive protein (CRP), serum ferritin and D-dimer level were evaluated and recorded at day 5, 11, 17 of symptoms.

Results: Totally, 82 patients in the intervention group and 76 patients in the control group have completed the treatment. In comparison with the control group, the level of CRP, serum ferritin and D-dimer have significantly improved ($P < 0.05$) in melatonin group in the second week of infection.

Conclusions: The adjuvant use of melatonin in COVID-19 patients has a potential to improve CRP, ferritin, and D-dimer.

Keywords: Clinical trial, COVID-19, Melatonin.

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INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a virus which cause Corona Virus Disease 2019 (COVID-19).[1] This disease emerged from Wuhan city in China and spread worldwide. As of June 2021, the COVID-19 pandemic had infected nearly more than 171 million people and more than 3 million people was killed.[2]

While COVID-19 continues to spread around the world, numerous therapeutic agents were tested and others are under clinical trials. However, these therapeutic agents have not yet been confirmed effective in treating COVID-19 patients. Numerous papers have reported using of drugs with insufficient therapeutic effects and numerous side effects.[3] Hence, at present there is no definitive treatment for this new coronavirus. Due to the high contagiousness and rapid spread of SARS-CoV-2 infection, the requirement for safe and effective medications for the treatment of COVID-19 is becoming increasingly important.

The pathophysiology of COVD-19 is caused by the coronavirus cell entry via binding spike protein to angiotensin converting the enzyme 2 (ACE2) host receptor, which be present in different human tissues and cell types.[4] The COVID-19 infection presentations a cytokine storm and an inflammatory cascade that can significantly contribute to disease progression and even leads to death.[5,6] Thus, modulation of the excessive inflammatory responses is thought to be important in improving the treatment outcomes for COVID-19 patients.

Melatonin is a multifunctional molecule that is well-known as an antioxidant, anti-inflammatory, and immune modulator. It has also been confirmed that melatonin participates in regulating sleep, blood pressure, and vascular function and

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improves viral respiratory disorders. Due to these properties, recent publications have recommended the possible beneficial effects of melatonin on improving clinical outcomes of COVID-19 patients.[7,8] However, there are few clinical and laboratory data on the usage of melatonin as an adjunctive therapeutic agent in COVID-19 infection. Hence, in this study a randomized clinical trial was designed and performed to evaluate the efficacy of oral melatonin in combination with standard treatment on C-reactive protein, serum ferritin and D–dimer level in adult patients hospitalized with severe COVID-19.

METHODS

This study was an open labeled, single-center, randomized clinical trial to evaluate the effects of melatonin on C-reactive protein, serum ferritin and D-dimer level in adults with COVID-19 admitted to Al-Shifaa Hospital, Mosul, Iraq from December 1, 2020 to June 1, 2021. The diagnosis of COVID-19 was established by Reverse Transcription-Polymerase Chain Reaction (RT-PCR), as well as Computed Tomography (CT) or chest radiography findings consistent with COVID-19 pneumonia. A total of 200 patients were screened, from them 42 patients were excluded, due to the absence of inclusion criteria and nonacceptance to participate in the study. Thus, 158 patients were randomly chosen to join in the trial. Eligible patients fulfilled inclusion and exclusion criteria. The inclusion criteria were hospitalized patients with confirmed sever COVID-19, aged ≥18 and ≤ 80 years. The exclusion criteria were as follows: Age < 18, > 80 years, history of known allergy to melatonin, pregnancy, lactating female, renal impairment, liver impairment, cancer, autoimmune disease, and terminal medical illness. The study protocol was approved by the ethical committee of the college of medicine, Baghdad University.

The patients were randomly divided into two groups of intervention and control using a random block design. The control group received standard therapy (oxygen, antiviral, antibacterial, dexamethasone and anticoagulants), and the intervention group received standard of therapy plus melatonin at a dose of 10 mg once daily for 14 days from diagnosis. laboratory parameters CRP, serum ferritin and D-dimer tests were performed at day 5, 11 and 17 of symptoms. All recorded data have been entered into an electronic database.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 20.0; IBM). Normality distribution of variables was assessed by the Kolmogorov Smirnov test. Continuous variables were reported as mean ± standard deviation (SD) and they were compared with the Mann-Whitney U test. Categorical ones were expressed as frequencies and percentages (%) and they were compared by the Fisher exact test or $\chi 2$ test and p-value < 0.05 was considered significant.

RESULTS

In this study the males percentage were 72.2% of the patients and 27.8% were females and there was no significant difference between melatonin group and control group in gender ($p > 0.05$).

The mean age in the study was 56.31 years, range (32–78 years), and there was no significant difference between the two groups ($p > 0.05$).

70.3% of the patients have had other comorbidities and no significant difference were seen between the two groups in having hypertension, ischemic heart disease, diabetes mellitus and asthma ($p > 0.05$) (Table 1).

1. Effect of Melatonin on C-reactive Protein (CRP) in COVID-19 Patients

In the base line reading (day 5 of symptoms) the non-parametric test (mann-whitney U test) indicated that there was no significant difference between the two groups regarding C-reactive protein ($p = 0.772$).

In day 11 of symptoms the CRP test was significantly greater in control group than in melatonin group, ($P = 0.001$).

In day 17 of symptoms also a very highly significant difference in the CRP test was seen between melatonin group and control group (greater in control group than in melatonin group) ($p = 0.000$) (Table 2 and Figure 1).

2. The Effect of melatonin on serum ferritin in COVID 19 patients

No significant difference ($P = 0.918$) in serum ferritin test was seen between melatonin group and control group in the base line reading (day 5 of symptoms), while in day 11 of symptoms the serum ferritin test was significantly greater for control group than for melatonin group ($P = 0.008$).

Table 1: Demographic and Clinical Characteristics of COVID-19 patients in melatonin group and control group.

Patient characteristics	*All patients (n = 158)*	*Melatonin group (n=82)*	*Control group (n=76)*	*df*	*p-value*
Age (mean ± SD)	56.31 ± 7.788	56.84 ± 7.578	55.74 ± 8.019	.	0.393 [a]
Gender					
Male no.(%)	114(72.2%)	58(70.7%)	56(73.7%)	1	0.725°
Female no. (%)	44(27.8%)	24(29.3%)	20(26.3%)		
Other comorbidities					
Hypertension	84 (53.2%)	50(61.0%)	34(44.7%)	1	0.055°
Ischemic heart disease	25(15.8%)	15(18.3%)	10(13.2%)		0.394°
Diabetes mellitus	47(29.7%)	25(30.5%)	22(28.9%)		0.863°
Asthma	16(10.1%)	5(6.1%)	11(14.5%)		0.113°

df= degree of freedom, SD= standard deviation, n= number of patient, %= percentage of patients in each group, °= no significant difference ($p > 0.05$) using Chi-square test, [a] = no significant difference ($P > 0.05$) using mann-whitney U test

Table 2: Effect of Melatonin on C-reactive Protein in COVID-19 Patients

C –reactive protein	*Melatonin group (mean ± SD) mg/L*	*Control group (mean ± SD) mg/L*	*Melatonin group (mean rank)*	*Control group (mean rank)*	*p–value*
Day 5	16.16 ± 13.768	14.84 ± 11.195	78.51	80.57	0.772°
Day 11	21.57 ± 10.763	27.78 ± 13.51	67.80	92.13	0.001**
Day 17	21.60 ± 13.837	35.7 ± 18.91	60.00	100.54	0.000***

SD = standard deviation, ***= very highly significant difference ($p < 0.001$)using Mann - Whitney U test,**= highly significant difference ($p < 0.01$), °= no significant difference ($p > 0.05$)

Table 3: The Effect of melatonin on serum ferritin in COVID 19 patients

Serum ferritin	*Melatonin group (mean ± SD) ng/mL*	*Control group (mean ± SD) ng/mL*	*Melatonin group (mean rank)*	*Control group (mean rank)*	*p–value*
Day 5	649.89 ± 607.2	568.45 ± 388.402	79.14	79.89	0.918°
Day 11	850.28 ± 538.6	1003.57 ± 480.358	70.25	89.48	0.008*
Day 17	802.93 ± 452.4	1215.97 ± 515.085	60.40	100.11	0.000**

SD = standard deviation, *= highly significant difference ($p < 0.01$)using Mann - Whitney U test,**= very highly significant difference ($p<0.001$), °= no significant difference ($p > 0.05$)

Table 4: Effect of melatonin on D-dimer level in COVID 19 patients

D-dimer	*Melatonin group (mean ± SD) ng/mL*	*Control group (mean ± SD) ng/mL*	*Melatonin group (mean rank)*	*Control group (mean rank)*	*p-value*
Day 5	532.1 ± 604.09	543.0 ± 356.536	73.96	85.47	0.112°
Day 11	820.4 ± 569.15	1022.9 ± 460.29	67.59	92.35	0.001*
Day 17	813.7 ± 556.84	1121.5 ± 499.24	64.20	96.01	0.000**

SD= standard deviation, *= highly significant difference ($p < 0.01$) using Mann - Whitney U test, **= very highly significant difference ($p<0.001$), °= no significant difference ($p > 0.05$)

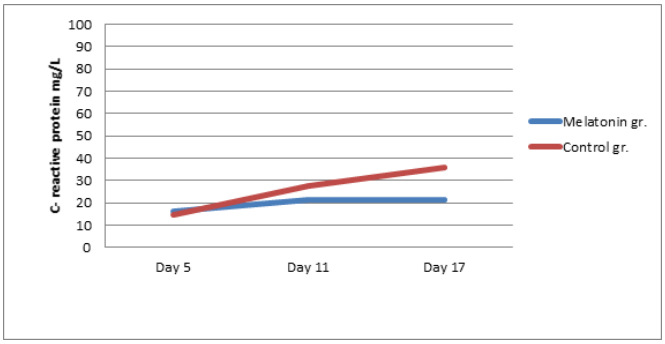

Figure 1: Effect of melatonin on CRP in COVID 19 patients

In day 17 of symptoms the serum ferritin test was highly significantly greater in control group than in melatonin group ($P = 0.000$) (Table 3)(Figure 2).

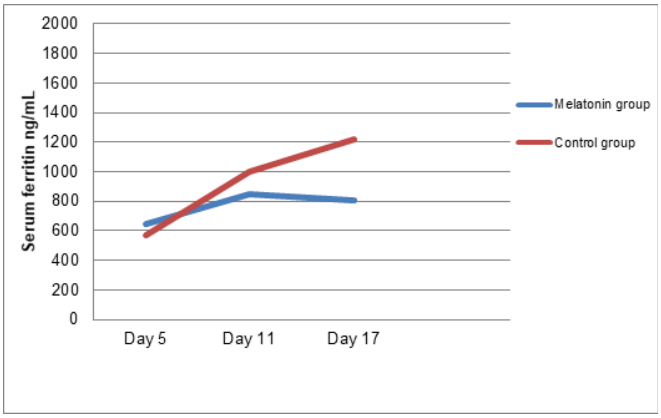

Figure 2: The Effect of melatonin on serum ferritin in COVID 19 patients

3. Effect of Melatonin on D-dimer in COVID-19 Patients:

In the base line reading (day 5 of symptoms) the non-parametric mann-whitney U test indicated that there was no significant difference between the two groups in D-dimer level ($p =0.112$).

In day 11 of symptoms high significant difference was seen in D- dimer level between melatonin group and control group (higher in control group than in melatonin group) ($p = 0.001$).

In day 17 of symptoms also a very highly significant difference in the D-dimer level was seen between melatonin group and control group (greater in control group than in melatonin group) ($p =0.000$) (Table 4 and Figure 3).

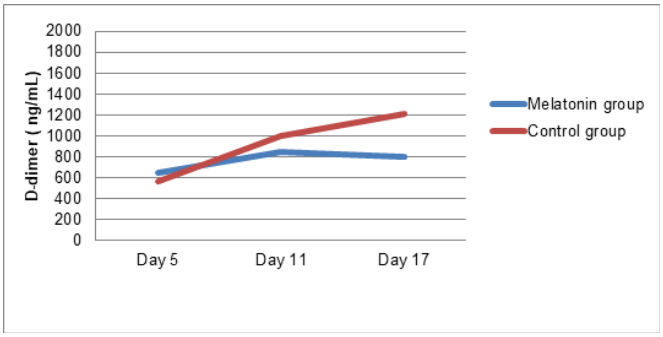

Figure 3: Effect of melatonin on D-dimer level in COVID 19 patients

DISCUSSION

There are large numbers of people infected with COVID19 with large numbers of death worldwide.[2] Which warranty urgent study to accelerate clinical trials with therapies that may reduce the worryingly high death rate. Since COVID-19 was became a pandemic, a variety of drugs have been investigated on patients with COVID-19.

The efficacy of melatonin as an adjunctive therapy was demonstrated in numerous diseases[9,10] and this is randomized trial to evaluate the efficacy of 10 mg oral melatonin on C-reactive protein, serum ferritin and D-dimer in patients hospitalized with severe COVID-19.

The CRP is an acute-phase protein and act as indicator of inflammation, infection, and tissue damage, and increased levels of CRP relate with high mortality rate.[11,12] More severe cases revealed a more evident rise in CRP levels 81.5% compared to non-severe cases 56.4%, respectively.[13] In this study, at day 11 and 17 of symptoms, increase in CRP was observed in most patients with a significant difference between the two groups, showing the effective impact of melatonin in inhibiting inflammation under the COVID-19 infection. The published clinical literature about the effects of melatonin supplementation on inflammatory markers is scarce. In a study conducted by Pakravan H,[14] melatonin supplementation for 12 weeks to patients with non-alcoholic fatty liver disease significantly reduced CRP concentrations. Conversely, melatonin administration in dose (10 mg/day) for 1 month to the patients who have severe and advanced atherosclerosis did not affect CRP levels.[15] Different study designs, different baseline values of dependent variables, different doses of melatonin used along with characteristics of study participants might explain the discrepancies among included studies.

Serum ferritin has been long studied as a indicator of iron metabolism,[16] however, its application as biomarker of inflammation has far presented high importance in the context of COVID-19 progression, as demonstrated by previous clinical studies in the field.[17] Chen *et al.* analyzed the clinical characteristics of 99 patients, in which 63 of them had serum ferritin way above of the normal range.[18] Increased level of ferritin was also found in autopsies of 12 patients who died with SARS-CoV-2 infection.[19] Thus, it was concluded that serum ferritin levels were closely related to the severity of COVID-19 infection.[20] This study at day 11 and 17 of symptoms increase in serum ferritin was observed in most patients with a significant difference between the two groups (greater in control group than melatonin group) ($p< 0.05$). This effect may be due to the modulation effect of melatonin on the levels of iron, iron binding proteins and cellular antioxidant levels and prevented the accumulation of a potentially toxic excess of iron and reactive oxygen species (ROS). This is also since melatonin possesses a broad spectrum of antioxidative mechanisms and likely offers cytoprotection at molecular and biochemical levels.[21]

D-dimer is a fragment produced when fibrin cleaved by plasmin to break down the clot. This assay is routinely used in diagnostic algorithm to exclude thrombosis. However, any pathologic or non-pathologic process that cause increase in fibrin production or breakdown also increases the plasma D-dimer level.[22] Thrombosis can occur in different organs in severe COVID-19 infection with subsequent organ failure. In patients with COVID-19, the presence of a concomitant disease such as diabetes, cancer, stroke, and physiological condition such as pregnancy can contribute to higher levels of D-dimer.[23] This study demonstrates an increase in D-dimer was observed in control group patients significantly higher than that in melatonin group ($p < 0.05$). There was no study evaluating the direct effect of melatonin on D-Dimer in COVID-19 patients except one study which compared the effect of single dose melatonin (3 mg) on D-dimer level induced by exercise only which showed an attenuated increase in D-dimer in response to exercise but was not statistically significant.[24] This apparent discrepancy may be due to a small single dose of melatonin used and the fact that this increase in D-dimer was a normal physiological response rather than a pathological one. However, melatonin and several of its metabolites have antioxidant effect and the capacity to regulate the vascular tone. These effects alone or in combination might be related to endothelial anticoagulant function.[25]

CONCLUSIONS

This study showed the efficacy of oral melatonin as an adjuvant therapy added to the standard of care compared with standard of care alone in hospitalized patients with severe COVID-19. Improving CRP, ferritin and D-dimer are in favor of the efficacy of this adjuvant medication in mitigating this infectious disease. Considering the high performance of melatonin as an inexpensive, affordable, highly safe to human and readily available medication, its prescription is strongly recommended to be addressed in future studies.

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