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The Effect of Low-Dose Atorvastatin on Inflammatory Factors in Patients with Traumatic Brain Injury: A Randomized Clinical Trial

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Abstract

Background: Traumatic brain injury (TBI) is the leading cause of morbidity and mortality. Each year near 1.5 million Americans experience a TBI. Of which about 235,000 are hospitalized. Also, TBI claims 50 000 American lives each year. TBI causes mechanical damage to the blood-brain barrier and white blood cells (WBCs) entry to the brain.

Objectives: The current study aimed to evaluate the efficacy of low-dose Atorvastatin on inflammatory factors in patients with traumatic brain injury (TBI).

Methods: This double-blind, randomized clinical trial study was conducted in the ICU ward of Golestan Hospital in the city of Ahvaz (Iran) from April 2019-May 2020. Sixty patients with moderate to severe TBI were studied. Patients were randomly assigned into two groups of Atorvastatin and control. The main outcomes included the amount of CRP and ESR as well as white blood cells in the first 14 days of hospitalization. Glasgow Coma Score, the length of ICU stay, and the duration of mechanical ventilation were secondary outcomes.

Results: The amount of CRP in the Atorvastatin group on the 14th day of hospitalization was significantly lower than those in the control group (31.99 ± 8.38 vs 59.65 ± 10.43) ($P < 0.0001$). On the same day, the Atorvastatin group had lower levels of ESR than the control group (14.28 ± 4.18 vs 25.57 ± 5.18) ($P < 0.0001$). The Atorvastatin group had significantly lower levels of white blood cells than the control group (5247.53 ± 751.93 vs 7143.94 ± 907.64, $P < 0.0001$). Glasgow Coma Score at the time of discharge from the ICU in the Atorvastatin group was more than control (14.06 ± 1.45 and 11.85 ± 0.75, respectively) ($P < 0.05$). A significant difference was found concerning the ICU stay between the two groups ($P = 0.03$).

Conclusions: This study demonstrated that Atorvastatin could reduce the rate of inflammatory factors in TBI patients. The inflammatory condition of TBI patients heavily determines their prognosis. Inflammation leads to several reactions as well as interactions between different cells and chemical mediators. The Atorvastatin could reduce the rate of inflammatory factors and improved GCS in TBI patients.

Keywords: Traumatic Brain Injury, Atorvastatin, Inflammatory Factors

1. Background

Traumatic brain injury (TBI) is the leading cause of morbidity and mortality. Each year near 1.5 million Americans experience a TBI. Of which about 235,000 are hospitalized. Also, TBI claims 50 000 American lives each year (1). TBI causes mechanical damage to the blood-brain barrier and white blood cells (WBCs) entry to the brain (2). WBCs (neutrophils and activated macrophages) can release reactive oxygen molecules that eventually lead to cell destruction; They also stimulate the release of cytokines such as TNF alpha and Interleukins (IL-1, IL-6, IL-10, and IL12) (3). The inflammatory condition has an important role in the prognosis of patients with severe TBI. Inflammation leads to several reactions and interactions between different cell types and chemical mediators. During the acute phase reaction, the synthesis of C-reactive protein (CRP) increases so that its concentration increases significantly by 1000 times within 4-6 hours after tissue damage or acute inflammation (4, 5). ESR is the main indicator of acute-phase protein plasma reaction, which leads to a viscosity blend index and inflammation. Statins have anti-inflammatory effects and can reduce plasma CRP concentration (6, 7).

Statins also have anti-inflammatory and immunogenic properties (5, 6, 8, 9). Atherovstatin modulates

the immune system by affecting the endothelial cells, macrophages, Natural killer cells, and neutrophils (10). Statins are widely using to treat TBI due to their anti-inflammatory effects (11). Several studies have reported the effects of statins on the TBI pattern and its related processes, including cerebral ischemia, intracranial hemorrhage, and Sub Arachnoid Hemorrhage (12). However, there are studies that reported controversial results (13).

2. Objectives

The ratio of risk and benefit of statin treatment in the TBI ICU patients is not yet known. Moreover, the findings of studies on the effectiveness of statins for TBI patients are controversial. Hence, the current study aimed to investigate the effect of low-dose oral Atorvastatin on inflammatory factors in TBI patients.

3. Methods

3.1. Trial Design

This double-blind, randomized clinical trial study was conducted on TBI patients in the ICU ward of Golestan Hospital in the city of Ahvaz (Iran) from April 2019-May 2020. The study is approved by the Ethics Committee of Jundishapur University of Medical Sciences (code: IR.AJUMS.REC.1398.589). It's also registered by code: IRCT20191016045127N1. Written informed consent was obtained from the patient's legal representative.

3.2. Sample Size

The sample size was calculated using the relevant formula by considering a 95% confidence interval (CI). The study population consisted of 60 patients, based on the previous data (14).

Inclusion criteria: being aged 18 to 50 years, a moderate decrease in GCS (9-13), or severe decrease in GCS (5-8), having isolated TBI, and having brain contusions less than 30 cc volume in brain CT scan.

Exclusion criteria: GCS of 3 and 4, needing surgical evacuation, having spinal cord injury (SCI), history of renal or hepatic diseases, history of brain tumors, stroke, previous craniotomy, international normalized ratioc (INR) above 1.5, coagulopathy or treatment with anticoagulants a week before to admission, and baseline systolic BP < 90 mm Hg without responding to fluid administration.

3.3. Randomization

Random allocation was performed using a computer-generated eight-patient block randomization procedure. After eligibility assessment at ICU admission, each patient received a sealed brown envelope containing the random Atorvastatin or control treatment (the envelopes were opened on the evening of the first ICU day).

Blinding: two physicians, according to the requirements of the Ethics Committee, were aware of patients allocations and were continuously monitoring patients' health status, without participating in clinical decisions about sedative administration or interrupting the treatment.

In this study, patients in the intervention group received atorvastatin 40 mg tablet (Sobhan Darou, Iran) daily until hospitalization in ICU, and those in the control group received placebo (Placebo capsules filled by cornstarch powder (Beijing, China (by NG tube. The intervention began within 10 h of injury for all of the patients.

Primary Outcomes: CRP, ESR, and WBC serum levels changes (as inflammatory factors) in the first 14 days of hospitalization were the primary outcomes.

Secondary Outcomes: Glasgow Coma Score (GCS), the duration of mechanical ventilation, the length of ICU stay, and mortality rate in the ICU were examined. Data regarding these variables were collected using a questionnaire by a researcher who was blind to the study. The liver function was checked every day of ICU stay.

3.4. Statistical Analysis

Data were analyzed using SPSS version 22 (Chicago, IL). The results are expressed as mean± standard deviation, percentages (%), and numbers (n). The independent samples t-test was used to analyze the parametric data, and discrete (categorical) variables were analyzed using the x^2 test. The Repeated Measures ANOVA parametric test was also used to compare the two groups at different study times for CRP, ESR, and WBC. Statistical significance was considered when P value < 0.05.

4. Results

During the study period (April 2019-May 2020) 80 eligible patients were admitted to the ICU ward of the Golestan Hospital. After initial screening, 70 cases agreed to participate, and their legal representatives signed the informed consent. After further investigation, 10 patients were excluded due to not having inclusion criteria. Finally, 60 patients were enrolled in the study. Participants were divided into two groups of Atorvastatin and placebo (each with 30 subjects) (Figure 1). There was no statistically significant

difference between the two groups concerning the demographic characteristics (Table 1).

Comparing the CRP levels on the 14th day of the study and the baseline revealed a significant decrease (53%) in the intervention group compared to the control group (16%) ($P < 0.0001$) (Table 2 and Figure 2). In the Atorvastatin group, the changes in CRP between the first, 10th, and 14th days were significant ($F=239.102$, $P < 0.0001$). In the Control group, the changes in CRP between the first,10 th and 14 th days were significant ($F=10.655$, $P < 0.003$).

Regarding the ESR level, significant changes were observed at both groups on the 14th day of ICU admission ($P < 0.0001$). The observed decline in the intervention and control groups was 55 and 14%, respectively (Table 2 and Figure 3). In the Atorvastatin group, the changes in ESR between the first, 10th, and 14th days were statistically significant ($F=113.790$, $P < 0.0001$). In the Control group, the changes in ESR between the first, 10th, and 14th days were significant ($F=12.924$, $P=0.001$). Concerning the WBC level, while both groups were similar at the baseline and on the 7th day, a significant difference was observed at the 10th and 14th days of admission. Also, a significant decrease in WBC was observed in the Atorvastatin group ($P < 0.0001$) (Table 2 and Figure 4). In the Atorvastatin group, the changes in WBC between the first, 10th, and 14th days were significant ($F=209.851$, $P < 0.0001$). In the Control group, the changes in WBC between the first, 10th, and 14th days were significant ($F=78.895$, $P < 0.001$).

The clinical status of patients in both groups in terms of duration of mechanical ventilation, the length of ICU stay, and the mortality rate is provided in Table 3. A significant difference was observed concerning the length of ICU stay between the two groups ($P = 0.03$) (Table 3).

GCS of patients at the baseline and on the seventh day of admission was not significantly different between the two groups, but on the tenth (12.31 ± 3.47 vs. 10.16 ± 2.64, $P=0.04$) and fourteenth days (14.06 ± 2.1 vs. 11.85 ± 3.08, $P=0.01$), those in the intervention group had higher GCS than the control group, which was statistically significant ($P < 0.05$) (Figure 5). The changes in the liver function were not significant between the two groups. We did not find any signs of infection during the ICU stay.

5. Discussion

In the present study, we investigated the effect of low-dose of oral Atorvastatin on inflammatory factors in TBI patients admitted to the ICU. In this study, we compared the level of CRP, ESR, and WBC in the first, 10th, 14th days after receiving Atorvastatin. Also, a low dose of this drug was used to evaluate its effects; the literature review revealed that previous studies only have investigated the effects of Atorvastatin after treatment.

In the present study, Atorvastatin significantly reduced the rate of inflammatory factors (CRP and ESR) on the 14th day of ICU admission. The CRP level was decreased by 53% and 16% in the Atorvastatin and control groups, respectively. Also, the ESR rate in the intervention and control groups decreased by 55% and 14%, respectively.

There are trials on the effectiveness of Atorvastatin in TBI patients that completed phase II. Statin can prevent cholesterol biosynthesis and facilitates functional recovery following TBI in rodents. Simvastatin inhibits caspase-3 activation and apoptotic cell death, thereby improves neuronal rescue after TBI. Simvastatin improves the appearance of several growth factors and stimulates neurogenesis. Besides, it can support functional improvement after TBI (16).

Claudia S Robertson et al. showed that atorvastatin administration in patients with moderate TBI could reduce post-concussion symptoms. They also argued that atorvastatin administration for patients with one-week post-injury would be safe; nevertheless, no significant difference was observed concerning the neurological recovery post-mTBI with atorvastatin (17). Rongai Jiang et al. investigated the effect of Atorvastatin on Chronic Subdural Hematoma in phase II clinical trials. They found that Atorvastatin may be a safe and efficacious nonsurgical alternative for treating patients with chronic subdural hematoma (18).

Naqib et al. reported a significant decrease in CRP level 48 hours after administration of Simvastatin in TBI patients; however, they didn't report a significant difference concerning the Interleukin 6 levels (19). Zhang et al. reported a significant reduction in CRP during the six-month administration of oral atorvastatin 20 mg in patients with stroke (20). Aguilar et al. didn't report a statistically significant anti-inflammatory effect for statin in TBI patients, which is not in line with the findings of the present study. This discrepancy can be attributed to the short period of their study (three days) (13). In most cases, CRP and ESR are co-administered in hospitalized patients suspected of inflammatory or infectious disorders. ESR is higher in females than males. Besides, its concentration also increases with age, an issue which is neglected by most laboratory references. It's not clear whether there is a gender difference concerning the CRP or whether its concentration changes with age. However, in this study, age and gender factors were not taken into account while investigating the effects of Atorvastatin on inflammatory factors.

Inflammation of brain tissue after TBI may result in severe consequences such as changes in protein function, increased cell membrane permeability, leukocyte infiltra-

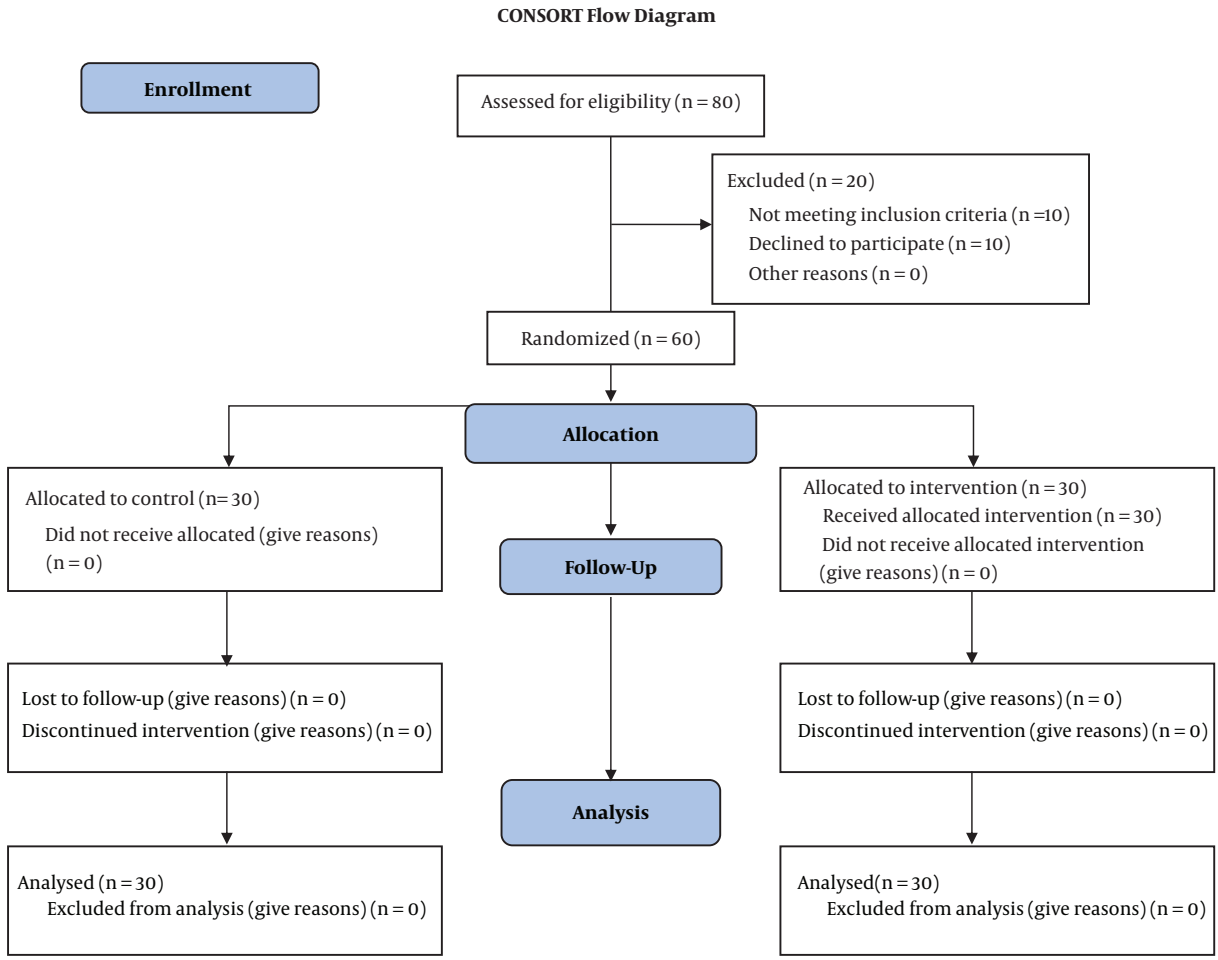


Figure 1. CONSORT Flow Diagram

Table 1. Demographic Characteristics of Participants Separated by the Group

Variable	Control (N=30)	Atorvastatin (N=30)	P Value
Sex M/F	14/16	17/13	0.54[a]
Age (Year), mean ± SD	52.36 ± 21.45	45.42 ± 18.52	0.36[b]
Weight (Kg), mean ± SD	69.56 ± 17.12	73.10 ± 24.50	0.74
Height (Cm), mean ± SD	172.45 ± 22.54	176.44 ± 18.50	0.38
APACHE II Score, mean ± SD	27.56 ± 10.82	29.73 ± 9.41	0.29[b]

Abbreviations: SD, standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation.
[a] x^2 test.
[b] student t-test.

tion, and cerebral tissue edema (21). Therefore, treating these side effects is necessary. Some studies on patients with other problems have also examined the anti-inflammatory effects of statins; for example, in an eight-week study on patients with congestive heart failure who were receiving these drugs every day, inflammatory factors (e.g. CRP) were reduced by 37% (22). In patients with ischemic stroke, rheumatoid arthritis, acute coronary syndrome, hyperlipidemia, etc., these anti-inflammatory effects have brought positive results (23, 24). Investigating

Table 2. Comparison of Inflammatory Factors (CRP and ESR) Between the Two Groups

Variable	Atorvastatin Group, Mean ± SD (15)	Control Group, Mean ± SD (15)	P Value
CRP (mg/L)			
First day	69.68 ± 9.52	68.76 ± 11.97	0.2500
10th day	50.48 ± 19.18	64.46 ± 9.55	0.0003[a]
14th day	31.99 ± 8.38	59.65 ± 10.43	< 0.0001[a]
ESR (mm/hr)			
First day	31.42 ± 7.50	30.36 ± 4.58	0.1732
10th day	22.37 ± 6.32	26.91 ± 5.86	0.0027[a]
14th day	14.28 ± 4.18	25.57 ± 5.18	< 0.0001[a]
WBC (cells × $10^3/\mu L$)			
First day	10666.08± 1647.93	10334.19 ± 1722.95	0.1511
10th day	6471.05 ± 446.76	8356.13 ± 1221.90	< 0.0001[a]
14th day	5247.53 ± 751.93	7143.94 ± 907.64	< 0.0001[a]

Abbreviations: CRP, C - reactive protein; ESR, erythrocyte sedimentation rate; WBC, White Blood Cell.
[a] significant. Independent samples t-test was used.

Table 3. Comparison of Secondary Outcomes

Variable	Control, Mean ± SD (15)	Atorvastatin, Mean ± SD (15)	P Value
Mechanical ventilation time (Days)	8.7 ± 3.4	6.1 ± 2.5	0.73
ICU staying time (Days)[a]	11.2 ± 3.5	9.4 ± 2.1	0.03
Mortality rate (No.)	4	3	0.26

Abbreviations: ICU, Intensive Care Unit.
[a] Independent samples t-test was used.

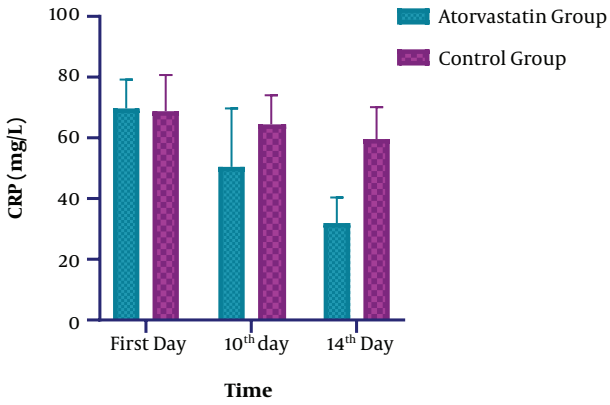


Figure 2. The changes of CRP after administration of Atorvastatin or Placebo

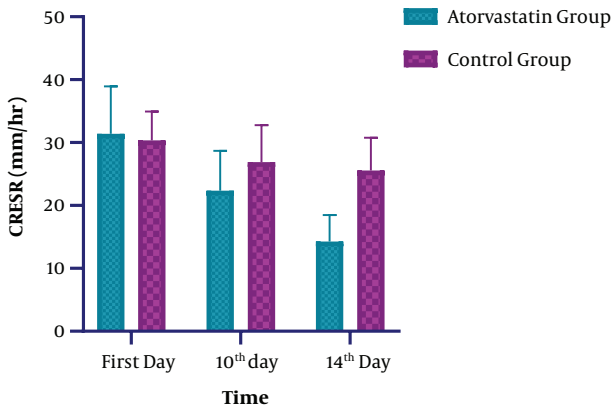


Figure 3. The changes of ESR after administration of Atorvastatin or Placebo

the effects of statins on the inflammatory status of gonadotropins showed that inflammatory problems present with symptoms such as trauma, sepsis, heart problems, etc., and change the permeability of the blood-brain barrier and activate the microglia or over-stimulation of inflammatory responses in the brain. Therefore, consuming medicines such as atorvastatin may alter the pathophysiological responses of the central nervous system to inflammation in TBI patients (25).

Another important finding of the present study was

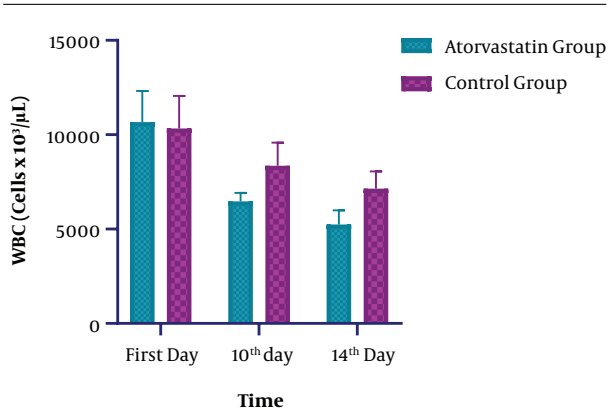
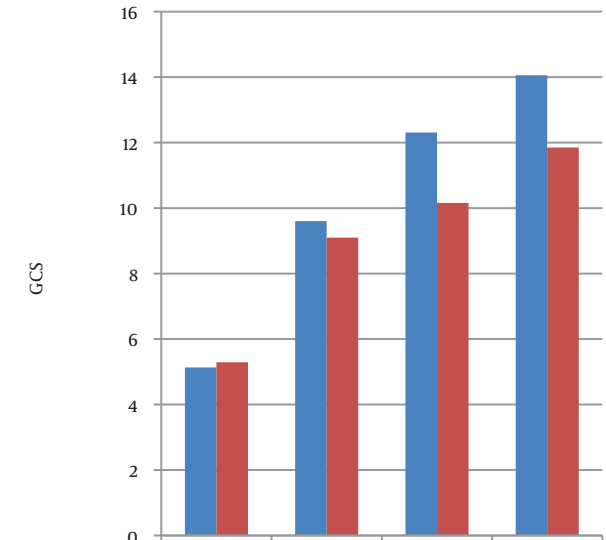


Figure 4. The changes of WBC after administration of Atorvastatin or Placebo



	Baseline	Day 7	Day 10	Day 14
Atorvastatin	5.13	9.6	12.31	14.06
Placebo	5.29	9.1	10.16	11.85

Figure 5. The changes of GCS after administration of Atorvastatin or Placebo

the significant decline of WBCs on the 14th day of ICU admission in the intervention group. Xu et al., in an animal study, have investigated changes in different types of WBCs, such as T-cells and NK cells, in the post-traumatic brain injury period and reported a significant increase in the number of T-cells and NK cells three days after consuming atorvastatin. There are studies that reported the destructive effect of increasing T-cells on neurotransmitters, which in turn may cause increased inflammation in brain tissue and neuronal damage (26). They argued that the destructive effects of NK Cells could be attributed to their toxic effects on brain cells. The destructive effects of NK Cells can cause inflammation and neuronal damage in the acute phase of brain damage through IFN-γ (27, 28).

In this study, the GCS of patients was assessed several times, from the onset of the study to the 14th day. Those in the intervention group had a significantly higher level of consciousness than the control group. This finding may be due to the protective effects of atorvastatin on the brain, which can prevent the release of inflammatory factors as well as neutrophil infiltration in the brain parenchyma to some extent with its anti-inflammatory effects on cerebral edema (29). Similar findings are reported by Rongcai Jiang and colleagues that investigated the effects of Atorvastatin on patients with chronic subdural hematoma in their clinical trial. They concluded that Atorvastatin could effectively increase the GCS (18). Naqibi also reported a significant increase in the consciousness level of patients in the intervention group compared to the control group after a week from ICU discharge (19).

In a clinical trial similar to ours on TBI patients, the authors reported that using statin could improve anti-inflammatory effects and patient recovery and reduced the disability scores (13). Morandi also examined critically ill patients with brain damage and reported that systematic inflammation could cause neuronal apoptosis, damage to the blood-brain barrier, and cerebral ischemia. They reported that statins could reduce such consequences and the occurrence of cognitive complications (25). In studies conducted on animals with traumatic induced brain injury, seven days of oral administration of atorvastatin was associated with effective preservation of brain neurons because of increasing the number of vessels in those areas after a traumatic brain injury (15).

In the present study, the length of ICU stay and the mortality rate were similar between the two groups, but the duration of mechanical ventilation was significantly lower in the atorvastatin group. The shorter duration of mechanical ventilation in the intervention group can be attributed to the anti-inflammatory effects of atorvastatin, which could reduce inflammatory factors such as CRP and ESR, as well as the number of WBCs during the study period. Besides, it was associated with reduced inflammation in the body and, in particular, in the brain and could improve the function of the alveoli and the patient's breathing by reducing the infiltration of neutrophils in the lungs, which in total decline the period of mechanical ventilation.

5.1. Limitation

The current study had limitations, including its small sample size, being a single-center, and not considering other inflammatory markers. Also, failure to evaluate the

patients' 3-month GOS is another important limitation of the present study. The authors suggest performing a multicenter study with a larger sample size, which contains patients' GOS and other inflammatory factors.

In conclusion, this study demonstrated that administration of Atorvastatin for TBI patients could reduce inflammatory factors over fourteen days, and despite not decreasing the ICU length of stay, it could improve GCS after ICU discharge and reduced the time spent on mechanical ventilation.

Acknowledgments

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Footnotes

Authors' Contribution: F. S. contributed in concept, study design, the definition of intellectual content; N. N. contributed in study design; F.J.z. drafted the manuscript; K. T. conceived of the study and participated in its design; A. K. provided study materials and patients' information; H. Z collected data. All authors read and approved the final manuscript.

Clinical Trial Registration Code: IRCT20191016045127N1.

Conflict of Interests: The authors declare no conflict of interest.

Ethical Approval: The study is approved by the Ethics Committee of the JUMS: (IR.AJUMS.REC.1398.589). Also, the study is registered as a RCT (IRCT20191016045127N1).

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Informed Consent: Written informed consent was obtained from the patient's legal representative.

References

1. Miri MM, Mofrad Taheri N, Soloki M. Survey of the Complications and Clinical Outcomes in Patients with Ventilator-Associated Pneumonia in Two Groups with and without Traumatic Brain Injury Admitted to ICU. *Journal of Military Medicine*. 2016;**18**(1):307–14.
2. Radhakrishnan M, Ghosh I, Dash HH. Evaluation of an indigenous ventilator for weaning in intensive care unit. *Journal of Anaesthesiology Clinical Pharmacology*. 2007;**23**(3):297.
3. Salmani F. The effect of discontinuation protocol on the duration of mechanical ventilation. *Iran Journal of Nursing*. 2013;**26**(82):62–73.
4. Thongtang N, Diffenderfer MR, Ooi EM, Asztalos BF, Dolnikowski GG, Lamon-Fava S, et al. Effects of atorvastatin on human C-reactive protein metabolism. *Atherosclerosis*. 2013;**226**(2):466–70. doi: 10.1016/j.atherosclerosis.2012.11.012. [PubMed: 23218801]. [PubMed Central: PMC5489069].
5. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170000 participants in 26 randomised trials. *The Lancet*. 2010;**376**(9753):1670–81. doi: 10.1016/s0140-6736(10)61350-5.
6. Ehrenstein MR, Jury EC, Mauri C. Statins for atherosclerosis–as good as it gets? *N Engl J Med*. 2005;**352**(1):73–5. doi: 10.1056/NEJMe048326. [PubMed: 15635116].
7. Javaherforooshzadeh F, Monajemzadeh SA, Soltanzadeh M, Janatmakan F, Salari A, Saeed H. A Comparative Study of the Amount of Bleeding and Hemodynamic Changes between Dexmedetomidine Infusion and Remifentanil Infusion for Controlled Hypotensive Anesthesia in Lumbar Discopathy Surgery: A Double-Blind, Randomized, Clinical Trial. *Anesthesiology and Pain Medicine*. 2018;**8**(2). doi: 10.5812/aapm.66959.
8. Comper P, Bisschop SM, Carnide N, Tricco A. A systematic review of treatments for mild traumatic brain injury. *Brain Inj*. 2005;**19**(11):863–80. doi: 10.1080/02699050400025042. [PubMed: 16296570].
9. Javaherforooshzadeh F, Amirpour I, Janatmakan F, Soltanzadeh M. Comparison of Effects of Melatonin and Gabapentin on Post Operative Anxiety and Pain in Lumbar Spine Surgery: A Randomized Clinical Trial. *Anesthesiology and Pain Medicine*. 2018;**8**(3). doi: 10.5812/aapm.68763.
10. Jorge AM, Lu N, Keller SF, Rai SK, Zhang Y, Choi HK. The Effect of Statin Use on Mortality in Systemic Autoimmune Rheumatic Diseases. *J Rheumatol*. 2018;**45**(12):1689–95. doi: 10.3899/jrheum.171389. [PubMed: 30173155]. [PubMed Central: PMC6289699].
11. Peng W, Yang J, Yang B, Wang L, Xiong XG, Liang Q. Impact of statins on cognitive deficits in adult male rodents after traumatic brain injury: a systematic review. *Biomed Res Int*. 2014;**2014**:261409. doi: 10.1155/2014/261409. [PubMed: 25157352]. [PubMed Central: PMC4135130].
12. Tseng MY, Participants in the International Multidisciplinary Consensus Conference on the Critical Care Management of Subarachnoid H. Summary of evidence on immediate statins therapy following aneurysmal subarachnoid hemorrhage. *Neurocrit Care*. 2011;**15**(2):298–301. doi: 10.1007/s12028-011-9596-6. [PubMed: 21826581].
13. Sanchez-Aguilar M, Tapia-Perez JH, Sanchez-Rodriguez JJ, Vinas-Rios JM, Martinez-Perez P, de la Cruz-Mendoza E, et al. Effect of rosuvastatin on cytokines after traumatic head injury. *J Neurosurg*. 2013;**118**(3):669–75. doi: 10.3171/2012.12.JNS121084. [PubMed: 23289819].
14. Sakpal T. Sample size estimation in clinical trial. *Perspectives in clinical research*. 2010;**1**(2):67.
15. Qu C, Lu D, Goussev A, Schallert T, Mahmood A, Chopp M. Effect of atorvastatin on spatial memory, neuronal survival, and vascular density in female rats after traumatic brain injury. *J Neurosurg*. 2005;**103**(4):695–701. doi: 10.3171/jns.2005.103.4.0695. [PubMed: 16266052].
16. Crupi R, Cordaro M, Cuzzocrea S, Impellizzeri D. Management of Traumatic Brain Injury: From Present to Future. *Antioxidants (Basel)*. 2020;**9**(4). doi: 10.3390/antiox9040297. [PubMed: 32252390]. [PubMed Central: PMC7222188].
17. Robertson CS, McCarthy JJ, Miller ER, Levin H, McCauley SR, Swank PR. Phase II Clinical Trial of Atorvastatin in Mild Traumatic Brain Injury. *J Neurotrauma*. 2017. doi: 10.1089/neu.2016.4717. [PubMed: 28006970].
18. Jiang R, Zhao S, Wang R, Feng H, Zhang J, Li X, et al. Safety and Efficacy of Atorvastatin for Chronic Subdural Hematoma in Chinese Patients: A Randomized ClinicalTrial. *JAMA Neurol*. 2018;**75**(11):1338–46. doi: 10.1001/jamaneurol.2018.2030. [PubMed: 30073290]. [PubMed Central: PMC6248109].

19. Naghibi T, Madani S, Mazloomzadeh S, Dobakhti F. Simvastatin's effects on survival and outcome in traumatic braininjury patients: a comparative study. *Turk J Med Sci*. 2016;**46**(1):1–5. doi: 10.3906/sag-1404-125. [PubMed: 27511325].
20. Zhang Y, Gao Z, Wang D, Zhang T, Sun B, Mu L, et al. Accumulation of natural killer cells in ischemic brain tissues and the chemotactic effect of IP-10. *J Neuroinflammation*. 2014;**11**:79. doi: 10.1186/1742-2094-11-79. [PubMed: 24742325]. [PubMed Central: PMC4039314].
21. Strandberg TE, Pyörälä K, Cook TJ, Wilhelmsen L, Faergeman O, Thorgeirsson G, et al. Mortality and incidence of cancer during 10-year follow-up of the Scandinavian Simvastatin Survival Study (4S). *The Lancet*. 2004;**364**(9436):771–7. doi: 10.1016/s0140-6736(04)16936-5.
22. Mozaffarian D, Minami E, Letterer RA, Lawler RL, McDonald GB, Levy WC. The effects of atorvastatin (10 mg) on systemic inflammation in heart failure. *Am J Cardiol*. 2005;**96**(12):1699–704. doi: 10.1016/j.amjcard.2005.07.092. [PubMed: 16360360].
23. Zheng P, Tong W. Understanding the neurotransmitter changes underlying cognitive dysfunction in traumatic brain injury and possible therapeutic targets: a review. *Arch Med Sci*. 2015;**11**(3):696–8. doi: 10.5114/aoms.2015.52380. [PubMed: 26170868]. [PubMed Central: PMC4495167].
24. Khurana S, Gupta S, Bhalla H, Nandwani S, Gupta V. Comparison of anti-inflammatory effect of atorvastatin with rosuvastatin in patients of acute coronary syndrome. *J Pharmacol Pharmacother*. 2015;**6**(3):130–5. doi: 10.4103/0976-500X.162011. [PubMed: 26311995]. [PubMed Central: PMC4544133].
25. Morandi A, Hughes CG, Girard TD, McAuley DF, Ely EW, Pandharipande PP. Statins and brain dysfunction: a hypothesis to reduce the burden of cognitive impairment in patients who are critically ill. *Chest*. 2011;**140**(3):580–5. doi: 10.1378/chest.10-3065. [PubMed: 21896517]. [PubMed Central: PMC3168859].
26. Xu X, Gao W, Cheng S, Yin D, Li F, Wu Y, et al. Anti-inflammatory and immunomodulatory mechanisms of atorvastatin in a murine model of traumatic brain injury. *J Neuroinflammation*. 2017;**14**(1):167. doi: 10.1186/s12974-017-0934-2. [PubMed: 28835272]. [PubMed Central: PMC5569493].
27. Li M, Li Z, Yao Y, Jin WN, Wood K, Liu Q, et al. Astrocyte-derived interleukin-15 exacerbates ischemic brain injury via propagation of cellular immunity. *Proc Natl Acad Sci U S A*. 2017;**114**(3):E396–405. doi: 10.1073/pnas.1612930114. [PubMed: 27994144]. [PubMed Central: PMC5255606].
28. Gan Y, Liu Q, Wu W, Yin JX, Bai XF, Shen R, et al. Ischemic neurons recruit natural killer cells that accelerate brain infarction. *Proc Natl Acad Sci U S A*. 2014;**111**(7):2704–9. doi: 10.1073/pnas.1315943111. [PubMed: 24550298]. [PubMed Central: PMC3932858].
29. Beziaud T, Ru Chen X, El Shafey N, Frechou M, Teng F, Palmier B, et al. Simvastatin in traumatic brain injury: effect on brain edema mechanisms. *Crit Care Med*. 2011;**39**(10):2300–7. doi: 10.1097/CCM.0b013e3182227e4a. [PubMed: 21666443].