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ORIGINAL RESEARCH ARTICLE

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

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Abstract

Background and Objective Early management of traumatic brain injury (TBI) is essential. We aimed to evaluate the efficacy of vitamin D over early clinical outcome and serum cytokine levels in patients with moderate to severe brain injury.

Methods Thirty-five patients with moderate to severe traumatic brain injury who were admitted to the ICU unit were recruited into the study. Subjects were randomly allocated to a treatment regimen comprising either a one-time oral dose of 120,000 IU (two tablets of 60,000 IU each) of vitamin D ($n = 20$) or 8 mg of saccharide (two tablets of 4 g each) as placebo ($n = 15$). The main parameters evaluated included duration of mechanical ventilation and ICU stay, Glasgow Coma Scale (GCS) and cytokine levels (interleukin (IL)-6, tumour necrosis factor (TNF)-α, interferon (IFN)-γ, IL-2).

Results The results indicated an improvement in the level of consciousness after 7 days in the vitamin D-treated group compared with placebo. An elevation in GCS score by 3.86 units in the vitamin D-treated group with a 0.19-unit descent in the control group was recorded. Duration of mechanical ventilation was reduced in the vitamin D-treated group compared with the control group (4.7 days vs. 8.2 days, p value 0.0001). A noticeable reduction was recorded in inflammatory biomarkers (cytokines) in the vitamin D-treated group (IL-6 $p = 0.08$, TNF-α $p = 0.02$, IL-2 $p = 0.36$) with notable elevation in IFN-γ ($p = 0.65$) compared to the control group.

Conclusion In the acute phase of moderate to severe traumatic brain injury, vitamin D supplementation plays a vital role and has a favourable effect on the consciousness level of patients.

Clinical trial Registry (CTRI) No. CTRI/2019/05/019259.

1 Introduction

Traumatic brain injury (TBI) can be defined as any impact on the brain that could lead to an alteration in the mental state of an individual. It is also known as intracranial injury, which occurs when an external force injures the brain. TBI is one of the major causes of death and disability in individuals aged 40 years and younger. Worldwide each year around 6.2 million people are exposed to TBI. In India, 1.6 million people sustain head injury, of which 200,000 die and 1 million require rehabilitation services at any point in time after injury. According to a recent report, the total prevalence of head injuries in India is estimated as 9.7 million, of which almost 16% of individuals receive severe TBI [1, 2].

A person with acute TBI shows a range of effects, for example affected psychomotor ability, memory deficit, attention deficit, altered information processing speed (IPS), cognitive deficit with altered general fluid intelligence (Gf), difficulty in understanding language, altered hearing and altered visual-spatial skills. These effects have a significant influence on the personal, social, occupational and personal life of an individual [3].

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Key Points

Lack of vitamin D leads to various inflammatory conditions and affects the physiology of the central nervous system.

Vitamin D supplements exhibit neuroprotective effects via amelioration of cytokine level traumatic brain insult.

Vitamin D therapy may be considered a rational strategy in the management of traumatic brain injury.

TBI is one of the most common extracranial mechanical insults leading to brain dysfunction with marked morbidity and mortality [4–7]. The conventional management protocol for the injured brain includes adequate delivery of oxygen along with sufficient metabolic substrates and prevention of secondary brain insult due to hypoxia and hypotension [8]. This secondary brain injury may cause excitotoxicity, oxidative stress, disruption of the blood-brain barrier (BBB), episodes of cortical-spreading depression, impairment of mitochondria and subsequent cell death in the brain [9, 10].

Inflammatory biomarkers are useful in determining the severity and outcome of the injury and act as prognostic factors in estimation of recovery [11]. It is well established that cytokine levels, i.e. tissue necrosis factor (TNF)-α interleukin (IL)-1β and IL-6, increase after severe injury and indicate the extent of systemic inflammation. It has also been observed that an elevated level of IL-6 in the acute period of injury acts as a key marker for severe complications and organ failure [12–14].

Since it is difficult to prevent primary stress, investigations are primarily focused on reversing the secondary insults. As seen in recent studies, oxidative stress is found to have a central role in the pathogenesis of traumatic brain injury [15, 16]. Oxidative stress augments free radical generation, which in turn activates apoptotic cascade resulting in cell death. After TBI, neurodegeneration may continue for months [17, 18]. Cytokine-mediated inflammation plays a key role in developing secondary pathology after brain injury [19].

Vitamin D deficiency establishes a higher baseline level of inflammation even prior to injury, in effect priming the system for an increased immune response after TBI. This elevated acute-phase response correlates with increased cell death and DNA damage, leading to more severe secondary injury processes after injury even with progesterone treatment. Both TNF-α and IL-6 are significantly increased in vitamin D deficiency and both cytokines demonstrate a strong correlation between deficiency and treatment [20]. A recent study showed that elevation in IL-6 was most evident when comparing vitamin D-deficient and vitamin D-normal animals after TBI and progesterone treatment. While most of the other cytokines were elevated in vitamin D-deficient animals two- or threefold, IL-6 was increased nearly fivefold by 72 h after injury, suggesting that IL-6 may be the primary cytokine involved in the detrimental effects of vitamin D deficiency after TBI [21]. Various aspects of vitamin D deficiency are presented in Fig. 1.

Vitamin D, a hormone, has secosteroidal, neuroactive and neurosteroidal actions in the central neuronal system. Neuroprotective effects of vitamin D have recently been shown in a variety of animal models, including ischaemic and traumatic brain-insult models [22, 23]. Experimental evidence suggests that post-injury treatment with vitamin D decreases brain oedema, attenuates free radical damage, reduces neuronal loss in TBI animal models, reduces the inflammatory cytokines TNF-α, IL-6 and nitric oxide (NO), and attenuates neurological abnormalities after ischaemia [24–26]. In a rat model it was reported that the treatments enhanced the formation of new axons as well as increasing axon diameter and improving sensory responses to metabolic stimulation [27].

Despite these potential advantages and the good safety profile of vitamin D described in studies utilizing animals or humans as subjects, there is relatively little information available on assessing the neuroprotective properties of vitamin D in patients with acute brain trauma. The effects of vitamin D on the neurological outcome of TBI patients remain unclear. In light of these facts, the present study aimed to evaluate the efficacy of vitamin D on the early prognostic clinical outcome and serum levels of cytokines (IL-6, TNF-α, IF-γ and soluble IL-2 in patients suffering from moderate to severe TBI in a randomized clinical trial.

2 Patients and Method

A total of 35 patients of both genders between the ages of 16 and 65 years from the north India neighbouring areas of the Delhi region who had sustained moderate to severe traumatic brain insult were admitted to the referral trauma centre and intensive care unit (ICU) of Dr. Ram Manohar Lohia Hospital, New Delhi, India. The subjects were enrolled for a period of 1 year from April 2018 to April 2019 as per the inclusion and exclusion criteria.

The inclusion criteria of this study were a Glasgow Coma Scale (GCS) score of 4–12, started on enteral nutrition within 24 h after admission and on mechanical ventilation in the ICU. Patients with GCS >12 or <4; internal organ bleeding; limb fractures; history of underlying neurologic, metabolic, or psychiatric disorders; and alcohol or drug abuse were excluded from the study.

The procedure and protocol of this study were approved by the ethics committee of the Postgraduate Institute of Medical Science and Research, Dr. Ram Manohar Lohia Hospital (197/EC(16/2017)/PGIMER/RMLH)237/18) and registered

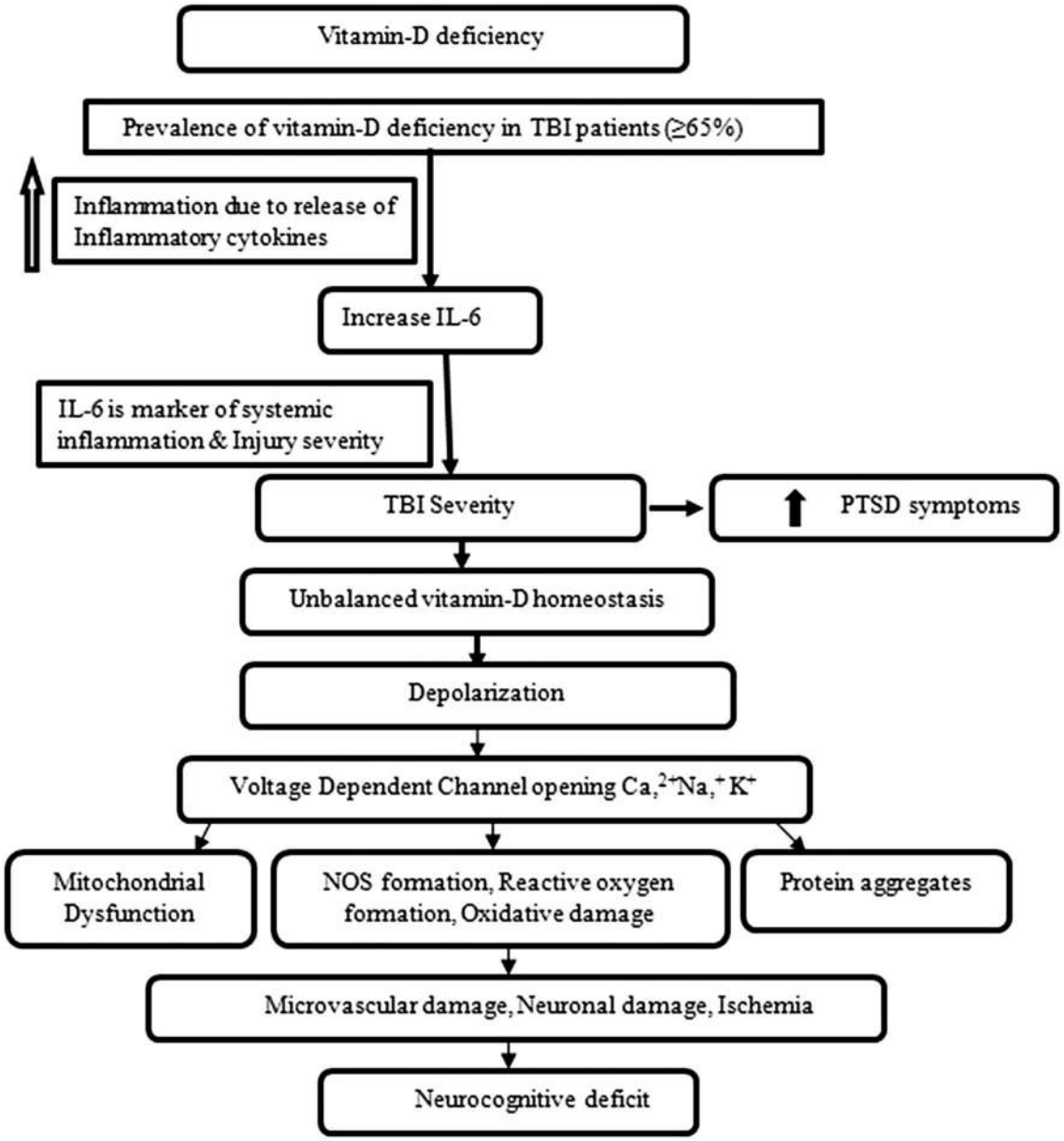

Fig. 1 Various aspects of vitamin-D deficiency in neuronal damage. Role of Vitamin-D in regulation of inflammatory response associated with traumatic brain injury (TBI) severity with Post traumatic stress disorder (PTSD) and Neurocognitive Impairment, *IL-6* interleukin-6, Ca^{2+} calcium, Na^{+} sodium, K^{+} potassium, *NOS* nitric oxide synthase, *PTSD* post-traumatic stress disorder

on the Clinical Trial Registry-India (CTRI/2019/05/019259). Written informed consent was obtained from patients' relatives.

2.1 Randomization and Allocation Concealment

A computer-generated balanced block randomization in a 1:1 ratio was carried out after exclusion and after obtaining the appropriate consent from first-degree relatives. The patients were randomized into two groups: placebo control (Group 1) and vitamin D (Group 2). For ensuring allocation concealment, the sequentially numbered, opaque, sealed-envelope technique was used.

2.2 Data Collection and Supplementation

After anamnesis, the patients underwent essential emergency care in the neurosurgery department of the Dr. Ram

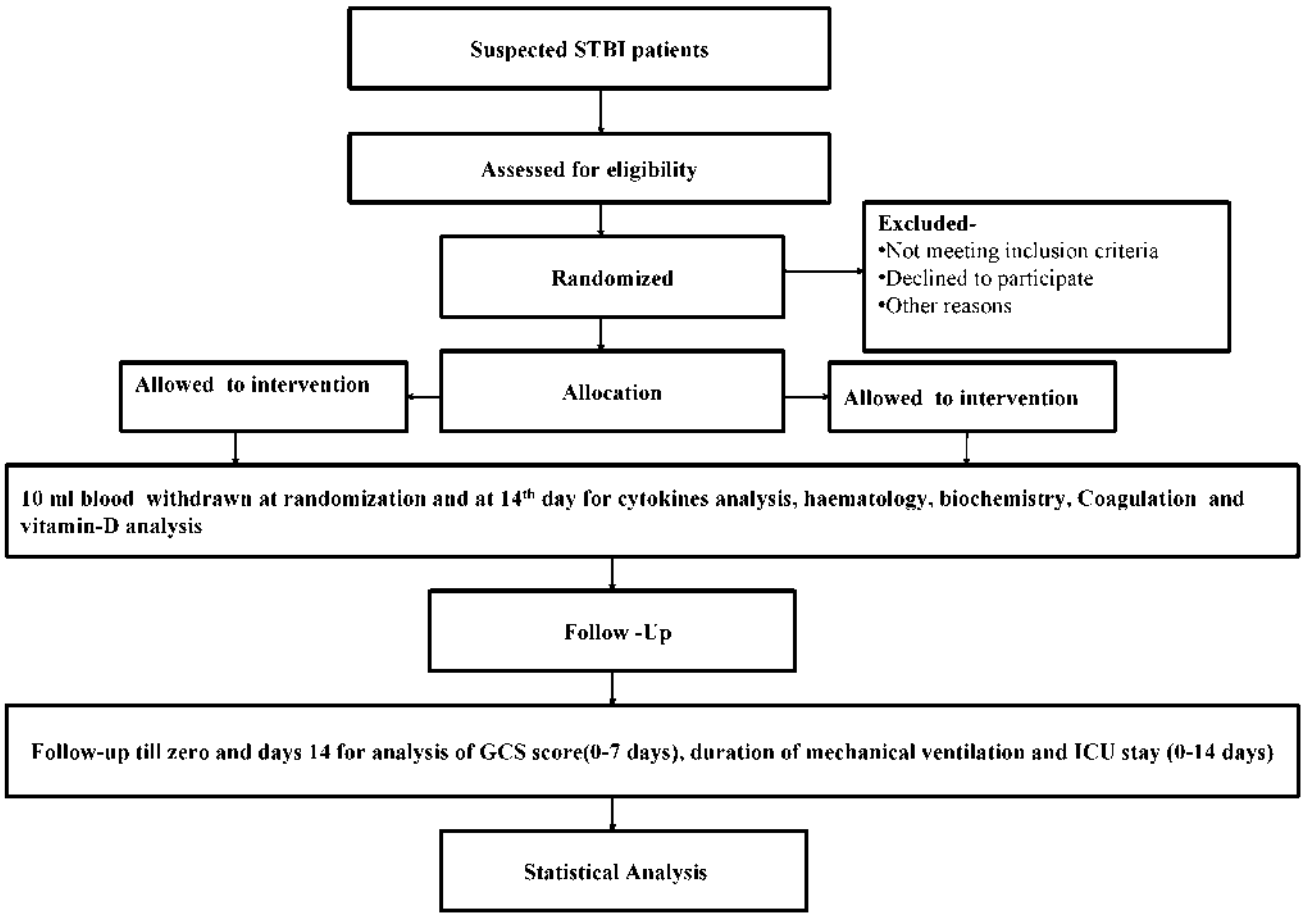

Fig. 2 CONSORT flow diagram of the study. Study protocol for the enrolment, assessment and treatment of severe traumatic brain injury (STBI) patients with oral vitamin-D at onset

Manohar Lohia Hospital. Obligatory information including demographic and clinical history at the time of admission was recorded. Patients meeting the inclusion criteria were divided into two groups using random balanced blocks: (1) receiving vitamin D (case group, $n = 20$) and (2) receiving placebo (control group, $n = 15$).

The enrolled subjects were supplemented with vitamin D at a dose of 120,000 units (two tablets of 60,000 IU each) after trituration in a mortar and pestle. This was administered via Ryle's tube immediately after randomization by a nurse who was blinded to the treatment. The control group were given 8 mg (two tablets, 4 mg each) of saccharide tablets in the same way as the vitamin D by the same nurse. During the course of test drug administration, the GCS of the patient was recorded by a neurosurgery resident every day on their rounds at 8:00 a.m.; the resident was blinded to the intervention. Vital signs along with serum levels of glucose, haematocrit/haemoglobin and platelets were recorded every day at 9:00 a.m. for 1 week. The blinded patient's relative, neurosurgery residents and nurses were unblinded at the end of the study. In addition, the duration of mechanical ventilation and ICU stay were also noted.

In order to analyse serum cytokine levels, a 10-mL peripheral venous blood sample was taken from patients on admission (T1), and 14 days after the last lavage with vitamin D, the serum was centrifuged and stored at − 80 °C. Human cytokines (TNF-α, IL-6, IL-2, IFN-γ) were assayed using in vitro, Diaclone-France kits using ELISA reader according to the manufacturer's protocol (Fig. 2).

2.3 Statistical Analysis

Statistical analysis was carried out using statistical software SPSS version 17.0 (IBM Corporation, Armonk, NY, USA). The data were represented as no. (%), mean ± SD, and median interquartile ranges. The baseline and clinical characteristics were compared between the groups using the following statistical tests: Chi-square test for categorical variables, Student's independent *t* test, *t* test for parametric continuous variables and Mann-Whitney *U* test for nonparametric continuous variables. The primary outcome

mortality rate was compared using the t-test for proportion. The results are reported as a difference in proportion (95% confidence interval (CI)).

3 Results

Thirty-five subjects (vitamin D 20, placebo control group 15) were analysed as per the demographic characteristics (age/sex) depicted in Table 1. The enrolled subjects were young (mean age 36.4 years, 71.4% male and female 28.6%) and all suffering TBI. The causes of TBI were as follows: road traffic accidents (62.9%), falls (22.9%) and assault (14.3%). All these patients were deficient in vitamin D levels. After treatment, there was a significant rise ($p \leq 0.001$) in the level of vitamin D in the intervention group. Seven days after TBI, from day 2 to day 7, improvement in the GCS score was found to be higher in the vitamin D group ($p \leq 0.0001$) compared to the control group. The mean GCS level increased by 3.86 units in the vitamin D group while having an average 0.19-unit deterioration in the control group. The overall duration of ICU stay and mechanical ventilation was lower in the vitamin D group (6.19 vs. 9.07 days) (Table 2). The GOSE (Glasgow Outcome Score Extended) score analysed after 14 days was found to be higher in the vitamin D group than in the control group. Cytokine (IL-6, TNF-α, IL-2, IFN-γ) analysis showed that the median values showed a reduction of IL-6 ($p = 0.08$), TNF-α ($p = 0.02$) and IL-2 α ($p = 0.36$) and improved level of IFN-γ ($p = 0.65$) in the vitamin D group compared to the control group (Table 3). During the study, pre (at the time of admission) and post (14 days after the last lavage with vitamin D) haematological, biochemical (Table 4) and coagulation parameters (Table 5) were analysed to record any discrepancies. Coagulation parameter values were noted as normal or abnormal, for which normal values are presented in unit % in both the groups in Table 5. The prevalence of these potentially confounding factors was found to be satisfactory in both the groups (Tables 4 and 5).

Table 1 Clinical characteristics of the study groups

Variable	Value (units)
Mean age	36.4 (years)
Male	71.4 (%)
Female	28.6 (%)
Road traffic accident	62.9 (%)
Fall from height	22.9 (%)
Assault	14.3 (%)
Undergone surgery	62.8 (%)

Baseline characteristic were assessed by applying the Mann-Whitney *U* test for nonparametric continuous variables

Table 2 Comparison of clinical severity, duration of intensive care unit (ICU) stay, mechanical ventilation duration and mortality rate between the two groups

Parameter	Case	Control	*p* value
Pre GCS	7.09 ± 2.21	6.28 ± 2.36	0.310
First day GCS	7.00 ± 2.14	5.66 ± 1.82	0.080
Second day GCS	7.85 ± 2.45	5.33 ± 1.07	< 0.0001
Third day GCS	8.66 ± 2.26	5.91 ± 1.31	0.001
Fourth day GCS	10.20 ± 2.74	6.36 ± 1.50	< 0.0001
Fifth day GCS	11.05 ± 1.95	7.45 ± 1.86	< 0.0001
Sixth day GCS	12.05 ± 1.61	8.63 ± 2.06	< 0.0001
Seventh day GCS	12.63 ± 1.42	8.72 ± 1.84	< 0.0001
ICU stay (days)	6.19 ± 2.29	9.07 ± 2.95	0.003
Ventilator support (days)	4.70 ± 2.17	8.23 ± 2.65	< 0.0001
GOSE	4.80 ± 2.52	2.21 ± 0.89	< 0.0001
Mortality during study	2 (14.3%)	3 (14.3%)	0.797

GCS Glasgow outcome scale, *ICU* Intensive care unit, *GOSE* Glasgow Outcome Scale Extended, *SD* standard deviation

Values are expressed as the mean ± SD. Analysis was done between case and control group. The results values were reported as difference in proportion (95% CI). Mortality rate is expressed in % compared using *t* test for proportion

4 Discussion

The present study evaluated the effects of vitamin D on the clinical outcomes (consciousness, duration of ICU stay, mechanical ventilation and serum cytokines (IL-6, TNF-α, IL-2, IFN-γ levels)) of patients who had sustained severe TBI. Our results showed a strongly beneficial effect of vitamin D supplementation with improvement of GCS and shortening of the mechanical ventilation period in these patients. These results are in agreement with earlier studies. One study reported that 7 days of oral supplementation with 0.1% of vitamin D in water administered for brain injury in rats could decrease the chances of neuronal damage [28]. Another study by Wei et al., reported that administration of a secosteroid injection showed neuroprotective effects in brain damage in rats by shielding them from oxidative reparation of the brain. This effect is achieved by reducing the free radical damage and preventing apoptosis in damaged neurons [29]. A plethora of studies have highlighted the fact that IL-6, TNFα, IL-2 and IFN-γ serum levels are accurate biomarkers for brain damage [30].

A lack of vitamin D indicates a higher baseline inflammation level even prior to insult as suggested by previous studies. This elevated acute response may contribute to increased cell death and damage to DNA potentially leading

Table 3 Comparison of the cytokine levels between the two study groups

Parameter	Case	Control	*p* value
Vitamin D pre	18.30 (14.50–22.95)	15.15 (11.80–26.90)	0.661
Vitamin D post	39.15 (36.75–44.58)	27.30 (14.60–30.80)	0.001
Pre IL-6	93.80 (45.63–192 92)	221.90 (69.25–240.10)	0.131
Post IL-6	8.43 (3.61–75.24)	45.09 (21.85–139.41)	0.088
Pre TNF-α	11.97 (6.89–18.99)	12.25 (3.13–26.03)	0.845
Post TNF- α	5.01 (0.69–6.79)	8.93 (4.05–17.71)	0.028
Pre IFN-γ	0.87 (0.73–1.44)	1.83 (0.99–5.85)	0.026
Post IFN-γ	4.01 (2.34–6.17)	3.35 (0.28–17.20)	0.658
Pre IL-2	2206.80 (1872.10–2278.5)	1891.80 (1820.09–2050.75)	0.109
Post IL-2	1836.90 (1341.50–2335.0)	2149.60 (1875.25–2201.00)	0.368

IL interleukin, *TNF*-α tumour necrosis factor, *IFN*-γ interferon-gamma

Values are expressed as median (interquartile range). Analysis was done between the case and control groups. The result values are reported as difference in proportion (95% confidence interval)

Pre-value at the time of admission

Post-value at 14 days after the last lavage with vitamin-D

Table 4 Comparison of biochemical and haematological parameters between the two groups

Parameter	Case	Control	*p* value
Pre Hb	9.80 (9.20–11.45)	10.25 (9.40–12.57)	0.649
Post Hb	10.40 (9.65–11.75)	10.30 (9.20–11.40)	0.425
Pre ESR	12.00 (4.50–21.50)	12.00 (3.50–14.00)	0.510
Post ESR	20.00 (18.00–24.00)	23.00 (21.50–34.25)	0.102
Pre RBS	107.0 (98.0–170.50)	105.0 (96.75–153.25)	0.469
Post RBS	122.0 (105.0–147.50)	113.0 (102.0–164.50)	0.859
Pre calcium	8.20 (7.55–8.90)	7.20 (6.37–8.72)	0.089
Post calcium	8.30 (7.60–9.30)	7.90 (7.65–8.45)	0.523
Pre sodium	138.0 (134.0–141.0)	138.50 (135.50–142.0)	0.761
Post sodium	141.0 (137.50–145.0)	140.0 (136.0–142.50)	0.403
Pre potassium	3.40 (3.23–3.95)	3.45 (3.20–3.75)	0.566
Post potassium	3.90 (3.38–4.40)	3.80 (3.27–4.60)	0.986
Pre SGOT	46.0 (34.00–50.0)	40.50 (34.0–48.0)	0.388
Post SGOT	48.0 (31.0–54.50)	48.0 (39.0–55.50)	0.958
Pre SGPT	25.0 (18.50–46.0)	23.0 (18.75–35.25)	0.555
Post SGPT	29.0 (25.50–51.0)	32.0 (22.0–43.0)	0.929
Pre D/B	0.30 (0.19–0.41)	0.20 (0.19–0.42)	0.748
Post D/B	0.37 (0.20–0.44)	0.39 (0.21–0.45)	0.859
Urine calcium	0.76 (0.38–4.3)	0.41 (0.26–1.42)	0.102
U-Cr/Ca	0.43 (0.04–0.53)	0.42 (0.10–0.52)	0.873

Hb haemoglobin, *ESR* erythrocyte sedimentation rate, *RBS* random blood sugar, *SGOT* serum glutamic oxaloacetic transaminase, *SGPT* serum glutamic pyruvic transaminase, *D/B* direct bilirubin, *U-Cr/Ca* urine creatinine/calcium

– Values are expressed as median (interquartile range). Analysis was done between case and control group. The results values were reported as difference in proportion (95% CI)

Pre-value at the time of admission

Post-value at 14 days after the last lavage with vitamin-D

Table 5 Comparison of normal coagulation profiles between the two groups after treatment

Parameter	Case	Control	*p* value
PT	14 (70%)	9 (64.3%)	0.726
APTT	18 (94.7%)	11 (91.7%)	0.735
ABG	21 (100%)	13 (92.9%)	0.214

PT prothrombin time, *aPTT* activated partial thromboplastin time, *ABG* arterial blood gas

Values are expressed in unit %. Coagulation profile values were analysed by the Chi-square test followed by Student's independent *t* test, *t* test for parametric continuous variables and Mann-Whitney *U* test for nonparametric continuous variables

to a more severe secondary insult after injury even after treatment with progesterone. Both TNF-α and IL-6 were found to be significantly increased with a decrease in the levels of vitamin D. However, both these cytokines show a direct relationship between insufficient levels and treatment [31]. Our data clearly depicted an elevation of IL-6 levels after TBI in both groups. However, other cytokines were also found to be elevated in the vitamin D-deficient group, with nearly two- or three-fold increases; IL-6 was found to have a fivefold increase 72 h after the traumatic event. This suggests that IL-6 is the main cytokine responsible for detrimental effects due to a lack of vitamin D after traumatic brain injury.

The results of our study demonstrated that supplementation with vitamin D resulted in a reduction in the levels of cytokines compared with the control group. Pro-inflammatory cytokines such as TNF-α play an important role in the pathophysiology of cerebral oedema and brain damage after traumatic brain injury. Anti-inflammatory properties

aid in augmenting the amount of blood flow to damaged tissues thereby ameliorating the wound-healing cascade and recovery [32]. As suggested by many studies, vitamin D is capable of inhibiting TNF-α gene expression. A study with an animal model of multiple sclerosis showed that the combination of glatiramer acetate (GA) and vitamin D had favourable effects on neuronal survival and axonal growth through reduced reactive oxygen species generation and a reduction in the nuclear factor kappa light-chain enhancer of activated B cells (NFkB) [33].

The ultimate goal of TBI research is to prevent, diagnose and treat TBI by avoiding secondary brain insult. Several important aspects need to be understood, including the mechanism through which the primary injury initiates the secondary injury and the sensitivity/specificity of different biomarkers to injury severity. In addition, identifying and evaluating novel targets for pharmacological protection and developing reliable extrapolation methods to predict pharmacological responses in humans are necessary. Despite advances in research and development, healthcare costs associated with TBI are escalating by billions of dollars [34], thus identifying novel pharmacological strategies that effectively restrain and manipulate the evolution of injury pathways are a primary research focus for addressing the immediate need for better TBI treatments. Furthermore, the precise path of secondary injury progression, i.e., the trajectory of injury and its dynamic progression in the human brain, remains unknown. This non-deterministic nature of the injury trajectory limits the design of rational TBI pharmacotherapies; consequently, clinical trials have failed to demonstrate the efficacy of treatments. This lack of success is likely attributed to several factors, including the complexity and heterogeneity of TBI pathophysiology; the choice of drugs, dosages, delivery routes, dosing regimens, and/or treatment durations; and an incomplete understanding of the pharmacokinetics (PK), dose-response relationships and therapeutic windows [35]. Therefore, there is currently no 'magic bullet' for delaying the progression of secondary injury.

To the best of our knowledge, to date no human study has assessed the effect of vitamin D supplementation on the secondary neural damage caused by trauma. In order to control the confounding effect of underlying mental illness and/or internal organ injury on the clinical outcome of TBI, we excluded these patients from our study. The other probable causes of confounding include haematologic and metabolic impairment, which in turn may affect the clinical outcome in the patients with traumatic brain insult. However, statistical analysis revealed that prevalence of these parameters was not significant and may not serve as a confounding source.

4.1 Limitations of the Study

The major limitation of this study is the small sample size. Another limitation is that, due to the overwhelming predominance of TBI among men in our country, more male patients were included than females. Further studies with long-term follow-up are needed to provide a wider spectrum of knowledge about the clinical outcomes in TBI patients.

4.2 Conclusion

The results of this study showed that supplementation with vitamin D in the acute phase of the injury has favourable effects on the level of consciousness and duration of mechanical ventilation in the early phase of severe TBI. Due to ease of availability, optimum cost and its crucial pharmacological role, early treatment with vitamin D can be considered rational therapy in the management of TBI in patients admitted to the ICU.

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Author Contributions All authors had full access to all data presented in this study and take responsibility for the accuracy of the data analysis. Concept and design: AK and AC. Acquisition, analysis, or interpretation of data: AC and SS. Drafting of the manuscript: AK and LK. Collected the data: SS, AB and VK. Supervision: AC, NS and SS.

Compliance with Ethical Standards

Funding There was no significant financial support for this work

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics approval The procedure and protocol of this study were approved by the Ethics Committee of the Postgraduate Institute of Medical Science and Research, Dr. Ram Manohar Lohia Hospital(197/EC(16/2017)/PGIMER/RMLH)237/18) and registered by Clinical Trial Registry-India (CTRI/2019/05/019259).

Consent to participate Written informed consent was obtained from all concerned patients' relatives.

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