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Effect of a Combinational therapy of Erythropoietin, Pentoxifylline, and Vitamin D on the Glasgow Outcome Scale in Patients with Traumatic Brain Injury

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Abstract

Background: Diffuse axonal injury (DAI) is the most prevalent nerve lesion in brain trauma. Given the known effects of erythropoietin and pentoxifylline on the reduction of cell death following hypoxia.
Objectives: The current study assessed the possible effects of a combination of erythropoietin, pentoxifylline, and vitamin D on patients' consciousness level.
Methods: The present research is a double-blind clinical trial with parallel groups addressing 64 DAI patients (average age: 26.5). The intervention group included 32 patients who, apart from the routine treatment, received a combination of erythropoietin, pentoxifylline, and vitamin D3. The control group also encompassed 32 patients who only received routine treatment. The effects of the interventions were assessed based on Glasgow Coma Scale (GCS), Glasgow Outcome Scale (GOS) scores, and hospitalization duration.
Results: The findings of the current research revealed that the GCS scores of the supplement (13.6±0.5) and control groups (12.4±1.6) were significantly different (P=0.043), being higher in the supplement group. The supplement group gained a higher GOS score compared to the controls (4.62±0.15 and 3.8±0.16, respectively, P=0.045). Moreover, a significant difference was observed in the hospitalization duration of the supplement (26.5±5.2) and control groups (21.4±3.2) (P=0.020). Nonetheless, the analysis of the extubation time and blood pressure of the two groups exhibited no substantial difference.
Conclusion: In this study, GCS and GOS improved after supplementation. The patients in the supplement group displayed a significantly shorter hospitalization duration. No significant difference was, however, detected when the tube was removed.

Keywords: Brain trauma, Diffuse brain injury, Erythropoietin, Pentoxifylline, Vitamin D

1. Background

Traumatic Brain Injury (TBI), defined as a modification in the function of the brain due to an external force, has a considerable effect on public health (1). A total of 10 million cases are annually under the influence of a new TBI event. The TBI is an important source of long-term debility in children and adults younger than 35. These diseases can exert serious impacts on patients' lifestyles (2, 3). Furthermore, it has been established that brain damage leads to poor cognitive performance, such as planning, language, memory, perception, attention, and even brain death. The Centers for Disease Control and Prevention (CDC) has asserted that during 2002-2006, on average, 1.7 million people annually experienced TBI, either alone or in conjunction with other damages and/or medical conditions (4).

In other words, TBI is predicted to be the third leading cause of global disease burden in 2020. Immediately following the initial damage, brain edema, the formation of free radicals, and inflammatory factors result in secondary brain damage, known as the main reason for mortality and morbidity (5, 6). Diffuse axonal damage (DAI) occurs in 30% of severe concussions. Today, this type of injury is considered the most common nerve lesion in brain trauma. The DAI usually affects white matter tracts complicated in the corpus callosum and brainstem. Clinically, persons with DAI can present a wide spectrum of neurological dysfunctions (7).

Since the primary damage is permanent, avoidance of secondary damage is the core of TBI management measures, necessitating the development of effective and safe neuroprotective agents to avoid secondary brain injury (8). Although various types of anti-inflammatory and antiapoptotic drugs have different neuroprotective effects on brain injury, a specific drug capable of suppressing brain injury progression has not yet been introduced.

Due to the effects of erythropoietin and pentoxifylline on improving the outcomes of brain trauma (9, 10), the present research assessed the possible influence of their combination with vitamin D on the consciousness level of patients suffering a diffuse brain injury.

2. Objectives

The current study assessed the possible effects of

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a combination of erythropoietin, pentoxifylline, and vitamin D on patients' consciousness level.

3. Methods

This controlled, double-blind, randomized clinical trial was conducted on parallel groups at Kermanshah University of Medical Science in 2018. The study protocol was approved by the Ethical Committee of Kermanshah University of Medical Science (IR.KUMS.REC.1399.813), and it is registered in the Iranian clinical trial registration site with code of IRCT20130812014333N157. The Inclusion criteria entailed an age range of 16-65 years, brain injury patients admitted to intensive care units (ICUs) with moderate (GCS 9-12) and severe severity (3<GCS< 8), admission within 24 hours after brain injury, no spinal cord injury. On the other hand, the exclusion criteria were as follows: a prior history of amblyopia or deep vein thrombosis (DVT), cancer, administration of erythropoietin in the last 30 days, pregnancy, uncontrolled hypertension (systolic above 200 or diastole above 110 mm Hg), myocardial infarction in the last year, a history of epilepsy, patient's withdrawal from the study, patient death, and GCS=3. The participants were selected via available random sampling. The sample size was calculated according to Abrishamkar et al. (2012) regarding the level of consciousness variable as follows (10).

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (\partial_1^2 + \partial_2^2)}{(\mu_1 - \mu_2)^2} = 32 \quad per \quad group$$

$\mu_2 = 3.4$ $\sigma_2 = 0.5$ $\mu_1 = 3.9$ $\sigma_1 = 0.7$ $\alpha = 0.05$ $\beta = 0.1$

The investigated population consisted of patients with brain injury who were assigned to two groups. The intervention group (n=32) received a combination of erythropoietin (2000 U for six doses in two weeks), pentoxifylline (400 mg per oral three times per day with meals for two weeks, reduce dose to 400 mg twice a day if gastrointestinal intolerance is observed), and vitamin D3 (2000 U for six doses in two weeks), in addition to the routine treatment (supplement group), while the control group (n=32) only recived routine treatments. The assessed variables included GCS (Glasgow Coma Scale), GOS (Glasgow Outcome Scale), hospitalization duration, blood pressure, extubation time, red blood cell, hematocrit, and thrombocytes. Patients were evaluated at different time intervals: the beginning of the study (before the intervention), one week, as well as one, three, and six months post-intervention.

For blinding, a pharmacist prepared and numbered identical vials containing either routine treatment or a combination of erythropoietin, pentoxifylline, and vitamin D3. In addition, the partner physician of the project, who did not know how to group the patients, gave the medicines to the patients in the specified packages; moreover, the patients admitted to the ICU did not know how to treat them. The limitation of the present study was the occurrence of adverse drug reactions that made it impossible for the patient to continue taking the medicines.

3.1. Statistics

Mann-Whitney U test and Independent sample t-test were employed for comparing the quantitative variables. Moreover, the qualitative variables were compared by the Chi-square test. Data were reported as mean±standard error. A p-value less than 0.05 was considered statistically significant.

4. Results

In this clinical trial, 64 DAI patients were screened (Figure 1), out of whom four cases died, two in the control group (reasons: malignant brain edema and sepsis) and two in the treatment group (due to pneumonia, multi-organ failure, and sepsis). The administration of intravenous heparin in the two groups showed no difference. Follow-up neurologic results are presented in Table 1. A significant difference can be detected between the GCS score of the supplement (13.6±0.5) and control groups (12.4±1.6) (P=0.043). The supplement group (4.62±0.15) had a higher GOS score than the controls (3.8±0.16) (P=0.045). Moreover, a significant difference was observed in the hospitalization duration of the supplement (26.5±5.2) and control groups (21.4±3.2) (P=0.020). Nevertheless, the extubation time and blood pressure of the two groups (P>0.05) revealed no significant difference. Table 2 illustrates hematocrit, red blood cell (RBC) counts, as well as the thrombocytes of the supplement and control groups. The results of the statistical analysis demonstrated no

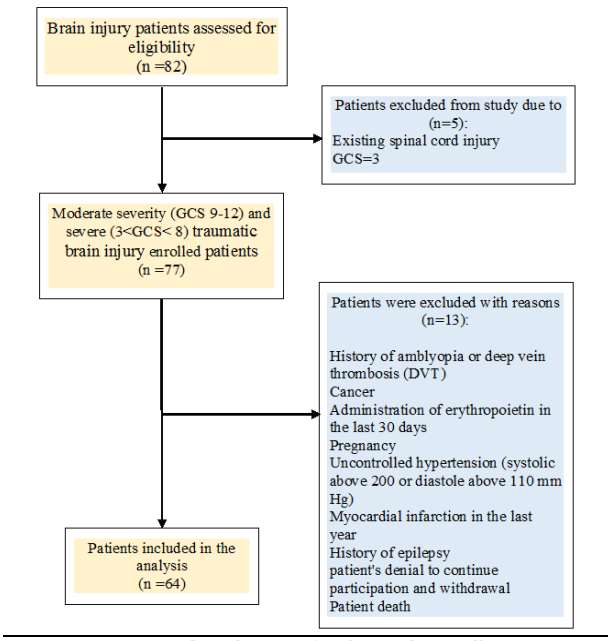

Figure 1. Consort flow diagram for the study enrollment

Table 1. Comparison of outcomes between the supplement and control groups

Variables	Control	Supplement	p-value
Age (year)	26.2±4.7	29.1±5.3	0.53 *
GCS at admission	6.3±0.5	5.6±0.7	0.120 **
GCS at discharge	12.4±1.6	13.6±0.5	0.043 **
GOS at admission	3.3±0.5	3.9±0.07	0.240 **
GOS at discharge	3.8±0.16	4.62±0.15	0.045 **
Hospitalization duration (day)	26.5±5.2	21.4 ± 3.2	0.020 **
Extubation time (day)	13.12±7.8	12.6±3.1	0.080 **
Blood pressure (mmHg)	133±7	127±6	0.070 *

GCS = Glasgow Coma Scale, GOS = Glasgow Outcome Scale
*Independent sample t-test, ** Mann-Whitney U test

Table 2. Hematocrit, red blood cell counts, and thrombocyte of patients in the consecutive estimation

	1 week		1 month		3 months		6 months	Supplement
	Control	Supplement	Control	Supplement	Control	Supplement	Control	
Hematocrit (%)	41.9±1.8	42.10 ±0.8	40.5±0.39	41.8 ±1.25	38±1.7	41.58±1.46	40.6±2.6	41.3±1.06
Red Blood Cell (10^6/ml)	4.5±0.8	4.7±0.16	4.5±0.3	4.7±0.6	4.3±0.31	4.6 ±0.5	4.5±0.18	4.8±0.5
Thrombocyte(*10^3)	223±13.1	226.4±17.1	271.6±4.2	257.3±17.4	228.4±16.1	243.4 ±3.1	281.3±5.8	242.4±17.4

significant change in the hematocrit, RBC counts, and thrombocytes of the treatment group compared to those reported in the control group(P>0.05).

5. Discussion

The present study investigated the possible neuroprotective action of a combination of erythropoietin, pentoxifylline, and vitamin D in TBI patients. The results revealed a significant enhancement in the GCS and GOS scores of the supplement group compared to those of the controls. Moreover, the hospitalization duration of the supplement group was decreased. Traumatic brain injury, which is known as a complex and multidimensional nerve injury, is accompanied by acute and chronic changes in neural functions (11, 12).

Both erythropoietin, a glycoprotein hormone with pleiotropic cytokine-like activities, and its receptor exist in the central nervous system (13). Erythropoietin and its receptor could also be found in the peripheral nervous system (14). Although erythropoietin and its receptors are present in the normal adult brain, their level will increment in neurons, glial, neuronal progenitor cells, and endothelial cells in response to injury (15). Elevated erythropoietin rates are also found in Schwann cells following peripheral nerve damage (14). Evidence suggests that peripherally administered erythropoietin can pass across the blood-brain barrier via special transport mechanisms, which might be modulated during cerebral hypoxia (16).

The related investigations have pointed out that erythropoietin improves neurological results after traumatic brain injury; therefore, it could be a potential candidate to improve secondary brain damage in traumatic brain injury (17). Previous studies clarified that in patients with acute TBI, the administration of an erythropoiesis-stimulating factor can significantly improve the survival rate with no rise in morbidity (18). Tsai et al. reported that erythropoietin therapy enhanced long-term neural effects in acute ischemic stroke patients (19). Intravenous erythropoietin was well tolerated in diffuse axonal injury (DAI) (10) and acute ischemic stroke (20); moreover, it was related to an improvement in patient outcomes.

On the other hand, Nirulaet al. indicated that erythropoietin did not decrease neuronal cell death in comparison with placebo. According to Nichol et al., erythropoietin did not change the total number of patients with proper recovery or moderate disability six months after moderate or severe TBI (21). A clinical trial on stroke illustrated no beneficiary influence of erythropoietin (greater mortality than the placebo-receiving control samples) (22). Despite its antiapoptotic effects and ability to reduce the inflammatory response, as well as its neurotrophic action, erythropoietin is actively involved in neuroprotection in various neurological trauma types (23).

Pentoxifylline (PTX) is a methylxanthine derivative and a nonspecific type 4 phosphodiesterase blocker with extensive clinical applications in the treatment of intermittent claudication (24). The neuroprotective impacts of PTX were documented among experimental models of global and focal cerebral ischemia (25). The beneficial effects of PTX are associated with modifications in the cell function and enhancement of microcirculatory perfusion in peripheral and cerebral vascular beds (26). David et al. pointed out that Pentoxifylline improves cerebral blood flow in patients suffering from cerebrovascular disease, irrelevant to "intracerebral steal" (27).

The neuroprotection of PTX against Subarachnoid hemorrhage (SAH)-induced early brain injury (EBI) has been recently reported, probably due to the elimination

of inflammation and apoptotic neural cell death. The present study revealed that PTX lowers EBI after SAH by the elimination of brain edema, decreasing BBB permeability, and reducing TNF-alpha expression, reactive nitrogen metabolites, and apoptosis (9). Bhat et al. confirmed the positive impacts of pentoxifylline in cases suffering from traumatic brain edema by antagonizing malondialdehyde as a product of lipid peroxidation (28).

Several animal studies have pointed to the positive influences of vitamin D administration on the enhancement of recovery from TBI (29). In vivo experimentations denoted that 1,25OH2D3 administration after an ischemic incident increased cell survival (30). In a previous study, Arabi et al. reported that vitamin D decreased mortality and inflammation among TBI cases (31). Moreover, vitamin D administration in TBI patients suffering from high vitamin D deficiency during the acute stage of the injury managed to increase long-term performance and cognitive functions (32). The prior administration of exogenous vitamin D could reduce infarct development and induce rapid anti-inflammatory effects in the ischemic and reperfused brain (33).

Vitamin D can increase NR3 expression and subsequently activate the CREB transcription factor by provoking pro-inflammatory extracellular signal-regulated kinase (ERK) MAPK pathways, thereby improving glutamate-induced cell degradation. The CREB expression can stimulate neurogenesis and exert a protective effect against glutamate excitotoxicity due to its antiapoptotic effects (34). Furthermore, vitamin D is involved in protecting close junctions of endothelial cells in the BBB through numerous pathways via its negative impact on matrix metalloproteinase activity. After hypoxia, Vitamin D can reduce the expression of MMP-9, thereby stimulating BBB impermeability (35).

5.1. Limitations

The notable limitation of the present study was the occurrence of unwanted drug reactions that make it impossible for the patient to continue taking the drug.

6. Conclusion

The present research clarified the therapeutic effect of a combinational therapy comprising erythropoietin, pentoxifylline, and vitamin D in TBI patients by reducing inflammation and mortality from infection. The clinical results encourage the application of a combinational therapy, including erythropoietin, pentoxifylline, and vitamin D for TBI patients.

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None.

Footnotes

Conflicts of Interest: The authors declare no conflicts of interest.
Author contributions: Akrami M.R. and Bagheri R. designed the experiments; Akrami A. performed experiments; Saeidi Borojeni H., Akrami M.R. and Bagheri R. analyzed data; Saeidi Borojeni H. wrote and edited the manuscript. All authors reviewed the manuscript.
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Ethical considerations: The protocol of the clinical trial, which was conducted according to the ethical principles of the Declaration of Helsinki (version 2002), was approved by the Ethics Committee of Kermanshah University of Medical Sciences.

References

1. Menon DK, Schwab K, Wright DW, Maas AI. Position statement: definition of traumatic brain injury. *Arch Phys Med Rehabil*. 2010;**91**(11):1637-40. doi: 10.1016/j.apmr.2010.05.017. [PubMed: 21044706].
2. Hyder AA, Wunderlich CA, Puvanachandra P, Gururaj G, Kobusingye OC. The impact of traumatic brain injuries: a global perspective. *NeuroRehabilitation*. 2007;**22**(5):341-53. [PubMed: 18162698].
3. Feigin VL, Theadom A, Barker-Collo S, Starkey NJ, McPherson K, Kahan M, et al. Incidence of traumatic brain injury in New Zealand: a population-based study. *Lancet Neurol*. 2013; **12**(1):53-64. doi: 10.1016/S1474-4422(12)70262-4. [PubMed: 23177532].
4. Faul M, Wald MM, Xu L, Coronado VG. Traumatic brain injury in the United States; emergency department visits, hospitalizations, and deaths, 2002-2006. CDC; 2010.
5. Gentleman D. Preventing secondary brain damage after head injury: a multidisciplinary challenge. *Injury*. 1990;**21**(5):305-8. doi: 10.1016/0020-1383(90)90047-x. [PubMed: 2242917].
6. Matthews DS, Matthews JN, Aynsley-Green A, Bullock RE, Eyre JA. Changes in cerebral oxygen consumption are independent of changes in body oxygen consumption after severe head injury in childhood. *J Neurol Neurosurg Psychiatry*. 1995;**59**(4):359-67. doi: 10.1136/jnnp.59.4.359. [PubMed: 7561912].
7. Feng D, Ma Y, Zhang Y, Plets C, Goffin J, Chen J. Controlled study of nimodipine in treatment of patients with diffuse axonal injury. *Chin J Traumatol*. 2000;**3**(2):85-8. [PubMed: 11874647].
8. Liu WC, Wen L, Xie T, Wang H, Gong JB, Yang XF. Therapeutic effect of erythropoietin in patients with traumatic brain injury: a meta-analysis of randomized controlled trials. *J Neurosurg*. 2016;**127**(1):8-15. doi: 10.3171/2016.4.JNS152909. [PubMed: 27367243].
9. Goksu E, Dogan O, Ulker P, Tanrıover G, Konuk E, Dilmac S, et al. Pentoxifylline alleviates early brain injury in a rat model of subarachnoid hemorrhage. *Acta Neurochir (Wien)*. 2016; **158**(9):1721-30. doi: 10.1007/s00701-016-2866-5. [PubMed: 27311763].
10. Abrishamkar S, Safavi M, Honarmand A. Effect of erythropoietin on Glasgow Coma Scale and Glasgow Outcome Sale in patient with diffuse axonal injury. *J Res Med Sci*. 2012;**17**(1):51-6. [PubMed: 23248657].
11. Stein DG. Embracing failure: what the phase III progesterone studies can teach about TBI clinical trials. *Brain Inj*. 2015;**29**(11):1259-72. doi: 10.3109/02699052.2015.1065344. [PubMed: 26274493].
12. Daneshvar DH, Goldstein LE, Kiernan PT, Stein TD, McKee AC.

Post-traumatic neurodegeneration and chronic traumatic encephalopathy. *Mol Cell Neurosci.* 2015;**66**:81-90. doi: 10.1016/j.mcn.2015.03.007. [PubMed: 25758552].
13. Buemi M, Cavallaro E, Floccari F, Sturiale A, Aloisi C, Trimarchi M, et al. The pleiotropic effects of erythropoietin in the central nervous system. *J Neuropathol Exp Neurol.* 2003;**62**(3):228-36. doi: 10.1093/jnen/62.3.228. [PubMed: 12638727].
14. Campana WM, Myers RR. Erythropoietin and erythropoietin receptors in the peripheral nervous system: changes after nerve injury. *FASEB J.* 2001;**15**(10):1804-6. doi: 10.1096/fj.00-0857fje. [PubMed: 11481236].
15. Grasso G. Neuroprotective effect of recombinant human erythropoietin in experimental subarachnoid hemorrhage. *J Neurosurg Sci.* 2001;**45**(1):7-14. [PubMed: 11466502].
16. Lykissas MG, Korompilias AV, Vekris MD, Mitsionis GI, Sakellariou E, Beris AE. The role of erythropoietin in central and peripheral nerve injury. *Clin Neurol Neurosurg.* 2007;**109**(8):639-44. doi: 10.1016/j.clineuro.2007.05.013. [PubMed: 17624659].
17. Yatsiv I, Grigoriadis N, Simeonidou C, Stahel PF, Schmidt OI, Alexandrovich AG, et al. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. *FASEB J.* 2005;**19**(12):1701-3. doi: 10.1096/fj.05-3907fje. [PubMed: 16099948].
18. Talving P, Lustenberger T, Inaba K, Lam L, Mohseni S, Chan L, et al. Erythropoiesis-stimulating agent administration and survival after severe traumatic brain injury: a prospective study. *Arch Surg.* 2012;**147**(3):251-5. doi: 10.1001/archsurg.2011.1838. [PubMed: 22430906].
19. Tsai TH, Lu CH, Wallace CG, Chang WN, Chen SF, Huang CR, et al. Erythropoietin improves long-term neurological outcome in acute ischemic stroke patients: a randomized, prospective, placebo-controlled clinical trial. *Crit Care.* 2015;**19**(1):1-9. doi: 10.1186/s13054-015-0761-8. [PubMed: 25888250].
20. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, et al. Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med.* 2002;**8**(8):495-505. [PubMed: 12435860].
21. Nichol A, French C, Little L, Haddad S, Presneill J, Arabi Y, et al. Erythropoietin in traumatic brain injury (EPO-TBI): a double-blind randomised controlled trial. *Lancet.* 2015;**386**(10012):2499-506. doi: 10.1016/S0140-6736(15)00386-4. [PubMed: 26452709].
22. Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, et al. Recombinant human erythropoietin in the treatment of acute ischemic stroke. *Stroke.* 2009;**40**(12):647-56. doi: 10.1161/STROKEAHA.109.564872. [PubMed: 19834012].
23. Gorio A, Gokmen N, Erbayraktar S, Yilmaz O, Madaschi L, Cichetti C, et al. Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. *Proc Natl Acad Sci U S A.* 2002;**99**(14):9450-5. doi: 10.1073/pnas.142287899. [PubMed: 12082184].
24. Doyle J, Creager MA. Pharmacotherapy and behavioral intervention for peripheral arterial disease. *Rev Cardiovasc Med.* 2003;**4**(1):18-24.
25. Vakili A, Mojarrad S, Akhavan MM, Rashidy-Pour A. Pentoxifylline attenuates TNF-α protein levels and brain edema following temporary focal cerebral ischemia in rats. *Brain Res.* 2011;**1377**:119-25. doi: 10.1016/j.brainres.2011.01.001. [PubMed: 21219888].
26. Neves KRT, Nobre Jr HV, Leal LKA, de Andrade GM, de Castro Brito GA, de Barros Viana GS. Pentoxifylline neuroprotective effects are possibly related to its anti-inflammatory and TNF-alpha inhibitory properties, in the 6-OHDA model of Parkinson's disease. Parkinsons Dis. 2015;**2015**:1-15. doi: 10.1155/2015/108179. [PubMed: 26491600].
27. Bowton DL, Stump DA, Prough DS, Toole JF, Lefkowitz DS, Coker L. Pentoxifylline increases cerebral blood flowin patients with cerebrovascular disease. *Storke.* 1989;**20**(12):1662-6. doi: 10.1161/01.str.20.12.1662. [PubMed: 2512690].
28. Bhat AR, Wani MA, Kirmani A, Raina T, Alam S, Ramzan A. Traumatic brain edema and survival-effective role of pentoxifylline. *Biomed Res J.* 2008;**19**(3):161-7.
29. Cekic M, Cutler SM, VanLandingham JW, Stein DG. Vitamin D deficiency reduces the benefits of progesterone treatment after brain injury in aged rats. *Neurobiol Aging.* 2011;**32**(5):864-74. doi: 10.1016/j.neurobiolaging.2009.04.017. [PubMed: 19482377].
30. Atif F, Yousuf S, Sayeed I, Ishrat T, Hua F, Stein DG. Combination treatment with progesterone and vitamin D hormone is more effective than monotherapy in ischemic stroke: the role of BDNF/TrkB/Erk1/2 signaling in neuroprotection. *Neuropharmacology.* 2013;**67**:78-87. doi: 10.1016/j.neuropharm.2012.10.004. [PubMed: 23154302].
31. Arabi SM, Sedaghat A, Ehsaei MR, Safarian M, Ranjbar G, Rezaee H, et al. Efficacy of high-dose versus low-dose vitamin D supplementation on serum levels of inflammatory factors and mortality rate in severe traumatic brain injury patients: study protocol for a randomized placebo-controlled trial. *Trials.* 2020;**21**(1):1-8. doi: 10.1186/s13063-020-04622-6.
32. Lee JM, Jeong SW, Kim MY, Park JB, Kim MS. The effect of vitamin D supplementation in patients with acute traumatic brain injury. *World Neurosurg.* 2019;**126**:1421-6. doi: 10.1016/j.wneu.2019.02.244. [PubMed: 30904798].
33. Evans MA, Kim HA, Ling YH, Uong S, Vinh A, De Silva TM, et al. Vitamin D 3 supplementation reduces subsequent brain injury and inflammation associated with ischemic stroke. *Neuromolecular Med.* 2018;**20**(1):147-59. doi: 10.1007/s12017-018-8484-z. [PubMed: 29476479].
34. Fu J, Xue R, Gu J, Xiao Y, Zhong H, Pan X, et al. Neuroprotective effect of calcitriol on ischemic/reperfusion injury through the NR3A/CREB pathways in the rat hippocampus. *Mol Med Rep.* 2013;**8**(6):1708-14. doi: 10.3892/mmr.2013.1734. [PubMed: 24141895].
35. Colón YM. Vitamin D clinical relevance in the recovery from traumatic brain injury among the military population. [Thesis]. University of Central Florida; 2016.