Original Article DOI: https://doi.org/10.18502/fem.v5i3.5894

Evaluation of the Effects of Melatonin Supplementation on Coagulation in Patients with Haemorrhagic Stroke; A Randomized, Double-Blind, Controlled Trial

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Published online: 2021-03-14

Abstract

Introduction: Considering that hemorrhagic stroke patients are at higher risk for bleeding, administration of higher doses of melatonin with a controversial coagulation profile is a serious concern.

Objective: This study aimed to investigate the possible effects of high doses of melatonin on bleeding parameters and blood hemostasis in hemorrhagic stroke patients.

Methods: This study is a randomized, double-blind, prospective, controlled trial. Confirmed hemorrhagic stroke patients were divided into two groups. Participants were randomly assigned into the melatonin group (30 mg daily via gastric tube gavage for 5 consecutive days) or the control group. Each patient was monitored for 5 days, and 2 blood samples were taken and the effect of the intervention on coagulation factors and blood hemostasis were investigated.

Result: In total, 30 patients were randomly assigned to melatonin (n=15) or control groups (n=15). there was no significant difference between the two groups in demographic and clinical characteristics. There was a significant decline in prothrombin time (PT) and fibrinogen levels in the melatonin group ($p=0.011$ & $p<0.001$, respectively). P-values for VII and VWB factors showed a significant increment in these two factors in the melatonin group after the intervention ($p=0.035$ & $p=0.002$, respectively). No significant changes in serum levels of D-dimer factor, APACHE II, and GCS scores were evident in the two groups after the intervention ($p>0.05$).

Conclusion: Considering the favorable changes in coagulation parameters observed in this study, it could be concluded that melatonin can have both procoagulant and antithrombin properties.

Key words: Blood Coagulation Disorders; Factor VII; Fibrinogen; Hemorrhagic Stroke; Melatonin; von Willebrand Factor

Cite this article as: Shohrati M, Mohammadi A, Najafi A, Sharifzadeh M, Sharifnia H, Abdollahi M, et al. Evaluation of the Effects of Melatonin Supplementation on Coagulation in Patients with Haemorrhagic Stroke; A Randomized, Double-Blind, Controlled Trial. Front Emerg Med. 2021;5(3):e32.

INTRODUCTION

Hemorrhagic stroke is a dominant cause of disability and even death worldwide (1). Notably, various molecular and cellular events such as inflammation, apoptosis, and oxidative stress are involved in hemorrhagic stroke's pathogenesis (2). Melatonin has been proven to have neuroprotective properties against brain injury in hemorrhagic stroke via multiple molecular mechanisms. This is assumed due to its antioxidant, anti-inflammatory, and anti-apoptosis properties (3). Melatonin is a broad-spectrum neuro-hormone and a potent free radical scavenger. It is centrally synthesized by the pineal gland and directly released into the blood (3, 4). This neuro-hormone is a ubiquitous agent in almost every living organism, from bacteria and

viruses to vertebrates (5). In the human body, the hypothalamic suprachiasmatic nucleus controls the synthesis and release of this substance.

There is evidence suggesting that melatonin, as an antithrombotic agent, can cause decrement in coagulation factors. However, some experiments indicated that melatonin affects fibrinolysis without affecting the coagulation process (6, 7). The effects of melatonin on platelets are also unclear. Melatonin may inhibit platelet aggregation by inhibiting arachidonic acid and thromboxane B2 (8). Furthermore, an increment in platelet levels was observed after administering melatonin in patients with different causes of thrombocytopenia (9). Melatonin enhances calcium entry to the endothelial and subsequently stabilizes the endothelium (10). Thereby, clinical studies about the coagulant or anticoagulant effects of melatonin are inconsistent and inadequate; and more comprehensive and further investigations are still needed.

It has been demonstrated that the administration of melatonin has beneficial neuroprotective effects in patients with hemorrhagic stroke in the intensive care unit (ICU) by improving clinical outcomes (11). Considering that hemorrhagic stroke patients are at higher risk for bleeding, there is a severe concern that the administration of higher doses of melatonin with a controversial coagulation profile may increase the risk of bleeding in these patients. This study aimed to investigate the possible effects of high doses of melatonin on bleeding parameters and blood hemostasis.

Methods

Study design

This randomized, double-blind, prospective, controlled trial evaluated the melatonin effect on coagulation following hemorrhagic stroke. It was carried out in the ICU of Sina hospital, Tehran, Iran. This clinical trial was registered by the Iranian Registry of Clinical Trials (IRCT201610225073N2). The ethical committee of Baqiyatallah University of Medical Sciences approved the study protocol (Ethics Code: IR.BMSU.REC.1395.89). Before enrollment, all patients' companion was provided with verbal information and written consent.

Study population

To be eligible for enrollment, participants met the following inclusion criteria: patients admitted to the ICU within 72 hours of hemorrhagic stroke confirmed by computed tomography (CT) scan. Exclusion criteria were evidence of hepatic or renal failure, brain tumor, Glasgow Coma Scale (GCS) more than 8, known hypersensitivity to melatonin, and rheumatologic or autoimmune diseases.

The sample size was estimated as 15 per group based on earlier experience and previous studies by using the sample size equation ($N=(z1-\alpha2/-z1-\beta)\ 2\ (SD1+\ SD2)\ 2/d2$) for comparing two means ($\alpha=0.05$; $\beta=0.2$) (12). The sample size was calculated based on the hemodynamic parameters, including prothrombin time (PT). The numbers replaced in the sample size equation were obtained through a pilot evaluation in the early stages of the study, which were all confirmed at the end.

Randomization

In this prospective study, double-blinded randomization was used. Thirty eligible patients were randomly enrolled in two groups using a permuted block randomization method. Blocks of four were used. Each patient was given a six-digit random number. Patients were allocated to the study group according to Random Number Table.

Intervention

Participants in the first group received 30 mg of melatonin (Melatonin tablets were purchased from Razak Company) daily via gastric tube gavage for 5 days, while patients in the second group did not receive this medicine. Patients in each group received the standard treatment of care for hemorrhagic stroke. Each patient was monitored for 5 days, and 2 blood samples were taken. The first one was before melatonin administration, and the second sample was obtained 5 days later. Blood samples were centrifuged, and the serum was kept at -80 °C for later assessments. The principal investigator, who had no clinical involvement in the trial, performed randomization and blinding. Patients, clinical personnel, and investigator of clinical responses were all blind to the study's arms.

Primary endpoints

The study's primary outcome criteria were serum biomarkers, including D-dimer, fibrinogen level, platelet count, PT, partial thromboplastin time (PTT), international normalized ratio (INR), von Willebrand factor (vWF), and factor VIIa. The serum levels of biomarkers were measured at baseline and five days after melatonin initiation.

Secondary endpoints

Secondary outcome criteria were age, mortality, length of ICU stay, and level of consciousness. Rate of mortality was evaluated by using Acute Physiology and Chronic Health Evaluation II (APACHE-II) criteria. Level of consciousness was measured by Glasgow Coma Scale (GCS).

Furthermore, lab data of patients, including blood urea nitrogen (BUN), serum creatinine (Cr), white blood cell (WBC), and hemoglobin (Hb) were recorded at baseline and then on day 5.

Statistical analysis

Descriptive baseline characteristics for the two groups comparisons were tabulated as mean ± SD, or as percentages. If the data distribution was normal, the parametric test (sample T-test) was used. If data distribution was not normal, non-parametric tests (Mann-Whitney test and Wilcoxon Test) were used. Since the comparison was before and after and we wanted to calculate the difference between the findings, in the comparison of before and after in each group, Wilcoxon Test was used. All statistical analysis was conducted using SPSS software version 16 and differences with a value of $p< 0.05$ were considered to be significant. Analysis was performed on an intention-to-treat basis.

RESULTS

Participants

The enrollment flow chart of the patients was displayed in figure 1. In total, 30 patients were randomly assigned to melatonin (n=15) or control groups (n=15). Demographic and baseline clinical characteristics of patients were presented in Table 1. As indicated, there was no significant difference between the two groups in demographic and clinical characteristics (age, gender, body mass index, urea, Ca, Na, K and the sequential organ failure assessment score).

Values of coagulation and blood parameters in melatonin and control groups before and after the intervention are reported in table 2. A significant rise in platelet count followed the intervention in the melatonin and control groups was observed ($p<0.05$), increasing from $167.33±51.2 \times 10^9$/L before administration of melatonin to $243.00±41.4 \times 10^9$/L at the end of study period ($p=0.005$). The same trend was followed for the control group, increasing from $173.73±65.8 \times 10^9$/L to $208.26±34.8 \times 10^9$/L after intervention ($p=0.008$). The mean difference for melatonin was much higher (75.66 vs 34.53), but the difference between the two groups was not statistically significant ($p=0.206$). There was a significant reduction in the PT in the melatonin group after the intervention, decreasing from 16.30±2.6 seconds to 14.98±2.2 seconds ($p=0.021$). Moreover, by comparing the mean of PT decrement between the two groups, the melatonin group had a significant decrease during

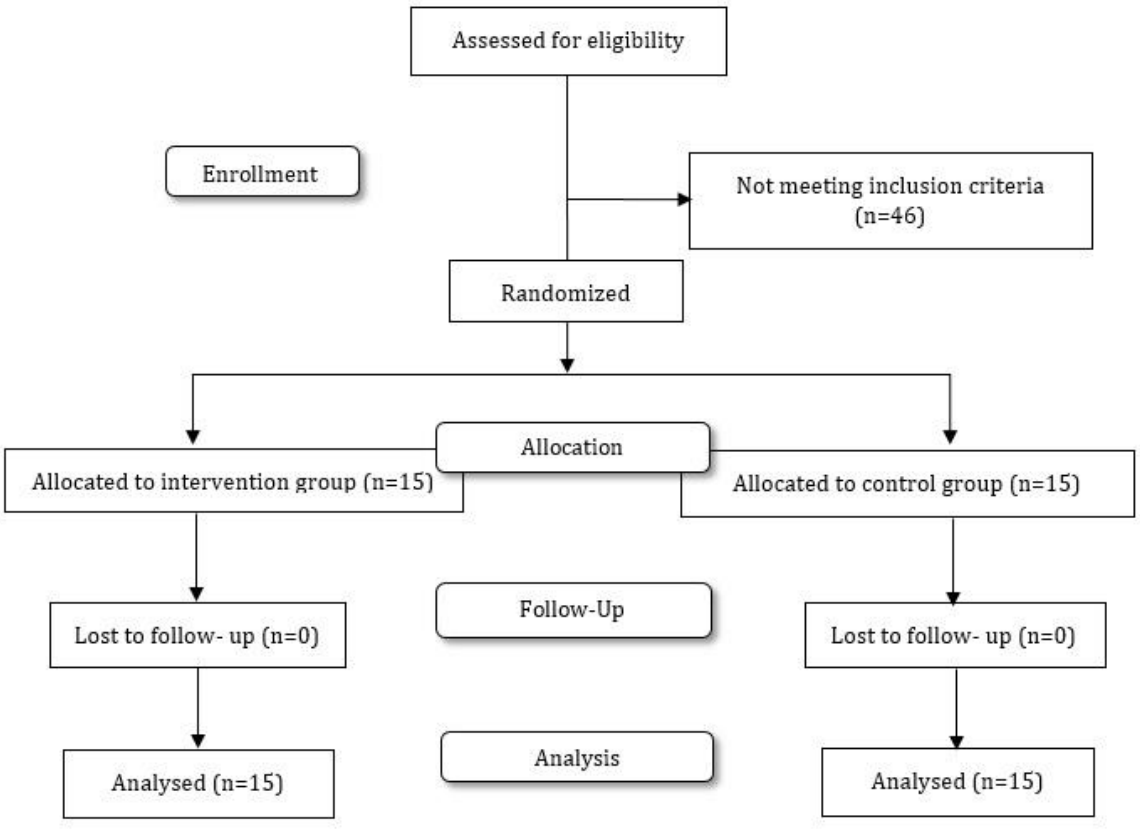

Figure 1: Flow diagram of the study

study period, based on the Mann–Whitney test (p=0.011). There were no significant changes in the level of other blood parameters like PTT, PTT-Ratio, Hb, INR, white blood cell (WBC), and hematocrit (HCT). Furthermore, we observed no statistical differences in these parameters within the study period between the melatonin and control groups (p>0.05).

Comparison of fibrinogen, D dimer, factor VII, vWB factor levels in melatonin and control groups before and after the intervention are reported in table 3. Based on the Wilcoxon test, there was a significant difference in fibrinogen level in the melatonin and control groups after the intervention. Before intervention, fibrinogen level was 459.9±73.9 mg/dL which changed to 380.9±71.9 mg/dL at the end of study. Based on the Mann-Whitney U test, the melatonin group had a statistically significant reduction in fibrinogen levels (p=0.0001). Based on the Wilcoxon test, p-values for factor VII and vWB factor suggested a significant increment in these two factors in the melatonin group after the intervention (p=0.035 & p=0.002, respectively). However, there was no significant change in the control group. Overall, taking melatonin, compared with the control, significantly increased factor VII and vWB factor, increasing from 116.3±34.3 µg/dL to 122.7±35.5 µg/dL for factor VII, and from 101.8±25.2 IU/dL to 113.7±21.8 IU/dL for vWB factor (p=0.858 &

Table 1: Demographic and clinical information of patients

Variable	Melatonin (n=15)	Control (n=15)	P-value
Age (year)	57.7±12.7	52.9±13.7	0.329
Sex (male)	12 (80)	9 (60)	0.232
BMI (kg/m²)	26.03±1.84	24.6±1.79	0.05
Urea (mg/dl)	33.9±14.1	25.7±9.27	0.215
Ca (mg/dl)	9.29±0.85	8.94±0.23	0.324
Na (mEq/l)	146.1±5.02	141.8±5.24	0.132
K (mEq/l)	4.01±0.20	4.18±0.31	0.220
SOFA score	6.27±0.70	6.64±0.93	0.228

BMI: body mass index; Ca: calcium; Na: sodium; K: potassium; SOFA score: The Sequential Organ Failure Assessment (SOFA) score. Numerical values were reported as mean±SD and nominal values as n(%). Student's t-test and Chi-square were used to compare these values, respectively.

Table 2: Values of coagulation and blood parameters in melatonin and control groups before and after the intervention

Variable	Melatonin Group				Control Group				P^
	Before	After	p*	Mean diff	Before	After	p*	Mean diff	
	Mean±SD				Mean±SD				
PLT (×10⁹/L)	167.33±51.2	243.00±41.4	0.005#	75.66	173.73±65.8	208.26±34.8	0.008#	34.53	0.206
PT (sec)	16.30±2.6	14.98±2.2	0.021#	-1.32	14.70±1.04	14.71±1.1	0.317	0.01	0.011#
PTT (sec)	36.07±4.6	35.60±5.1	0.729	-0.47	35.93±5.2	37.07±2.9	0.348	1.13	0.419
PTT-R	1.267±0.27	1.25±0.08	0.613	-0.02	1.28±0.28	1.20±0.13	0.717	-0.09	0.513
Hb (mg/dL)	11.89±4.5	10.83±1.7	0.348	-1.07	10.56±2.8	10.52±1.7	0.589	-0.05	0.262
INR	1.29±0.32	1.22±0.14	0.691	-0.07	1.329±0.29	1.21±0.17	0.088	-0.12	0.361
WBC (x10⁹/L)	10.08±2.2	10.29±3.9	0.820	0.22	9.13±2.7	9.13±2.7	0.977	-0.006	0.575
HCT (%)	32.45±7.2	31.19±5.7	0.410	-1.26	34.53±7.8	34.53±5.5	0.955	0.00001	0.604

PLT: platelet; PT: prothrombin time; PTT: partial thromboplastin time; PTT-R: PTT-Ratio; Hb: hemoglobin; INR: international normalized ratio; WBC: white blood cell; HCT: hematocrit.

* Based on Wilcoxon test; ^ Based on Mann–Whitney test; # significant difference

Table 3: Comparison of fibrinogen, D dimer, factor VII, VWB factor levels in melatonin and control groups before and after the intervention

Variable	Melatonin Group				Control Group				P^
	Before	After	p*	Mean diff	Before	After	p*	Mean diff	
	Mean±SD				Mean±SD				
Fibrinogen (mg/dL)	459.9±73.9	380.9±71.9	0.001#	-79.06	432.1±57.9	467.9±61.9	0.025#	35.80	0.0001#
D-dimmer (ng/mL)	3180.1±1614.1	2986.8±1195.6	0.100#	-193.93	3087.9±1298.5	2957.8±1143.2	0.061	-130.0	0.787
Factor VII (IU/dL)	116.3±34.3	122.7±35.5	0.035	6.46	126.5±33.1	126.5±33.4	0.858	-0.06	0.043#
vWB factor (IU/dL)	101.8±25.2	113.7±21.8	0.002	11.93	88.4±33.4	93.3±29.3	0.107	4.93	0.043#

vWB: von Willebrand factor

* Based on Wilcoxon test; ^ Based on Mann–Whitney test; # significant difference

Table 4: Comparison of GCS, APACHE-II, and renal indices in melatonin and control groups before and after the intervention

Variable	Melatonin Group				Control Group				
	Before	After	*P**	Mean diff	Before	After	*P**	Mean diff	*P**
	Mean±SD				Mean±SD				
GCS	6.5±2.7	7.5±2.8	0.142	0.93	7.6±3.2	7.73±2.89	0.972	35.80	0.235
APACHE-II	19.5±9.4	18.7±7.4	0.189	-0.73	19.7±7.6	17.80±7.33	0.067	-130.0	0.833
BUN	39.7±15.6	42.9±17.9	0.211	3.27	34.5±13.47	39.53±10.39	0.023#	-0.06	0.618
Cr	1.1±0.5	1.4±0.77	0.027#	11.93	0.97±0.33	1.12±0.302	0.035#	4.93	0.442

GCS: Glasgow Coma Scale; BUN: blood urea nitrogen; Cr: creatinine
* Based on Wilcoxon test; # significant difference

Table 5: Comparison of mechanical ventilation rate, length of hospitalization, mortality rate, and infection

Variable	Melatonin	Control	*P-Value*
Duration of mechanical ventilation (Days)	4 (2-16)	12 (4-20)	0.065
Length of ICU stay (Days)	8 (6-21)	12 (8-25)	0.041*
In ICU mortality	3 (15.0)	6 (30.0)	0.451
Sepsis	5 (25.0)	10 (50.0)	0.095

Numerical values were reported as median (Interquartile range), and nominal factors as number (%), Mann-Whitney U test and fisher exact test were used to compare these values.
* significant difference

p=0.107, respectively). D-dimer factor showed no significant change after intervention in both arms, and there was no significant difference between the two groups either (p=0.787).

Comparison of GCS, APACHE-II, and renal indices in melatonin and control groups before and after the intervention are provided in table 4. No significant changes in APACHE II and GCS parameters were evident in both groups after the intervention, and no significant differences were observed in the comparison of the melatonin group and the control group (p>0.05). Regarding the BUN and creatinine parameters, although there was a significant increment in both groups during the study period, no differences were found between melatonin and control groups.

Comparison of mechanical ventilation rate, length of hospitalization, mortality rate, and infection are presented in table 5. The length of ICU stay in the melatonin group was significantly shorter than the control group, 8 (6-21) days for melatonin group and 12 (8-25) days for (p=0.041). Although mechanical ventilation was shorter in the melatonin group compared to the control group, there was no significant difference between the two groups (p=0.065). Similarly, the rate of mortality and infection was lower in melatonin-consuming patients. However, it was not statistically significant (p>0.05).

Discussion

In recent years, melatonin's advantageous effects in reducing the complications following stroke and hypoxia have been proven (13, 14). Melatonin stabilizes endothelial and regulates vascular tone by its impact on the endothelium calcium signaling pathway. This agent improves calcium mobilization and its entrance to endothelial cells (10). Moreover, it has been shown that melatonin was able to decrease proinflammatory cytokines (15).

A study carried out by Dianatkhah et al. confirmed the beneficial neuroprotective effects of 30 mg of melatonin in patients with hemorrhagic stroke in the intensive care unit (11). Since hemorrhagic stroke patients are at higher risk for the occurrence of bleeding, we decided to evaluate the possible effects of high doses of melatonin on blood hemostasis in this group of patients. The results of melatonin on hemostasis are poorly studied, and published reports are also contradictory. The current study documented a significant increase in platelet count after the intervention in melatonin and control groups.

However, the difference was not statistically significant. In agreement with our study, Irina Pashalieva et al. found that melatonin, as an essential regulator of thrombocyte homeostasis in rats, can cause a significant elevation in thrombocyte count and markers of functional activity of thrombocytes, known as beta-thromboglobulin (β-TG) and platelet factor 4 (PF-4) (16).

Melatonin is a lipophilic molecule that readily crosses the cell membrane. The increase in platelet count can be due to the effects of melatonin on bone marrow cells. Furthermore, the impact of melatonin on platelets can be related to platelet production cytokines (17). In a study by Lissoni et al., 200 patients with various causes of

thrombocytopenia, 20 mg/day of melatonin were given orally for a month. Finally, 72% of patients experienced a rapid increment in mean platelet number, and no melatonin-related toxicity was reported in any of the patients. This study suggested that melatonin could stimulate platelet generation by modulating the cytokines involved in platelet production and promoting the fragmentation of the megakaryocytes (9). In another study performed on 5 individuals, melatonin inhibited the platelet activation induced by collagen and arachidonic acid. Furthermore, melatonin inhibits platelet function in the cyclooxygenase-dependent pathway. Besides, it has been proposed that the cyclooxygenase pathway involves platelet aggregation induced by melatonin, too (8).

Contrary to previous studies mentioned, in an in-vitro study by Kesturu Subbaiah Girish et al., melatonin enhanced apoptosis in platelets by releasing free radicals, particularly H_2O_2 (18). A significantly shortened PT in the melatonin group was observed in the current study, suggesting that this hormone has pro-coagulant properties. The results of an animal study performed on 52 male Wistar rats provided strong evidence that melatonin significantly shortened the activated partial thromboplastin time (aPTT), PT, and thrombin time. Indeed, melatonin promoted a tendency to hypercoagulability by activating the coagulation pathways and the conversion of fibrinogen to fibrin (19). Bekyarova et al. evaluated the effects of melatonin on burn-induced inflammatory responses and coagulation disorders in rats. Melatonin diminished the elevated c-reactive protein (CRP) and fibrinogen levels, normalized malondialdehyde levels, platelet morphology, and decreased prothrombin activity in rats with burn injury. Thus, melatonin acted as an anticoagulant substance (20).

Tugba Tunali et al. demonstrated that melatonin reduced oxidative damage to the skin and normalized blood coagulation induced by thermal injury. Melatonin limited the entrance of tissue factors to the bloodstream by reduction of skin oxidant damage. Intraperitoneal injection of melatonin to the thermal injury rat models caused PT normalization and fibrin degradation products (FDPs) decrement (21). In our study, plasma levels of fibrinogen decreased in the melatonin group after the intervention, which indicated the antithrombotic effect of melatonin.

A placebo-controlled human survey by Petra H. Wirtz and colleagues in 2008 showed that oral administration of melatonin was associated with decreased plasma levels of factor VIII and fibrinogen, while factor VII and D-Dimer did not change. Parallel with our findings, such decrement in coagulation factors indicated the antithrombotic effect of melatonin. There is probably a dose-response relationship between the plasma concentration of melatonin and coagulation activity (6). In another human study conducted by Wirtz et al. the effect of melatonin on stress-induced pro-coagulant response was investigated. Consequently, it demonstrated that melatonin reduced D-dimer plasma levels without affecting the VII, VIII and fibrinogen factors. Additionally, it concluded that melatonin did not affect the coagulation process but affected the following stage, fibrinolysis (7). Yuri Nyagolov et al. reported that melatonin shortened aPTT, increased factors V, XII, and XIII but did not affect factor XI. Thus, the melatonin application was accompanied by a tendency to hypercoagulability (22). Recent studies in COVID-19 patients indicate that the viral-induced systemic inflammatory responses trigger endothelial dysfunction, the waterfall coagulation cascade, and subsequent stroke and MI (23, 24). Thereby, melatonin's anti-inflammatory and endothelial stabilizing properties make this supplement a potential adjuvant in stroke with a favorable safety profile.

We observed a significant rise in vWF plasma levels in the melatonin group compared to the control group. Negrin Negrev and colleagues indicated that melatonin could increase the Tissue Factor and vWF factor and simultaneously reduced the level of tissue factor pathway inhibitor (TFPI). TF is involved in the external coagulation pathway via binding to the factor VIIa. TFPI blocks the external coagulation pathway by inhibiting TF. vWF also plays a significant role in the coagulation process by helping attach platelets to endothelium and inhibiting factor VIII proteolysis. Therefore, melatonin's mentioned effects on TF, TFPI, and vWF show that melatonin induces a pronounced tendency to hypercoagulability in rats (25).

Furthermore, in agreement with the previous study, Emiliya Stancheva et al. showed that melatonin lowered the plasma levels of protein C, protein S, and thrombomodulin, which involves the anticoagulant pathway. Inhibition of anticoagulant factors can be considered one of the mechanisms by which this neurohormone causes an evident tendency to hypercoagulability (26).

Limitations

The major limitation of the present study was the small number of patients. Although the results of this study showed positive effects of melatonin in

hemorrhagic stroke with acceptable coagulation safety, but due to the small sample size, the results of this study should be confirmed in a large clinical trial with predefined outcomes.

Conclusions

The significant increase in platelet level, reduction in prothrombin time, and VWF and VII factors in the melatonin group indicates its pro-coagulant properties. The statistically significant decrease in fibrin levels is suggestive of its anti-thrombin effects of melatonin. Further studies with a larger sample size could help make a conclusive decision to administer melatonin in intensive care patients with hemorrhagic stroke.

Acknowledgements

The results of this trial are a part of Majid Shohrati's post-graduate thesis of his fellowship course in Critical Care Pharmacotherapy from Tehran University of Medical Sciences.

Authors' contribution

MSH: collecting the clinical data; AM: drafting the manuscript; AN: head of treatment team, the physician providing clinical visit, and supervising on data records; MSH: conception and design of the study, submission guidance; HSH: the physician providing clinical visit; MA: conception and design of the study; MS: data analysis; AS: interpreting data, drafting the manuscript, submission; MM: the principal investigator and manager of the study, design and conduction the study.

Conflict of interest

None declared.

Funding

This study was financially supported by a grant from Vice Chancellor of Research and Technology affairs of Tehran University of Medical Sciences (grant number:95023332348). This grant providing funding to purchase medication, and data analysis.

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