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

CURRENT STATUS: UNDER REVIEW

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DOI:
10.21203/rs.2.16302/v2
SUBJECT AREAS
  Internal Medicine  Integrative & Complementary Medicine

KEYWORDS
  traumatic brain injury, vitamin D, inflammation, mortality
Abstract

Background

Traumatic Brain Injury (TBI) is the most common trauma worldwide and is a leading cause of injury-related death and disability. Inflammation is a major problem among TBI patients which is in association with severity of illness and mortality in brain trauma patients, especially in subdural hemorrhage and epidural hemorrhage cases. A high percentage of adults admitted to the intensive care unit with critical conditions are diagnosed with vitamin D-deficiency, this deficiency may induce impaired immune responses and increase the risk of infections. Vitamin D intervention has been shown to modulate pro- and anti-inflammatory cytokines in non-critically ill patients, but to date, there is no substantial data on the effectiveness of vitamin D for the improvement of immune function in traumatic brain injury patients.

Methods/design

A randomized clinical trial (RCT) will be performed on 74 Iranian adults 18-65 years old with brain trauma, and will be treated daily by vitamin D supplements (100000 IU oral drop) or a similar placebo (1000 IU) for 5 days.

Discussion

If this randomized clinical trial elucidates reduction in inflammatory cytokines, it would provide the evidence for multi-central clinical trials to evaluate the efficacy of vitamin D supplementation in neuro-critically ill patients. Since vitamin D supplements are inexpensive and safe, this clinical trial could have the potential to improve clinical outcomes in traumatic brain injury patients through reduction of inflammation and infection associated morbidity and mortality rates.

Background
Vitamin D is an essential hormone for calcium hemostasis and its intestinal absorption, it also has an important role in several neuromuscular activities and metabolic responses (1). Recent studies indicated that active form of vitamin D may have a crucial function in modulating immune responses to inflammatory conditions and infectious diseases (2, 3). According to previous data, in critically ill patients’ vitamin D deficiency has been associated with a high incidence of adverse events and mortality (4). Vitamin D can induce production of anti-inflammatory cytokines and some antimicrobial proteins such as cathelicidin by macrophages and neutrophils (3). Antimicrobial proteins are expressed significantly in the respiratory epithelium and other organs and they could reduce risk of infections in integumentary barrier sites (5, 6). Notably, infectious diseases such as pneumonia and sepsis are common in traumatic patients leading to more than 20 % rate of death in these patients, in addition there is a high prevalence of vitamin D deficiency in these patients, therefore vitamin D treatment is of high importance in traumatic brain injury (TBI) patients (7-9). Moreover, vitamin D deficiency is also related to serious complications such as coma, slow neurological recovery and critical illness polyneuropathy in TBI patients (TBI) (10, 11). Although, there has been a direct association between vitamin D deficiency and adverse clinical outcomes, the data regarding vitamin D supplementation from randomized clinical trials (RCT) are limited (12). It has not yet been shown that vitamin D supplementation can efficiently affect the endpoints of TBI patients (4, 9). The type of vitamin D supplement, rout of intervention and speed of normalization may be associated in clinical outcomes (4, 9, 12). As compared to vitamin D injection, enteral vitamin D administration seems to be more effective and is considered safe (13, 14). To date, studies on the effects of vitamin D on brain trauma patients have been inadequate and could not provide definitive conclusions (15-17). The purpose of the present study is to determine the effect of high dose vitamin D supplementation versus
low dose on mortality rate and inflammatory cytokines levels, in critically ill traumatic brain patients under enteral nutrition therapy at intensive care unit (ICU).

Methods/design

Study design

The treatment of vitamin D deficiency in neurocritical ill patients (VITdAL-ICU) protocol is designed according to the CONSORT guidelines for randomized, clinical trial (RCT) (18). The protocol of this RCT is approved by Medical Ethics Committee of Mashhad University of Medical Science (IR.MUMS.MEDICAL.REC.1397.381) and is registered at the Iranian Registry of Clinical Trials under IRCT2018061904151N3. The flow diagram illustrates details of the current study protocol in Figure 1. Moreover, the study time framework for screening, supplementation and monitoring are described in Figure 2.

Study objectives and rational

The primary goal of the present study is to investigate the effect of daily intake of high dose vitamin D supplements (100000 IU) versus low dose vitamin D supplements (1000 IU) on mortality rate and inflammatory markers through a double-blind, randomized, controlled clinical trial on traumatic brain injury (TBI) patients. According to previous observational studies, TBI patients with vitamin D deficiency had higher mortality rate and neuro-inflammation in comparison with TBI patients with vitamin D sufficiency (15, 17). Also, as vitamin D deficiency is highly prevalent in critically ill patients, rapid normalization of this deficiency may be beneficial to these patients (7, 22, 23). For this reason, we hypothesized that rapid correction of vitamin D deficiency in TBI patients may improve inflammation and decrease mortality rate. This study will also evaluate seven secondary outcomes that may be influenced by vitamin D administration in neurocritical patients. In order to determine the effects of high dose vitamin D intervention 1) Glasgow coma scale, 2) time of final weaning 3) time of discharge from ICU 4) SOFA score
(Sequential Organ Failure Assessment), 5) APACHE II Score (Acute Physiology and Chronic Health Evaluation II) 6) delirium severity and 7) parathyroid hormone (PTH) will be assessed in the treatment group compared to control group. The study participants will be recruited from adult wards at trauma referral hospitals namely Kamyab and Taleghani in Mashhad, Iran. This study is a phase II-RCT and it has started from August 2019 and will carry on for 5 months.

**Eligibility criteria**

Eligibility criteria for TBI patients are listed below. Participants will be allocated into four equal blocks by random in two groups using online randomization list.

**Inclusion criteria**

1- Patients: traumatic brain injury adults aged 18-65 years’ old
2- Admission to neurocritical care unit with EDH and SDH
3- Glasgow Coma scale 7-9
4- TBI patients with vitamin D levels lower than 20 ng/mL
5- Received informed consent from patient’s parents or family members prior to intervention

**Non-entry criteria**

1- Patients with hypercalcemia (Ca > 10.8 mg/dl)
2- TBI Patients who are NPO more than 48 hours or have started total parenteral nutrition (TPN).
3- Severe and active bleeding
4- Patients treated with inotropic and corticosteroid drugs.
5- Patients treated with therapeutic dose of any vitamins and minerals apart from the routine ICU protocol
6- Patients with BMI > 40kg/m² and BMI < 17kg/m²
7- History of any disease such as autoimmune disorders, cancer, sepsis, infection, liver
disorder, kidney disorder, diabetes, heart failure and metabolic diseases

8-Known pregnancy and lactation

9-Patients who are transferred from other ICUs after > 1week

**Exclusion criteria**

Death before 7 days of traumatic brain injury patients

Request to stop the study by patients’ parents or family members

Patients treated with different medication protocol

If item 1 to 5 of non-entry criteria are met

**Study design and setting**

We will include 74 TBI patients with Epidural hemorrhage (EDH) and subdural hemorrhage (SDH) as diagnosed according to computed tomography scan (CT) or Magnetic resonance imaging (MRI) findings by the neurosurgeon. Patients will receive vitamin D drop (100000 IU) or 1000 IU identical control drop in a 1:1 ratio through random assignment method. Vitamin D drops are manufactured by Zahravi Company under good manufacturing practice (GMP). Each vial of vitamin D drop is produced to be dissolved completely in 1ml of extra virgin olive oil (Familia Company), through a nasogastric tube (NGT) for 5 days in TBI patients. Both high dose and low dose drops will have the same bottle, flavor and aroma to the blinded.

**Sample size**

To calculate the sample size, in accordance with a previous study (7). interleukin 6 (IL-6) will be used as the main variable and the **type on error** is considered with an **alpha of 0.05**. We estimated 62 TBI patients with 80% power to detect an effect size of 0.5 for reduction of IL-6 between intervention and control groups as calculated with the next formula. Considering 20% dropouts in this study, the total sample size is estimated to be 37 participants in each group as calculated in below formula (7). (see Formula in the
**Randomization and blinding**

Block randomization method will be considered for this trial study. TBI patients will be randomized (in four blocks) into intervention group and control group based on a blinded randomization list generated by randomization and online databases for clinical trials (https://www.sealedenvelope.com) and will be managed by research director of Clinical Nutrition Department, Mashhad University of Medical Sciences. Patients will be allocated by random according to the severity of brain injury (GCS 7-8 and 8-9), type of brain injury (EDH and SDH) and gender to ensure match distribution of these factors in all four blocks. Given that ICU supervisor has no knowledge of which vial contains high dose or low dose of vitamin D, TBI patients will be randomized to group A or B. The label A or B on vials will be deleted by the research director before allocating the study supplements, thus the medication will appear similar to study researcher and nurses. The TBI patient’s study identification code will be documented and intervention group may only be determined by comparing the patient’s study number to the reference blinded list, which only research director will have access to until the trial is finished. Figure 2 indicates the SPIRIT schedule of evaluations and interventions.

**Proposed analysis**

To determine the normal distribution of variables, Kolmogorove Smirnov test will be conducted. The analyses will be performed according to intention-to-treat (ITT) test. One-way analysis of variance (ANOVA) (or multi-variable covariance analysis (ANCOVA) will be conducted to examine differences in severity of brain injury score (GCS), APACHE II score, SOFA score, delirium score and all variables at study baseline between the two groups. If the distribution of variables turns abnormal in this study, KruskaleWallis test will be performed to compare the case and control groups, and Wilcoxon test will be carried out.
for inter-groups comparison. The P values less than 0.05 will be considered statistically significant.

**Primary efficacy endpoint**

The primary efficacy outcome for this trial will be assessed by comparing the changes in vitamin D levels and inflammatory markers (IL-6, MCP-1 and CRP) between two groups. The rate of mortality in TBI patients until 28 day after admission (in ICU and regular ward) will be assessed in high and low dose vitamin D supplement groups. The impact of high dose versus low dose of vitamin D will be analyzed, with and without adjustment for age, sex, type of brain injury and severity of injury. If the results differ after adjusting for the variables, the results of both analyzes will be reported.

**Secondary efficacy endpoints**

All analyses will be conducted with and without adjustment for age, sex, type of brain injury and severity of the injury. Time to event analysis will be analyzed similarly as the primary endpoint. The secondary outcomes are listed below:

a. Comparison of GCS score changes between intervention and control groups during intensive care unit stay

b. Comparison of APACH II and SOFA score between intervention and control groups during intensive care unit stay.

c. Comparison of incidence of delirium between intervention and control groups during intensive care unit stay.

d. Comparison of changes in parathyroid hormone levels between intervention and control groups during intensive care unit stay.

e. Comparison of time of discharge from intensive care unit in patients treated with high dose and low dose vitamin D groups (during the first 28 day of admission).

f. Comparison of time to weaning from mechanical respiratory support in patients
treated with high dose and low dose vitamin D groups (during the first 28 day of admission).

g. Comparison of need for a tracheostomy between intervention and control groups during intensive care unit stay.

h. Comparison of occurrence of infections among intervention and control groups during intensive care unit stay.

Study assessments

At the beginning of the trial, demographic information, anthropometric parameters (weight (bed scale, Balas company), fat free mass, fat mass and mid arm circumference (bioelectrical impedance analysis s10, InBody company), vitamin D status (ELISA kit), IL-6 (ELISA kit), MCP-1(ELISA kit), quantitative CRP (ELISA kit), PTH (ELISA kit), Ca, APACH II score, SOFA score and delirium score will be measured and repeated on day 7 and 14 after intervention in groups A and B. All drugs will be ordered daily upon physician’s request during ICU stay will be recorded. For adjusting the effect of energy on final outcomes, based on ESPEN guideline for all patients 25kcal/kg actual body weight energy is considered (19). Moreover, the total kilocalories and macronutrient delivered by enteral feeding will be calculated daily for all the participants. Blood samples (5mL) will be collected at baseline (ICU admission) and 7 and 14 days after intervention at 10:00 am. Then, samples will be immediately stored at -80 °C for future inflammatory cytokines measurements. All routine biochemistry, hematology and urine test, until day 14, will be measured after sampling in Kamyab Hospital lab, Mashhad, Iran. Serum IL-6, MCP-1 and CRP levels will be analyzed in the baseline and day 7 and 14 at Immunology Lab of Bu Ali Research Institute, Mashhad, Iran. In addition, in case of occurrence of infections (blood stream, the urinary tract, the gastrointestinal tract, wounds and lung) an internist specialist will take responsibility (20). A summary of the schedule of registration,
supplementation, and study evaluation is shown in Figure 3.

**Safety**

Vitamin D deficiency treatment is required for patients with low vitamin D levels (< 20 ng/ml) [54]. However, the primary side effect of vitamin D supplementation is hypercalcemia (13). To prevent hypercalcemia in TBI patients, serum vitamin D and calcium levels are evaluated regularly, as adequate vitamin D levels are reached hypercalcemia is likely to be cured, therefore the intervention will be stopped (21). Moreover, to keep track of the patient's condition, daily vital signs and clinical examinations by neurosurgeon and ICU supervisor will be carried out and will be stopped if the patient's condition deteriorates.

**Discussion**

In the present study, if vitamin D significantly reduces the rate of mortality and inflammation in TBI patients, it can potentially provide the background for larger and well-designed multicenter clinical trial studies to establish the effect of vitamin D supplementation on TBI patients. Furthermore, vitamin D treatment is inexpensive and safe with minimal side effects, also could have positive impacts on critically ill patients (such as decrease mortality rate and hospital length) according to previous studies (7, 13, 14, 21). Potential limitations of the proposed study are as follows: blood transfusion (because of trauma and bleeding, some TBI patients may require blood transfusion during the study, which may affect the outcomes...Albumin injection in hypo-albuminemia patients (it can affect the levels of serum albumin). Also, TBI patients may need to undergo other surgeries than brain surgery thus, this factor could affect the study outcomes. For this reason, we will compare mortality rate and inflammatory cytokines levels in all participants (intervention and control) and separate patients with (anemia and hypoalbuminemia) in each group. If the rate of death or inflammation is different from
those in the case or control group, the results will be reported separately. Based on recent studies, we have considered the suggested vitamin D dose (100000 IU/day for 5 days) by Han, et al (21), as it is shown to be safe and efficacious in critically ill patients. Therefore, we believe that present study could elaborate the necessity of vitamin D treatment and its efficacy on TBI patients at ICU.

Conclusion

The present clinical trial has begun on June 2019 and will be completed in November 2019. If comprehensive results are observed in the clinical outcomes of TBI patients, vitamin D treatment could be conducted as a new approach for optimizing the traumatic brain injury patients' care at ICU.

Trial Status

This trial (protocol version number version 1.1, November 13, 2017) is ongoing. Participant recruitment began on August 11, 2019, and completed on December 21, 2019. The trial procedures are expected to be completed by the end of January 2020.

Abbreviations

**VITdAL-ICU**: The correction vitamin D deficiency in critically ill patients  
**ICU**: Intensive care unit  
**TBI**: Traumatic brain injury  
**EDH**: Epidural hemorrhage  
**SDH**: Subdural hemorrhage  
**EN**: Enteral  
**TPN**: Total parenteral nutrition  
**BMI**: Body mass index  
**NPO**: Nothing by mouth
RCT: Randomized controlled trial

GCS: Glasgow Coma Scale

APACHE II: Acute physiology and chronic health evaluation

SOFA: Sequential organ failure assessment

IL-6: Interleukin 6

MCP-1: Monocyte chemoattractant protein-1

CRP: C-reactive protein

PTH: Parathyroid hormone

Ca: Calcium

LOS: length of stay

Declarations

Acknowledgements

We would like to thank the vice chancellor of research center of Mashhad University of Medical Sciences for the sponsorship of this study, with thesis number: 961867 and ethical approval code: IR.MUMS.MEDICAL.REC.1397.381.

Authors’ contributions

ASM, NA, SA and EM all contributed to the design of the present study protocol. SM, RH and RR collaborated to perform of the clinical trial and TH and RG promoted the statistical plan, which was approved by all the authors, and drafted the manuscript. All authors read and approved the final manuscript.

Funding source

This trial is funded by Mashhad University of Medical Sciences (grant # 961867).

Availability of data and materials

Final data for the present clinical trial can be made accessible upon email request. Interested researchers should contact Dr. Abdolreza Norouzy at Norouzya97@gmail.com.
Ethics approval and dissemination

Mashhad University of Medical Sciences, School of Medicine Research Ethics Committee (IR.MUMS.MEDICAL.REC.1397.381), has approved the study protocol. Written informed consent will be obtained from the parents or family members of the TBI patients at ICU. Study participants will be monitored at Shahid Kamyab and Shahid Taleghani referral hospitals based on ICU protocols, including routine medical and nutrition therapy for severe neurocritical patients. If we intend to make any modifications on the protocol (e.g., changes to eligibility criteria, outcomes, analyses), we will inform the relevant parties (e.g., investigators trial registries, journals). The results of this trial will be published for the benefit of healthcare professionals and the public.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2011;96(7):1911-30.

2. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflammation Research. 2014;63(10):803-19.

3. Guillot X, Semerano L, Saidenberg-Kermanac’h N, Falgarone G, Boissier M-C. Vitamin D and inflammation. Joint Bone Spine. 2010;77(6):552-7.

4. Brenner ZR, Miller AB, Ayers LC, Roberts A. The role of vitamin D in critical illness. Critical Care Nursing Clinics. 2012;24(4):527-40.

5. White JH. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression:
past, present and future. The Journal of steroid biochemistry and molecular biology. 2010;121(1-2):234-8.

6. Hewison M. Antibacterial effects of vitamin D. Nature Reviews Endocrinology. 2011;7(6):337.

7. Miroliaee AE, Salamzadeh J, Shokouhi S, Sahraei Z. The study of vitamin D administration effect on CRP and Interleukin-6 as prognostic biomarkers of ventilator associated pneumonia. Journal of critical care. 2018;44:300-5.

8. Cariolou M, Cupp MA, Evangelou E, Tzoulaki I, Berlanga-Taylor AJ. Importance of vitamin D in acute and critically ill children with subgroup analyses of sepsis and respiratory tract infections: a systematic review and meta-analysis. BMJ open. 2019;9(5):e027666.

9. Williams S, Heuberger R. Outcomes of vitamin D supplementation in adults who are deficient and critically ill: a review of the literature. American journal of therapeutics. 2016;23(6):e1890-e902.

10. Lee P, Nair P, Eisman JA, Center JR. Vitamin D deficiency in the intensive care unit: an invisible accomplice to morbidity and mortality? Intensive care medicine. 2009;35(12):2028.

11. 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. Critical Care and Resuscitation. 2019;21(1):39.

12. Christopher KB. Vitamin D supplementation in the ICU patient. Current Opinion in Clinical Nutrition & Metabolic Care. 2015;18(2):187-92.

13. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. Jama. 2014;312(15):1520-30.
14. Quraishi SA, De Pascale G, Needleman JS, Nakazawa H, Kaneki M, Bajwa EK, et al. Effect of cholecalciferol supplementation on vitamin D status and cathelicidin levels in sepsis: a randomized, placebo-controlled trial. Critical care medicine. 2015;43(9):1928.

15. Guan J, Karsy M, Brock AA, Eli IM, Ledyard HK, Hawryluk GW, et al. A prospective analysis of hypovitaminosis D and mortality in 400 patients in the neurocritical care setting. Journal of neurosurgery. 2017;127(1):1-7.

16. 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. Clinical endocrinology. 2016;85(4):636-44.

17. Ardehali SH, Dehghan S, Baghestani AR, Velayati A, Shariatpanahi ZV. Association of admission serum levels of vitamin D, calcium, Phosphate, magnesium and parathormone with clinical outcomes in neurosurgical ICU patients. Scientific reports. 2018;8(1):2965.

18. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMC medicine. 2010;8(1):18.

19. Kreymann K, Berger M, Deutz Ne, Hiesmayr M, Jolliet P, Kazandjiev G, et al. ESPEN guidelines on enteral nutrition: intensive care. Clinical nutrition. 2006;25(2):210-23.

20. Health UDo, Services H. Common terminology criteria for adverse events (CTCAE) version 4.0. National Institutes of Health, National Cancer Institute. 2009;4(03).

21. Han JE, Jones JL, Tangpricha V, Brown MA, Hao L, Hebbar G, et al. High dose vitamin D administration in ventilated intensive care unit patients: a pilot double blind randomized controlled trial. Journal of clinical & translational endocrinology. 2016;4:59-65.

22. Alizadeh N, Khalili H, Mohammadi M, Abdollahi A, Ala S. Effect of vitamin D on stress-
induced hyperglycaemia and insulin resistance in critically ill patients. International journal of clinical practice. 2016;70(5):396-405.

23. Arabi SM, Ranjbar G, Bahrami LS, Vafa M, Norouzy A. The effect of vitamin D supplementation on hemoglobin concentration: a systematic review and meta-analysis. Nutrition Journal. 2020;19(1):11.

Figures
Figure 1

Participant flow diagram according to Consolidated Standards of Reporting Trials (CONSORT) 2010 statement
| TIMEPOINT** | Enrolment | Allocation | Post-allocation |
|------------|-----------|------------|-----------------|
|            | Day 1     | Day 1      | Day 1          |
|            |           |            | Day 5  | Day 7  | Day 14 | Day 28 | etc. |
| ENROLMENT: |           |            | Day 1     |         |        |        |      |
| Eligibility screen | X         |            |           |        |        |        |      |
| Demographic data and vitamin D levels | X         |            |           |        |        |        |      |
| Blood sample | X         |            | X         | X      |        |        |      |
| Allocation  |            |            |           |        | X      |        |      |
| INTERVENTIONS: |           |            |           |         |        |        |      |
| [high dose vitamin D] |             |            |           |         |        |        |      |
| [low dose vitamin D] |             |            |           |         |        |        |      |
| ASSESSMENTS: |           |            |           |         |        |        |      |
| Primary: *[Vitamin D level, IL-6, MCP-1, CRP, PTH]* |   |           | X         | X      | X      |        |      |
| Secondary: *[GCS, BMI, FFM, FM, APACH II, SOFA, delirium severity, infection rate, time to weaning from MV and discharge from ICU]* |   |           |           |         |        |        |      |
| [28 day mortality] |             |            |           |         |        |        |      |
| Adverse events (Ca) |           |            | X         | X      | X      |        |      |

Figure 2

SPIRIT figure showing schedule of interventions and assessments. IL-6: interlukine-6, MCP-1: monocyte chemoattractant protein-1, CRP: C-reactive
protein, PTH: parathyroid hormone, GCS: Glasgow coma scale, BMI: body mass index, FFM: fat free mass, FM: fat mass, APACHE II: acute physiology and chronic health evaluation, SOFA: Sequential Organ Failure Assessment, MV: mechanical ventilator
Figure 3

Trial procedures flow sheet

Supplementary Files
This is a list of supplementary files associated with the primary manuscript. Click to download.

Formula.jpg
SPIRIT-Checklist-download-8Jan13.pdf