



# Investigating the Effect of High-Dose Vitamin D3 Administration on Inflammatory Biomarkers in Patients with Moderate to Severe Traumatic Brain Injury: A Randomized Clinical Trial

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## What's Known

- Vitamin D deficiency is common in traumatic brain injury patients and may negatively affect the clinical course of the disease.
- Numerous studies have been conducted on vitamin D deficiency in patients with brain injury, but few clinical trials have focused on its administration, dosing, and anti-inflammatory role.

## What's New

- High-dose vitamin D administration resulted in a reduction in interleukin-1 $\beta$  level, as a relatively specific biomarker in traumatic brain injury patients.
- The reduction in neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) confirms the anti-inflammatory effect of vitamin D.

## Abstract

**Background:** Traumatic brain injury (TBI) is one of the most common neurological disorders worldwide. We aimed to investigate the efficacy of high-dose vitamin D3 on inflammatory biomarkers in patients with moderate to severe TBI.

**Methods:** Thirty-five moderate to severe TBI patients were randomly assigned to intervention and control groups. Patients in the intervention group received a single intramuscular (IM) dose of 300,000 IU vitamin D. The primary endpoints were interleukin levels (IL-1 $\beta$  and IL-6), and the secondary endpoints were changes in neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), Glasgow Coma scale (GCS), and Glasgow Outcome Scale-Extended (GOS-E) scores compared between intervention and control arms of the study. The linear Generalized Estimating Equations were used for trend analysis and evaluating the association of independent factors to each outcome.

**Results:** The results revealed a significant decrease in IL-1 $\beta$  levels (-2.71 $\pm$ 3.02, in the intervention group: P=0.001 vs. -0.14 $\pm$ 3.70, in the control group: P=0.876) and IL-6 (-88.05 $\pm$ 148.45, in the intervention group: P=0.0001 vs. -35.54 $\pm$ 175.79, in the control group: P=0.325) 3 days after the intervention. The improvement in the GCS score (P=0.001), reduction in NLR (P=0.001) and PLR (P=0.002), and improvement in the GOS-E score (P=0.039) was found to be greater in the vitamin D3 arm of the study than the control group.

**Conclusion:** Administration of high-dose vitamin D3 in the acute phase of TBI could be effective in lowering the inflammatory markers and improving the level of consciousness and long-term performance outcomes.

Trial registration number: IRCT20180522039777N2.

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**Keywords** • Vitamin D • Brain injuries, traumatic • Inflammation mediators

## Introduction

Traumatic brain injury (TBI) is a major worldwide neurological disorder that can be defined as any impact on the brain that disrupts the normal function of the brain.<sup>1</sup> TBI represents a significant cause of death and disability in patients with trauma,

and the annual incidence is variably estimated at 27 to 69 million cases.<sup>2</sup>

TBI has traditionally been categorized as mild, moderate, and severe based on a neurological scale named the Glasgow Coma Scale (GCS) and is classified as primary and secondary depending on timing and type of injury. Primary injury is reversible and occurs at the time of injury by mechanical force, while secondary damage results from oxidative stress caused by trauma, ischemia, edema, and inflammatory response, leading to neurological dysfunction.<sup>3</sup> Therefore, inflammation plays an important role in TBI physiology. Within minutes of trauma, a robust inflammatory response occurs in the injured brain. This inflammatory response after trauma is initiated and regulated by a set of pro- and anti-inflammatory cytokines. Tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 $\beta$ , and IL-6 are the most important proinflammatory cytokines.<sup>4</sup>

Serum level of IL-1 $\beta$  is correlated with initial TBI severity, increased intracranial pressure, and poor outcome<sup>5, 6</sup> and can be detected in blood for a maximum of 48 hours after TBI.<sup>7</sup> The result of a study indicated that by 72 hours post-injury, there was a nearly fivefold increase in IL-6 levels, thereby suggesting that deficient levels of vitamin D may have a significant impact on the pathogenesis of TBI.<sup>8</sup>

The role of various inflammatory cells in TBI, such as neutrophils, lymphocytes, and other blood cells in brain damage, has been investigated. The mentioned various ratios could be determined based on the blood count results that show ongoing inflammatory processes; among them, the neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are reliable and simple indicators of inflammation with great diagnostic power for short-term mortality, thereby being directly associated with poor clinical outcomes.<sup>9-11</sup>

There are numerous predictors of outcomes in patients with TBI. Glasgow Outcome Scale-Extended (GOS-E) has become one of the most widely used outcome measures for assessing overall disability and recovery following TBI.<sup>12</sup> It adequately presents clinical outcomes at discharge and in the months following patient discharge.<sup>13</sup> Another predictor is a Computed tomography (CT) scan, which is also increasingly being used as a major predictors of TBI. The international guideline on prognosis considers the CT classification as a significant predictor of prognosis, based on class 1 evidence.<sup>14</sup> Marshall<sup>15</sup> and Rotterdam<sup>16</sup> are two grading systems that are great in predicting early mortality and 30-day outcomes after TBI.

Vitamin D is a group of fat-soluble secosteroids whose role, in addition to calcium and phosphorus homeostasis, is to regulate proliferative and apoptotic activity. They play an immunomodulatory role also known as a neuroprotective and anti-inflammatory agent. In addition to these known effects, vitamin D also acts as an antioxidant throughout the expression of multiple molecules.<sup>17</sup> Vitamin D deficiency is a risk factor for states of high incidence of adverse events and mortality in patients, particularly in critically-ill ones.<sup>18</sup> Moreover in traumatic brain injury, low serum vitamin D levels are common and associated with several neurological disorders including coma, slow neurological recovery, cognitive impairment, and polyneuropathy.<sup>19, 20</sup> Studies have shown that vitamin D deficiency may negatively affect the clinical course of TBI.<sup>21, 22</sup>

In addition, numerous studies have been conducted on vitamin D deficiency in patients with brain injury, but few clinical trials have focused on its administration, dosing, and anti-inflammatory role.<sup>22, 23</sup> This study aimed to assess the effect of high-dose IM vitamin D3 administration on inflammatory biomarkers such as IL-1 $\beta$  and IL-6 after moderate to severe brain injury.

## Patients and Methods

### Study Design

This was a single-center, open-labeled, randomized controlled trial that took place in the intensive care unit (ICU) of Imam Hussein Medical Center, a 600-bed hospital affiliated with Shahid Beheshti University of Medical Science (SBMU) in Tehran, Iran, from June 2022 to April 2023. The study was approved by the Local Ethics Committee (IR.SBMU.PHARMACY.REC.1401.075) and registered at the Iranian Registry of Clinical Trials (IRCT20180522039777N2).

### Study Population

We evaluated the eligibility of all TBI patients admitted to Imam Hussein ICU. Inclusion and exclusion criteria are given below. Patients who did not allocated in one of the study groups during 24 hours after trauma, and who died within 72 hours of TBI, were excluded. The inclusion criteria of this study were TBI patients ranged between 18 to 65 years old, a GCS score between 3 to 12 within the first 24 hours after TBI, and a vitamin D3 level <30 ng/mL.

Non-entry criteria were hypercalcemia (Ca >10.5 mg/dL), administration of corticosteroids, Non-Steroid

Anti-Inflammatory Drugs (NSAIDs), and statins in the past 2 weeks, pregnancy and breastfeeding, history of vitamin D allergy, poor chance of survival, and history of cancer, autoimmune disorders, liver diseases (Child-Pugh C), and End-stage renal disease (ESRD).

#### *Study Protocol*

The block randomization method was considered for this study. TBI patients were allocated (in five blocks in size of 6, 8, 10) into the intervention group and control group. Permuted block randomization was carried out by blocks generated by the sealed envelope website (<https://www.sealedenvelope.com/simple-randomiser/v1/lists>).

Informed consent was obtained from each patient's next of kin, before they were recruited into the study. Patients in the intervention and control groups were managed with the 2020 TBI Guideline.<sup>24</sup> Once the patients were hospitalized and the inclusion criteria were met, they were randomly allocated to receive a single IM dose of 300,000 IU of vitamin D3 (DITHERCOL®, Daroupakhsh, Iran). During the study, patients' GCS was recorded daily for up to 7 days. The serum level of vitamin D3 was obtained by measuring 25-OH vitamin D3 at baseline by Imam Hussein Hospital Laboratory using a chemiluminescence immunoassay. NLR and PLR were calculated and recorded on the first day and for seven consecutive days after ICU admission. The serum levels of IL-1 and IL-6 in each patient were checked the first and third days after entry into the study. To analyze serum cytokine levels, a 10-mL peripheral venous blood sample was taken from patients on admission and 3 days later. The serum was separated by a centrifuge and transferred into a -70 freezer. Demographic data including age, sex, mechanism of trauma, and past medical and drug history were recorded on admission. Clinical and laboratory values were recorded on the first day following the assignment and for seven consecutive days. The acute physiology and chronic health evaluation (APACHE II) score was calculated based on the information retrieved from the patients in ICU at the start of the study.<sup>25</sup> We also assessed the Marshall and Rotterdam's scores for each patient in the beginning of the study.

Following the collection of all samples, the IL-1 $\beta$  serum level was measured using a human ELISA kit demeditec® (Demeditec Diagnostics GmbH company, Germany) and IL-6 using a chemiluminescence immunoassay. Other parameters such as neutrophils, lymphocytes, and platelets were retrieved from the Hospital

Information System (HIS). NLR was calculated as the ratio of absolute neutrophil numbers to lymphocyte numbers, and PLR was computed as the ratio of platelet counts to lymphocytes. Brain injuries were classified based on neuroradiological results of the admission computed tomography (CT) scans.

GOS-E is rated on an eight-point ordinal scale, from death (1) to good recovery (8). Three months after the patient was discharged from the hospital, the patient or their companion were called, and this score was evaluated.<sup>26</sup>

The primary endpoints in this trial were changes in IL-1 $\beta$  and IL-6 levels following intervention. Secondary outcomes were comparing changes in NLR, PLR, GCS and GOS-E scores between the intervention and the control group.

#### *Sample Size*

Based on the results of a previous study, mean and standard deviation of IL-6 in active group as 6.37 $\pm$ 4.25 and in control group as 11.04 $\pm$ 4.02 after intervention,<sup>27</sup> at 5% significance ( $\alpha=0.05$ ) with 80% power ( $\beta=0.2$ ), by a 15% probability of attrition rate, about 17 subjects in each group were employed (N total=34).

#### *Statistical Analysis*

The normality of the continuous variables was assessed using Shapiro-Wilk's test. Mean $\pm$ SD was used for reporting continuous variables, and frequency and percentage were used to describe categorical variables.

To compare the mean of continuous variables between vitamin D3 and control groups, parametric or non-parametric tests such as student's *t* test or Mann-Whitney U test were used. The equality of variances for continuous variables with normal distribution was assessed using Levene's test. Chi square test or Fisher's exact test was used to compare the difference between frequency of categorical variables. Paired *t* test or Wilcoxon signed-rank test based on normal distribution of continuous variables were employed to compare the paired continuous values such as IL-1 $\beta$  and IL-6 (before and after the intervention).

Ordinal regression model in univariate and multivariable levels was used for assessing effect of intervention on three-months Glasgow Outcome Scale-Extended (GOS-E) with considering confounder variables.

Linear Generalized Estimating Equations (GEE) with exchangeable correlation structure was considered as repeated measurements to assess the changes in NLR, PLR, and GCS in 7 days. Besides, for assessing the effect of

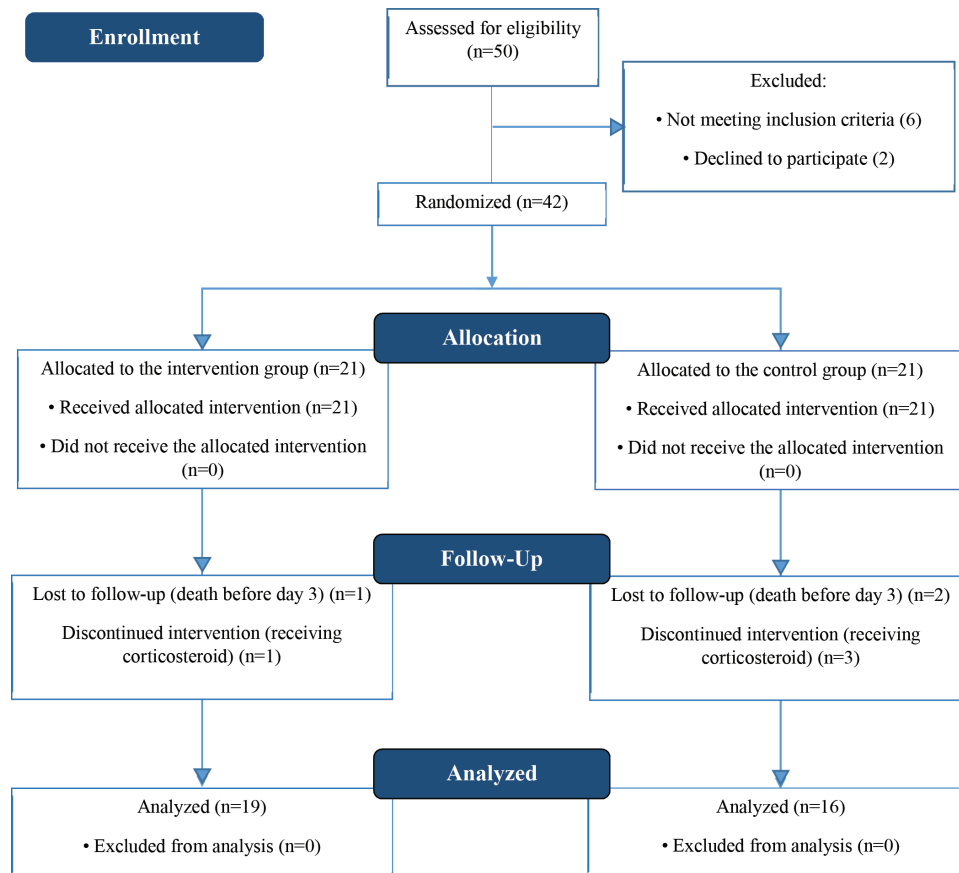
intervention on mean changes of NLR, PLR, and GCS, confounder variables and univariate and multivariable GEE with exchangeable correlation structure were used. For selecting the best variables to enter the last multivariable models, stepwise selection method with backward approach (with  $P \leq 0.2$ ) was used. Goodness of fit of last model was based on the smallest estimation of Quasi-likelihood under Independence Model Criterion (QIC). All of the statistical analysis performed at a significant level less than 0.05 with 95% confidence interval (CI) using STATA software version 14 (StataCorp LLC, College Station, TX 77845, USA).

## Results

Forty-two potential patients in the ICU were identified to participate in the study. The flow diagram of participants' progress through the study is shown in figure 1. Seven patients, two in the intervention and five in the study's control arms were excluded because of death before day 3 and receiving corticosteroids. Ultimately, 35 TBI patients based on inclusion criteria were recruited, 19 and 16 in intervention and control groups, respectively. All these patients were deficient in vitamin D3 levels. Table 1 shows the demographic, clinical, laboratory,

and radiological findings upon admission. There were no statistically significant differences in Marshall and Rotterdam scores and type of TBI in the two arms of the study.

Cytokine analysis showed no significant difference in IL-1 $\beta$  and IL-6 at baseline in two groups (table 1). But 3 days after vitamin D3 administration, the mean values showed a reduction of IL-1 $\beta$  ( $10.90 \pm 4.53$  in the vitamin D group and  $11.64 \pm 4.56$  in the control group,  $P=0.638$ ) and IL-6 ( $61.05 \pm 93.73$  in vitamin D group and  $123.53 \pm 243.60$  in the control group,  $P=0.389$ ) in the intervention group compared to the control group. Analysis of within-group changes in IL-1 $\beta$  revealed a significant decrease in IL-1 $\beta$  levels from baseline to the third day of the study only in the intervention group (in the intervention group:  $-2.71 \pm 3.02$ ,  $P=0.001$  vs. in the control group:  $-0.14 \pm 3.70$ ,  $P=0.876$ ). In the case of IL-6, these within-group changes showed a significant decrease in the intervention group 3 days after the intervention (in the intervention group:  $-88.05 \pm 148.45$ ,  $P=0.0001$  vs. control group:  $-35.54 \pm 175.79$ ,  $P=0.325$ ). Furthermore, a between-group analysis revealed that there was a statistically significant difference in the mean IL-1 $\beta$  levels in the vitamin D group following interventions compared with the control group ( $P=0.030$ ).



**Figure 1:** This figure represents the CONSORT flow diagram of the study.

**Table 1:** Baseline demographic, radiologic, and clinical characteristics

Variables	Vitamin D (n=19, 54.29%)	Control (n=16, 45.71%)	Total (n=35, 100%)	P value	
Age (years)	37.68±13.39	38.12±15.11	37.88±13.99	0.986	
Sex	Female	1 (5.26)	3 (18.75)	4 (11.43)	0.212
	Male	18 (94.74)	13 (81.25)	31 (88.57)	
Vitamin D level (ng/mL)	15.95±8.13	17.84±5.34	16.81±6.96	0.432	
Initial APACHE II score	7.57±1.80	9.87±3.03	8.62±2.66	0.013*	
Marshall score	2.68±1.56	2.43±1.82	2.57±1.66	0.682	
Rotterdam score	2.42±1.21	1.87±1.58	2.17±1.40	0.257	
Type of traumatic brain injury (TBI) (Yes)					
ICH	6 (31.58)	8 (50.00)	14 (40.00)	0.268	
EDH	6 (31.58)	2 (12.50)	8 (22.86)	0.244	
SDH	5 (26.32)	2 (12.50)	7 (20.00)	0.415	
SAH	1 (5.26)	2 (12.50)	3 (8.57)	0.582	
Contusion	2 (10.53)	1 (6.25)	3 (8.57)	1.000	
Depressed fracture	1 (5.26)	2 (12.50)	3 (8.57)	0.582	
DAI	2 (10.53)	1 (6.25)	3 (8.57)	1.000	
IVH	1 (5.26)	3 (18.75)	4 (11.43)	0.312	
Biomarkers	Baseline IL-1β (pg/mL)	13.62±5.24	11.78±4.55	12.78±4.95	0.282
	Baseline IL-6 (pg/mL)	149.11±230.07	159.08±251.81	153.66±236.69	0.642

Values described as mean±SD or n (%). \*Statistically significant, P<0.05; P value is based on student's *t* test or Mann-Whitney U test for comparing means and Chi square or Fisher's exact tests for comparing frequencies. APACHE II: Acute Physiology and Chronic Health Evaluation II; ICH: Intracranial Hemorrhage; EDH: Epidural Hemorrhage; SDH: Subdural Hemorrhage; SAH: Subarachnoid Hemorrhage; DAI: Diffuse Axonal Injury; IVH: Intraventricular hemorrhage

**Table 2:** Changes in IL-1β and IL-6 before and after the intervention between groups

Biomarkers	Mean±SD difference	Paired comparison, P value <sup>2</sup>	Between groups comparison <sup>3</sup> for IL-1β	Between groups comparison <sup>3</sup> for IL-6
Vitamin D group			0.030*	0.085
<sup>1</sup> Difference of IL-1β	-2.71±3.02	0.001*		
Difference of IL-6	-88.05±148.45	0.0001*		
Control group				
<sup>1</sup> Difference of IL-1β	-0.14±3.70	0.876		
Difference of IL-6	-35.54±175.79	0.325		

Values described as mean±SD. <sup>1</sup>Difference: Value after–value before; <sup>2</sup>Comparison of before intervention value with after intervention value (paired comparison) based on paired *t* test or Wilcoxon signed-rank test. <sup>3</sup>Comparison of difference values (IL-1β or IL-6) between two groups of intervention based on student's *t* test or Mann-Whitney U test. \*Statistically significant, P<0.05

**Table 3:** Effect of intervention on 3-month Glasgow Outcome Scale-Extended (GOS-E) based on univariate and multivariable ordinal regression

Variables	Crude OR <sup>1</sup> (95% CI)	P value	Adjusted OR (95% CI)	P value
Age (years)	0.98 (0.94–1.02)	0.409	0.97 (0.92–1.01)	0.229
Sex	Female	Reference	Reference	0.010*
	Male	0.12 (0.01–1.22)	0.04 (0.004–0.47)	
Groups	Control	Reference	Reference	0.039*
	Vitamin D	4.88 (1.33–17.85)	5.38 (1.08–26.71)	
Baseline Vitamin D (ng/mL)	1.04 (0.95–1.14)	0.326	1.08 (0.98–1.20)	0.110
Baseline APACHE II score	0.53 (0.38–0.73)	<0.001*	0.56 (0.40–0.78)	0.001*

<sup>1</sup>Statistically significant, P<0.05; <sup>1</sup>Odds Ratio, 95% Confidence Interval based on ordinal regression

However, the mean differences in IL-6 levels between the two arms after 3 days of the study were not statistically significant (P=0.085) (table 2).

The GOS-E score analysis after three months was higher in the vitamin D3 group than in the control group (P=0.017). Patients in the vitamin D3 group were found to improve on the GOS-E scale 3 months after discharge, roughly

five times more likely than the control group, according to the results in table 3 after adjusting for the effects of confounding variables. (OR=5.38, 95% CI=1.08-26.71, P=0.039).

As shown in figure 2, 7 days after TBI, changes in the mean of NLR, PLR, and GSC was observed. The mean of NLR in vitamin D3 group was statistically decreased (P=0.001). In addition, there was a significant interaction

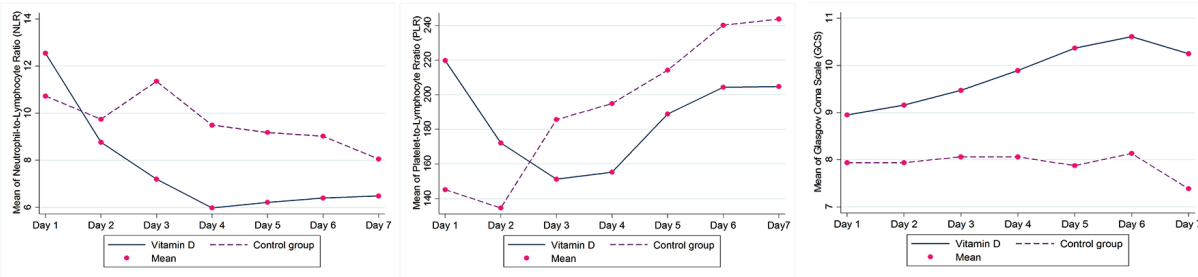


Figure 2: Changes in the mean of NLR, PLR, and GCS between the two groups during study.

Table 4: Results of univariate and multivariable linear generalized estimating equation about effect of intervention on mean changes of NLR, PLR, and GCS during study

Factors	Groups	Model 1 Crude $\beta$ (95% CI)	P value	Model 2 Adjusted $\beta$ (95% CI)	P value
NLR	Control	Reference	0.002*	Reference	0.002*
	Vitamin D	-3.07 (-5.02--1.12)		-3.78 (-6.21--1.36)	
PLR	Control	Reference	0.016*	Reference	0.001*
	Vitamin D	-45.95 (-83.35--8.55)		-64.63 (-103.96--25.30)	
GCS	Control	Reference	0.044*	Reference	0.102
	Vitamin D	1.12 (0.03--2.22)		0.97 (-0.19--2.13)	

\*Coefficient ( $\beta$ ), 95% Confidence Interval based on linear GEE; Model 1: Intercept, groups; Model 2: intercept, sex, age, groups, initial Vitamin D concentration, initial APACHE II score

effect between time and interventional groups in NLR factor ( $P=0.001$ ). Mean of PLR significantly changed in both groups through intervention period, and the interaction effect between two groups was significant ( $P<0.001$ ). Mean of GCS in vitamin D group was statistically increased ( $P=0.001$ ). Moreover, the interaction effect between time and interventional groups in GCS was statistically significant ( $P=0.001$ ) (figure 2).

Table 4 shows the effect of the intervention on changes in NLR, PLR, and GCS. In univariate model analysis, the average of NLR and PLR were significantly decreased, and the mean GCS was significantly increased in the vitamin D group compared with the control group. The results from multivariate model analysis revealed a statistically significant association between the intervention group and the changes in the average of NLR and PLR even after accounting for confounding factors such as age, sex, baseline vitamin D levels, and admission APACHE II score. Thus, the vitamin D group had a mean NLR reduction of approximately four units ( $\beta=-3.78$ , 95% CI=-6.21--1.36,  $P=0.002$ ) and a mean PLR reduction of approximately 65 units ( $\beta=-64.63$ , 95% CI=-103.96, -25.30,  $P=0.001$ ) compared to the control group. For the GCS factor, the mean of this scale increased by approximately one unit in the vitamin D group compared to the control group. Still, this increase was not statistically significant ( $P=0.102$ ).

There was no statistically significance difference in 28-day mortality rate (0% vs. 12.5%,

$P=0.202$ ), length of ICU stay ( $11.37\pm 5.95$  vs.  $12.70\pm 6.47$ ,  $P=0.571$ ), and need for mechanical ventilation (78.95% vs. 75%,  $P>0.999$ ) between the vitamin D and control groups, respectively.

## Discussion

In this open-labeled randomized controlled study, our main findings were a significant decrease in IL-1 $\beta$  and IL-6 levels from baseline to day 3 of the study in the intervention group compared to the control. Seven days after intervention, the improvement in the GCS score and reduction in NLR and PLR was found to be greater in the vitamin D3 group than in the control group. The improvement of GOS-E score compared to control group was recorded.

It is well accepted that cytokine levels, *i.e.*, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, increase after a severe injury and indicate the extent of systemic inflammation.<sup>6</sup> IL-1 $\beta$  and TNF- $\alpha$  induce activation of the inflammatory cascade after TBI and adversely affect improvement in trauma patients. Among these cytokines, IL-1 $\beta$  is a potent proinflammatory biomarker whose expression increases rapidly after injury.<sup>28</sup> Our study's findings demonstrated that vitamin D3 supplementation decreased cytokine levels. Although the decrease in the level of cytokines on day 3 of the study was not statistically significant, the difference in levels of IL-1 $\beta$  and IL-6 between baseline and day three in the supplement group was significantly different from the control group. The results of a study on TBI patients,

recorded a noticeable reduction in inflammatory biomarkers in the vitamin D-treated group (IL-6  $P=0.08$ , TNF- $\alpha$   $P=0.02$ , IL-2,  $P=0.36$ ).<sup>8</sup> After 7 days of vitamin D3 administration, a significant decrease in NLR and PLR was observed in the intervention group. In a preclinical trial, which demonstrated a protective effect of vitamin D3 supplementation on some inflammatory indices, there was a significant decrease in NLR after vitamin D supplementation in the normal diet rats.<sup>29</sup> These results demonstrated the anti-inflammatory and immunomodulatory effects of vitamin D during the acute phase of TBI.

Our study detected a significant increase in GCS levels. The results of a randomized clinical trial on 35 moderate to severe TBI patients in India showed an improvement in the level of consciousness after 7 days in the vitamin D3-treated group compared with the placebo.<sup>8</sup> In an analysis of the performance outcome, the vitamin D3 group were found to achieve a greater GOS-E score at 3 months post-TBI than the control group; these results agree with the data of a recent retrospective study. The study's authors indicated that the GOS-E score significantly improved from the first week to 3 months post mild to moderate TBI in the patients with vitamin D3 supplementation.<sup>22</sup> A prospective study of 497 ICU patients concluded that vitamin D deficient patients had significantly lower GOS scores than the vitamin D sufficient group 3 months after hospital discharge.<sup>30</sup> Another retrospective study by Jamall and colleagues indicated that vitamin D deficiency is common in TBI patients and is associated with cognitive impairment and more severe depressive symptoms.<sup>19</sup> All patients in our study had vitamin D deficiency. Vitamin D deficiency may be one of the several culprits for the long-term outcome and cognitive performance of TBI patients.

As far as we know, clinical studies of vitamin D in head injury patients are very limited, and the optimum dosage, treatment duration, and route of administration are unknown. Numerous studies have been done on the effects of high vitamin D3 doses in critically-ill patients. Research by Miri and colleagues showed that a 300,000-unit vitamin D3 injection given via the IM route could effectively reduce the duration of mechanical ventilation and mortality rate in ICU.<sup>31</sup> In another study, Miroliaee and colleagues revealed that vitamin D supplementation (300,000 units) could significantly lower procalcitonin in ventilator associated pneumonia (VAP) patients without any significant adverse events, and should be considered as a preventive and/or therapeutic strategy.<sup>32</sup>

In our study, a single IM dose of 300,000 units of vitamin D3 was administered, which is considered a high dose.

Finally, it must be noted that major limitation of this study was the single-center design. Additional research with a longer follow-up is advised.

## Conclusion

This study demonstrated that administration of high-dose vitamin D3 in the acute phase of TBI could effectively lower the inflammatory markers and favorably affect the level of consciousness and long-term performance outcomes. To bolster these findings, additional research with a multi-center design is needed.

## Authors' Contribution

F.M: data collection and writing the final manuscript; M.S: study design, conducting the study, and supervising the study; M.K, MM.M, S.S, and M.R: conducting the study; N.T: statistical analysis; M.K: study design; S.A: data collection. All authors have revised and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Data Sharing Policy

Data supporting the findings of this study are available from the corresponding author M.S. on request.

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**Conflict of Interest:** None declared.

## References

- 1 Mollayeva T, Mollayeva S, Colantonio A. Traumatic brain injury: sex, gender and intersecting vulnerabilities. *Nat Rev Neurol*. 2018;14:711-22. doi: 10.1038/s41582-018-0091-y. PubMed PMID: 30397256.

- 2 Peterson AB, Thomas KE, Zhou H. Surveillance Report of Traumatic Brain Injury-related Deaths by Age Group, Sex, and Mechanism of Injury—United States, 2018 and 2019. 2022.
- 3 Maas AIR, Menon DK, Manley GT, Abrams M, Akerlund C, Andelic N, et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol.* 2022;21:1004-60. doi: 10.1016/S1474-4422(22)00309-X. PubMed PMID: 36183712; PubMed Central PMCID: PMC6780814.
- 4 Ghaith HS, Nawar AA, Gabra MD, Abdelrahman ME, Nafady MH, Bahbah EI, et al. A Literature Review of Traumatic Brain Injury Biomarkers. *Mol Neurobiol.* 2022;59:4141-58. doi: 10.1007/s12035-022-02822-6. PubMed PMID: 35499796; PubMed Central PMCID: PMC9167167.
- 5 Kadhim HJ, Duchateau J, Sebire G. Cytokines and brain injury: invited review. *J Intensive Care Med.* 2008;23:236-49. doi: 10.1177/0885066608318458. PubMed PMID: 18504260.
- 6 Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. *Br J Pharmacol.* 2006;147:S232-40. doi: 10.1038/sj.bjp.0706400. PubMed PMID: 16402109; PubMed Central PMCID: PMC1760754.
- 7 Lu KT, Wang YW, Yang JT, Yang YL, Chen HI. Effect of interleukin-1 on traumatic brain injury-induced damage to hippocampal neurons. *J Neurotrauma.* 2005;22:885-95. doi: 10.1089/neu.2005.22.885. PubMed PMID: 16083355.
- 8 Sharma S, Kumar A, Choudhary A, Sharma S, Khurana L, Sharma N, et al. Neuroprotective Role of Oral Vitamin D Supplementation on Consciousness and Inflammatory Biomarkers in Determining Severity Outcome in Acute Traumatic Brain Injury Patients: A Double-Blind Randomized Clinical Trial. *Clin Drug Investig.* 2020;40:327-34. doi: 10.1007/s40261-020-00896-5. PubMed PMID: 32172522; PubMed Central PMCID: PMC7224135.
- 9 Li W, Deng W. Platelet-to-lymphocyte ratio predicts short-term mortality in patients with moderate to severe traumatic brain injury. *Sci Rep.* 2022;12:13976. doi: 10.1038/s41598-022-18242-4. PubMed PMID: 35978006; PubMed Central PMCID: PMC9385644.
- 10 Siwicka-Gieroba D, Malodobry K, Biernawska J, Robba C, Bohatyrewicz R, Rola R, et al. The Neutrophil/Lymphocyte Count Ratio Predicts Mortality in Severe Traumatic Brain Injury Patients. *J Clin Med.* 2019;8. doi: 10.3390/jcm8091453. PubMed PMID: 31547411; PubMed Central PMCID: PMC6780814.
- 11 Xu J, Li S, Lui KY, Song X, Hu X, Cao L, et al. The neutrophil-to-lymphocyte ratio: A potential predictor of poor prognosis in adult patients with trauma and traumatic brain injury. *Front Surg.* 2022;9:917172. doi: 10.3389/fsurg.2022.917172. PubMed PMID: 36081584; PubMed Central PMCID: PMC9445209.
- 12 Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use. *J Neurotrauma.* 1998;15:573-85. doi: 10.1089/neu.1998.15.573. PubMed PMID: 9726257.
- 13 Rubin ML, Yamal JM, Chan W, Robertson CS. Prognosis of Six-Month Glasgow Outcome Scale in Severe Traumatic Brain Injury Using Hospital Admission Characteristics, Injury Severity Characteristics, and Physiological Monitoring during the First Day Post-Injury. *J Neurotrauma.* 2019;36:2417-22. doi: 10.1089/neu.2018.6217. PubMed PMID: 30860434; PubMed Central PMCID: PMC6661910.
- 14 Ono J, Yamaura A, Kubota M, Okimura Y, Isobe K. Outcome prediction in severe head injury: analyses of clinical prognostic factors. *J Clin Neurosci.* 2001;8:120-3. doi: 10.1054/jocn.2000.0732. PubMed PMID: 11484659.
- 15 Marshall LF, Marshall SB, Klauber MR, Clark MvB, Eisenberg HM, Jane JA, et al. A new classification of head injury based on computerized tomography. *Journal of Neurosurgery.* 1991;75:S14-S20. doi: https://doi.org/10.3171/sup.1991.75.1s.0s14.
- 16 Maas AI, Hukkelhoven CW, Marshall LF, Steyerberg EW. Prediction of outcome in traumatic brain injury with computed tomographic characteristics: a comparison between the computed tomographic classification and combinations of computed tomographic predictors. *Neurosurgery.* 2005;57:1173-82. doi: 10.1227/01.neu.0000186013.63046.6b. PubMed PMID: 16331165.
- 17 Sistanizad M, Kouček M, Miri M, Salarian S, Shojaei S, Moeini Vasegh F, et al. High dose vitamin D improves total serum antioxidant capacity and ICU outcome in critically ill patients - A randomized, double-blind clinical trial. *European Journal of Integrative Medicine.* 2021;42:101271. doi: https://doi.org/10.1016/j.eujim.2020.101271.
- 18 Menger J, Lee ZY, Notz Q, Wallqvist J, Hasan MS, Elke G, et al. Administration of



- vitamin D and its metabolites in critically ill adult patients: an updated systematic review with meta-analysis of randomized controlled trials. *Crit Care*. 2022;26:268. doi: 10.1186/s13054-022-04139-1. PubMed PMID: 36068584; PubMed Central PMCID: PMCPMC9446655.
- 19 Jamall OA, Feeney C, Zaw-Linn J, Malik A, Niemi ME, Tenorio-Jimenez C, et al. Prevalence and correlates of vitamin D deficiency in adults after traumatic brain injury. *Clin Endocrinol (Oxf)*. 2016;85:636-44. doi: 10.1111/cen.13045. PubMed PMID: 26921561; PubMed Central PMCID: PMCPMC5053278.
  - 20 Viglianti EM, Zajic P, Iwashyna TJ, Amrein K. Neither vitamin D levels nor supplementation are associated with the development of persistent critical illness: a retrospective cohort analysis. *Crit Care Resusc*. 2019;21:39-44. PubMed PMID: 30857511; PubMed Central PMCID: PMCPMC6696918.
  - 21 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;21:685. doi: 10.1186/s13063-020-04622-6. PubMed PMID: 32727558; PubMed Central PMCID: PMCPMC7388115.
  - 22 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:e1421-e6. doi: 10.1016/j.wneu.2019.02.244. PubMed PMID: 30904798.
  - 23 Cekic M, Sayeed I, Stein DG. Combination treatment with progesterone and vitamin D hormone may be more effective than monotherapy for nervous system injury and disease. *Front Neuroendocrinol*. 2009;30:158-72. doi: 10.1016/j.yfrne.2009.04.002. PubMed PMID: 19394357; PubMed Central PMCID: PMCPMC3025702.
  - 24 Hawryluk GWJ, Rubiano AM, Totten AM, O'Reilly C, Ullman JS, Bratton SL, et al. Guidelines for the Management of Severe Traumatic Brain Injury: 2020 Update of the Decompressive Craniectomy Recommendations. *Neurosurgery*. 2020;87:427-34. doi: 10.1093/neuros/nyaa278. PubMed PMID: 32761068; PubMed Central PMCID: PMCPMC7426189.
  - 25 Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13:818-29. PubMed PMID: 3928249.
  - 26 Wilson L, Boase K, Nelson LD, Temkin NR, Giacino JT, Markowitz AJ, et al. A Manual for the Glasgow Outcome Scale-Extended Interview. *J Neurotrauma*. 2021;38:2435-46. doi: 10.1089/neu.2020.7527. PubMed PMID: 33740873; PubMed Central PMCID: PMCPMC8390784.
  - 27 Galea J, Ogungbenro K, Hulme S, Patel H, Scarth S, Hoadley M, et al. Reduction of inflammation after administration of interleukin-1 receptor antagonist following aneurysmal subarachnoid hemorrhage: results of the Subcutaneous Interleukin-1Ra in SAH (SCILSAH) study. *J Neurosurg*. 2018;128:515-23. doi: 10.3171/2016.9.JNS16615. PubMed PMID: 28298024.
  - 28 Bergold PJ. Treatment of traumatic brain injury with anti-inflammatory drugs. *Exp Neurol*. 2016;275:367-80. doi: 10.1016/j.expneurol.2015.05.024. PubMed PMID: 26112314; PubMed Central PMCID: PMCPMC6007860.
  - 29 Agh F, Mousavi SH, Aryaeian N, Amiri F, Jalilvand MR, Janani L, et al. Protective Effect of Vitamin D Supplementation on Some Hemogram Derived Inflammatory Indices in Normal and High-Fat Diet Fed Male Wistar Rats. *Int J Prev Med*. 2023;14:49. doi: 10.4103/ijpvm.ijpvm\_505\_20. PubMed PMID: 37351039; PubMed Central PMCID: PMCPMC10284218.
  - 30 Guan J, Karsy M, Brock AA, Eli IM, Manton GM, Ledyard HK, et al. Vitamin D status and 3-month Glasgow Outcome Scale scores in patients in neurocritical care: prospective analysis of 497 patients. *J Neurosurg*. 2018;128:1635-41. doi: 10.3171/2017.2.JNS163037. PubMed PMID: 28799870.
  - 31 Miri M, Kouchek M, Rahat Dahmardeh A, Sistanizad M. Effect of High-Dose Vitamin D on Duration of Mechanical Ventilation in ICU Patients. *Iran J Pharm Res*. 2019;18:1067-72. doi: 10.22037/ijpr.2019.1100647. PubMed PMID: 31531088; PubMed Central PMCID: PMCPMC6706749.
  - 32 Miroliaee AE, Salamzadeh J, Shokouhi S, Fatemi A, Ardehali SH, Hajiesmaeili MR, et al. Effect of Vitamin D Supplementation on Procalcitonin as Prognostic Biomarker in Patients with Ventilator Associated Pneumonia Complicated with Vitamin D Deficiency. *Iran J Pharm Res*. 2017;16:1254-63. PubMed PMID: 29201115; PubMed Central PMCID: PMCPMC5610782.