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Review article

Vitamin D Supplementation in Patients with Iron Deficiency Anaemia: A Systematic Review and a Meta-Analysis

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ABSTRACT

Recent studies exhibit low Vitamin D levels and iron deficiency anemia condition co-exist, but evidence on the impact of vitamin D supplementation on iron deficiency anemia is lacking. The objectives of the review were to estimate the efficacy of vitamin D supplementation on patients with iron deficiency anemia. This review included the Randomized Controlled Trials from PubMed, IndMed and Cochrane Database of Systematic Reviews. Trials presenting vitamin D supplementation with control groups administered placebo and published in a peer-reviewed journal were considered eligible. Four trials were included for the primary outcome analysis. Vitamin D supplementation had no statistically significant impact on the outcomes of Hemoglobin {p< 0.18; I^2: 36%; MD -0.05, 95% confidence interval (CI) -0.39 to 0.28} and Serum Ferritin {p< 0.18; I^2: 36%; MD -0.05, 95% confidence interval (CI) -0.39 to 0.28} However, there was a statistically significant impact for serum Vitamin D levels by supplementation of vitamin D {p<0.00001; MD 17.28, 95% confidence interval (CI) 5.64 to 28.92}. As the results depicted in this review are inconclusive, further randomized controlled studies are required to strongly conclude whether supplementation of vitamin D improves iron deficiency.

SYSTEMATIC REVIEW REGISTRATION

Prospero registration number: CRD42018082004.

Key words: Hemoglobin, Serum Ferritin, Serum Vitamin D, Supplementation, Randomized controlled trials.

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INTRODUCTION

Iron deficiency anemia is a condition deficit of iron in the body with resultant reduction in the production of red blood cells. The red blood cells play a foundation role in the human body with roles of oxygen carriage and storage.[1] Iron deficiency anemia affects more than 2 billion people worldwide. The worldwide effort so far has been largely based on iron supplementation, food fortification and diversification of the diet.[2] Iron is a trace element and iron-containing proteins are responsible for the storage and transport of oxygen (hemoglobin, myoglobin) and various biological activities such as catalyzing metabolic reactions, redox reactions and molecular signaling etc. The intake of iron through diet takes place through the divalent metal transporter DMT1, expressed on the brush-border membrane of duodenal enterocytes and erythrocyte precursors.[3]

Vitamin D deficiency or insufficiency is estimated to affect over 1 billion people globally. However, the role of international health organizations or governmental bodies in declaration of urgent needs of achieving sufficient vitamin D blood levels is very minimal.[4] Vitamin D as the name suggests is a vital organic compound playing significant roles in the development and growth of the human body. The sunshine vitamin is a product of the breakdown of 7-dehydrocholesterol corresponded by ultraviolet B radiation of the sunlight. Additionally, the active form of the mineral is Vitamin D3, which further uncovers the complex pathways from the livers to the kidneys and the consecutive production of calcitriol, stored in the body in the form of Cholecalciferol and Ergocalciferol. The requirement of Vitamin D supplementation depends upon various factors such as the regional climatic conditions, the key duration of sun exposure, lifestyle and co morbidities. Once ingested or synthesized in the body, both the parent vitamin D molecules are metabolized in the body, with the net production of 25-hydroxyvitamin D (25(OH)D), which is the key diagnostic indicator of vitamin D levels. Hydroxylation follows this stage in the hepatic and extra hepatic pathways with production of vitamin D_3 {1,25-dihydroxyvitamin D (1,25(OH) 2D). Vitamin D deficiency is defined as Serum 25(OH) D concentrations is lesser than 25 nmol/L while a concentration of 50 to 74 nmol/L indicates vitamin D insufficiency. Vitamin D deficiency condition is commonly found among the iron deficiency anemic population. Various studies have reported an association between vitamin D deficiency and iron deficiency anemia. Additionally the supplementation of vitamin D increases serum concentrations of 25(OH) D. The enzyme CYP27B1 catalyzes the 1-alpha hydroxylation step crucial for the synthesis of the active vitamin D and CYP27B1 is found predominantly in the kidney, pulmonary epithelium and the leucocytes[5] Studies suggest a relation between the up regulation of hepcidin in vitamin D deficiency whereas hepcidin plays a key role in the absorption of iron from the intestinal junction. Increased levels of hepcidin leads to decreased levels of ferroportin, which is a chief iron transport protein with net resultant deficiency of iron in the systemic circulation. Such findings lead to the possibility that supplementation of vitamin D may play a role in iron deficiency anemia through different biological pathways.[5]

This systematic review includes studies evaluating the effects of Vitamin D (vitamin D3, vitamin D2, 25(OH) D or 1,25(OH)2D) supplementation via any route and at any dose in patients with iron deficiency anemia. The aim of this systematic review is therefore to assess whether Vitamin D supplementation in iron deficiency anemia (IDA) patients presents outcome of interest (increase in hemoglobin concentration and serum ferritin levels).

METHODS

Electronic searches

We reviewed blinded, randomized and placebo-controlled trials comparing the supplementation of Vitamin D and placebo in IDA patients. We included studies where full texts were accessible. For studies where data were not available, we contacted the authors for the missing information of the trial and wherever full texts articles were unavailable, we excluded

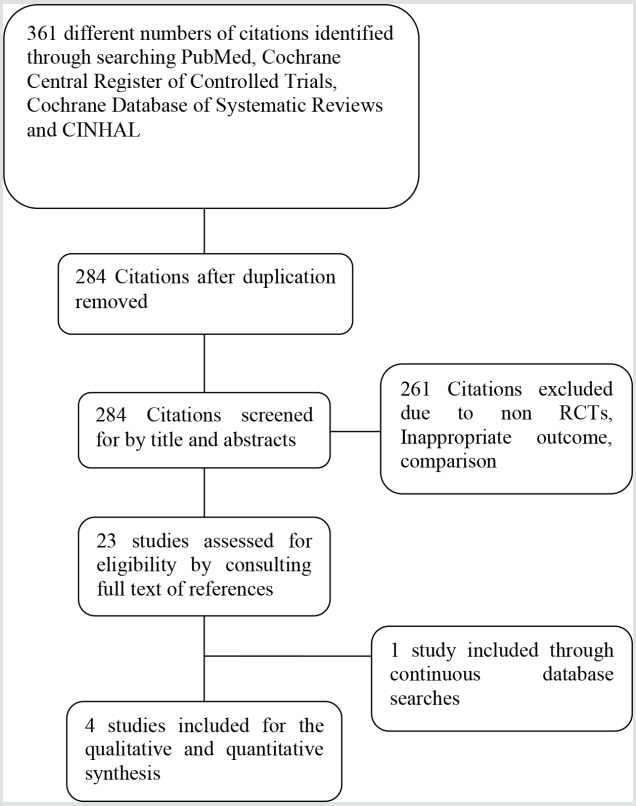

Figure1: Flow diagram of the study inclusion procedure.

the studies.

We identified trials from the Cochrane library, PubMed and IndMed. The search terms and strategy incorporated included were hydroxy vitamin D or hydroxyvitamin D or vitamin D or ergocalciferol or calcitriol or cholecalciferol or 25-hydroxy vitamin D or vitamin D deficiency or hydroxycholecalciferol or dihydroxycholecalciferol or calcifediol or calcidiol or calciol or alpha calcidol and iron deficiency anemia or iron deficiency or Total Iron Binding Capacity or red blood cell or mean cell volume or mean cell hemoglobin concentration or hematocrit. And the language was limited to English only. Furthermore, we also cross-checked the references indexed in all the primary included studies for additional sources of information in various online databases that could also be included in our analysis (Figure 1).

Selection of studies

The search strategy was carried out from 23rd December 2017 to 23rd of January 2018 and included trials conducted during the last 10 years. Three Reviewers RB, TT and SH independently screened all the potentially relevant studies identified for inclusion on the basis of the title and the abstracts using the online Rayyan platform where inclusion and exclusion for trials were carried out with assessments for the duplication of trials. We then retrieved the full text articles of the included studies and two reviewers (RB, TT) independently reviewed the full texts for inclusion and exclusion for the final meta-analysis.

Disagreements were sorted out through discussion with consultation with the third member of the review group (SH). The PRISMA flow diagram was considered for the selection process with sufficient details of the reviewing process. We included studies presenting adult patients with clinical diagnosis of IDA without any co morbidities and Vitamin D supplementation at any doses. The primary outcome was the evaluation of Vitamin D supplementation in IDA patients and the outcome changes in serum Vitamin D, serum ferritin and hemoglobin. Additionally, we excluded studies that were conducted in patients with multiple comorbidities.

Data extraction and management

For the included studies, two reviewers (RB and TT) independently extracted the key characteristics of the subjects and the interventions and reported the outcome data using the data extraction form. Which was prepared and validated by RB. Disagreements were sorted with consultation with a third review author (SH).

The data extraction form was designed by RB and constituted details pertaining to the study design, aim of study, study objective, year of publication, method of recruitment of participants, informed consent form obtained, study population, total number randomized, baseline imbalances, withdrawals and exclusions, primary and secondary outcomes, time points measured and reported, intervention and control, duration of study, risk assessment, inclusion and exclusion criteria, imputation of missing data, funding sources and conflicts of interest etc.

Assessment of the risk of bias in the included studies

The risk of bias was independently assessed in each included studies by two authors (TT and SH). Differences in opinions were resolved by consulting with a review author (AR). Risk of bias was estimated using the Cochrane Risk of bias assessment tool, including the following criteria; selection bias, allocation concealment, performance bias and detection bias, attrition bias, reporting bias and other potential sources of bias. The risk of bias was then categorized the risk of bias as 'low risk', 'high risk' or 'unclear risk'. A Risk of bias graph is presented with plausible summary of the risk of bias assessment (Figure 2). We carried the review according to a pre-set protocol assessing all the procedure requirements and management of deviations.

Statistical analysis

We conducted the statistical analyses following the recommendations set forth by the Cochrane Handbook for Systematic Reviews. We analyzed the data using Review Manager 5.3. The outcome impact of Vitamin D supplementation was calculated as mean difference or standardized mean difference (SMD) with 95 % confidence intervals (CIs) for continuous data using the random-effects model. Random effects model was used due to the high level of heterogeneity and the indecisive intervals of the estimate effects.

In case of missed data relating to the outcome, we contacted investigators to verify key study characteristics where possible. We applied the I^2 statistic to measure heterogeneity among the studies. Where possible (when the I^2 values were greater than 60%), we performed a subgroup analysis. However, limitations of subgroup analysis application were the large differences in sample size across the studies, which are mentioned separately as a plausible reason for the heterogeneity.

RESULTS

Our search strategy identified 361 potential citations for inclusion. After conducting the primary screening of titles and abstracts, 23 full-text studies were assessed for eligibility. The final screening of the full length articles lead to the inclusion of 4 RCTs. One study was included after the reference screening of the included studies as well. The laboratory value of serum Vitamin D post intervention exposure was sought after contacting the author.[6]

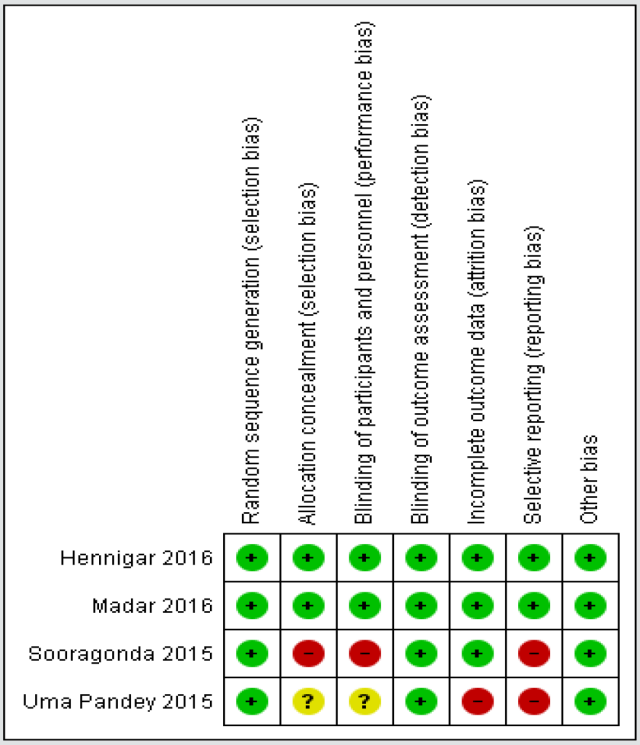

Figure 2: Risk of bias summary. The review author's judgments about each risk of bias item for each of the included studies.

Description of studies

We proceeded with the data extracted from the 4 included articles. The primary outcomes of interest were change in serum Vitamin D, change in serum Ferritin and change in hemoglobin levels, vitamin deficit, iron deficit and serum ferritin deficit. Where two studies were double blinded randomized controlled trials Hennigar *et al.*[7] Madar *et al.*[8] and one study single blinded Sooragonda *et al.*[9] and one pilot study Pandey and Divakar 2015.[10]

One study Hennigar *et al.* involved Vitamin D and calcium containing snack bar as the intervention. Another study Madar *et al.* had 10mcg and 25mcg of Vitamin D and the other 2 studies had Iron plus Vitamin D supplementation as the intervention.[9,10] The age ranged from 20 to 45 years and 56.51% (Data of females in Sooragonda is not mentioned) of the participants were women. The studies were conducted in Norway Madar *et al*. Natick, Massachusetts Hennigar *et al*. and India Sooragonda *et al*. The follow up procedure were not clear in these studies. However, one study was conducted in community centers Madar *et al.* 3 other studies were conducted in Outpatient settings Hennigar *et al*. Sooragonda *et al*. (Table 1) The studies which did not meet the inclusion criteria were excluded.

Methodological quality of included studies

These studies included were of variable methodological qualities. In particular, Sooragonda e*t al.* did not report allocation concealment. Therefore, we considered the risk of selection bias for this study as 'High' and the blinding method was unclear. Thus we concluded the risk of blinding bias for the above study as 'High'. However, the blinding process was quite clear in the other two studies Madar *et al.* and Hennigar *et al.* We therefore concluded the risk of performance and detection bias as low for these studies. 14.34% of participants were lost to follow up in the study by Madar *et al*. We have therefore rated the risk of attrition bias as low for this study. The study by Hennigar *et al* reported no lost to follow up and the data of all the 152 were included for final analysis. Therefore, it led us to assess the risk of attrition bias as being low for this study. We categorized the study by Sooragonda *et al.* as being at low risk of attrition bias since there was no loss to follow up. The study by Hennigar *et al.* did not report the outcome differential values of 25(OH) D pre and post analysis as it was reported in a previous publication. However, we did not find any evidence of selective reporting in any of the other studies. We identified that the sample collection was done via non-fasting venous blood Madar *et al*. while the other two studies included use of fasting blood sample only (Table 3). We did not find other potential sources of bias for the remaining of the studies (Figure 2).

Effects of interventions

We assessed the quality of evidence using the GRADE pro software. End-points relating to the comparison between Vitamin D versus placebo for patients with IDA relating to changes in serum Vitamin D, serum Ferritin and Hemoglobin are depicted in the Summary of findings (Table 2).

Outcomes

Four trials with a total of 429 participants contributed to the comparison for the outcome of Vitamin D, 407 participants for the outcome change in hemoglobin levels and 396 participants for the analysis of outcome changes in serum ferritin levels.

Serum Vitamin D

Consumption of vitamin D led to a statistically significant increase in the serum 25(OH) D levels (p<0.00001; MD 17.28, 95% confidence interval (CI) 5.64 to 28.92; 429 participants; 4 studies; high-quality evidence; (Figure 3a). However, the I^2 (Heterogeneity) was found 98%. This high level of heterogeneity may be due to the incomparable sample sizes between the studies. Additionally, the sample size calculation in one study Hennigar *et al.* was calculated for the estimation of calcium and Vitamin D supplementation in improving bone and muscle strength and not for the evaluation of outcome changes in the vitamin D, ferritin and hemoglobin etc. The study by Madar *et al.* was designed to evaluate two different doses of vitamin D supplementation. However, the results depicted were in combined values for both the doses with non-linearity in the sample sizes in the placebo to the intervention group. (Figure 3a)

Haemoglobin

Vitamin D supplementation was not statistically significant for a positive outcome in the change of blood haemoglobin levels. (p< 0.18; I^2: 36%; MD -0.05, 95% confidence interval (CI) -0.39 to 0.28; 407 participants; 4 studies; high-quality evidence; Analysis 1.2; Figure 3b). The study by Hennigar *et al.* depicted results separately for males and females, which we reported as two different outcome data. The inconclusive outcome in the mean pooled estimate can be a consequence of in the non-similar sample sizes as well as the different inter-study doses and sample size calculations. (Figure 3b)

Serum Ferritin

Supplementation of vitamin D did not produce a positive outcome on serum ferritin levels. {p<0.21; I^2: 33% (MD 1.70, 95% confidence interval (CI) -9.12 to 12.53; 396 participants; 3 studies; high-quality evidence; Analysis 1.3; Figure 3)}. The study by Hennigar *et al.*[7] presented results separately for males and females, which is depicted in our analysis as different comparators. The forest plot depicts an inconclusive outcome, which may be attributing to many factors such the Vitamin D doses,

Table 1: Characteristics of included studies.

Source	Design	Duration	Participants	Mean age	Sex	Interventions group	Outcome
Hennigar SR *et al.* (2016)	A randomised, double-blind, placebo-controlled trial Country: USA Intention to treat analysis	Period of study: February and April 2013	total number of subjects were 152 **Exclusion criteria** <18 years of age, pregnant or lactating women history of kidney disease or renal calculi Allergy to any component of the food product bars. **Inclusion criteria** Male and female subjects between the ages of 18 and 42 years who entered US Army basic combat training (BCT) during February 2013 were eligible to volunteer.	21.2	64.47% male 35.52% female	**Intervention group** 2000mg/d Ca and 25µg/d vitamin D (n=75) in a fortified food product during 9 weeks of military training affects Fe status in young adults. **Control group** Placebo (snack bar without Ca/vitamin D; n 77). The bars were individually labelled with volunteer ID numbers and packaged into 1-week allotments (fourteen bars each).	Change in levels of Serum Transferrin saturation, Serum Ferritin, Haemoglobin levels post Ca/Vit D supplementation. **Baseline** Male Hb: 15.4 g/dL Serum ferritin: 118 ng/ml Female Hb: 12.9 g/dL Serum ferritin: 54.5 ng/ml After 9 weeks Male Hb: 13.7 g/dL Serum ferritin: 53.6 ng/ml Female Hb: 12.7 g/dL Serum ferritin: 26.6 ng/ml
Madar AA *et al.* (2016)	A randomized, double-blind, placebo-controlled trial among ethnic minorities living in Norway Country: Middle East, Africa or South Asia. Protocol analysis	Period of study: The 16-week intervention study was carried out from January to June 2011	Randomized n-251 **Exclusion criteria** Not meeting inclusion criteria Declined to participate **Inclusion criteria** not pregnant non-breastfeeding not regularly using vitamin D-containing supplements not being treated for vitamin D deficiency not using medication that interfered with vitamin D metabolism (thiazides, anti-epileptics, prednisolone or hormone re- placement therapy) not suffering from any condition such as malabsorption, kidney diseases, cancer, tubercu- losis, sarcoidosis, osteoporosis or recent fractures.	37.3	Female 72.5% Males 72.5%	**Intervention group** 2 intervention group 25mcg vitamin D3 daily 16 weeks (n=84) 10 mcg vitamin D3 daily 16 weeks (n=85) **Control group** Placebo tablet daily 16 weeks (n=82) The tablets were similar in colour, size and packing. Each participant was given a box containing 120 tablets (a 16- week use corresponds to 112 tablets) at baseline along with a self-administered compliance form.	**Primary outcome**: the changes in the s-ferritin, haemoglobin, s-iron and transferrin saturation. status during the 16-week intervention between **Secondary outcome**:The effect of vitamin D supplementation on muscle strength and power among ethnic minorities in Norway **Baseline** 25(OHD): 28.7 nmol/l Hb: 13.7 g/dL Serum ferritin: 68.4 µg/L **After 16 weeks** 25 (OHD): 48.8 nmol/l Hb: 13.5 g/dL Serum ferritin: 68 µg/L

Continued....

Table 1: Characteristics of included studies. (Con)

Source	Design	Duration	Participants	Mean age	Sex	Interventions group	Outcome
Sooragonda B *et al.* (2015)	A Randomized, Single-Blinded, Placebo-Controlled Trial Country: Chandigarh, India Intention to treat analysis	Period of study: 12-week interventional	Randomized n-30 **Exclusion criteria** Pregnant patients Patients with CKD (defined as an eGFR <90 ml/min/1.73 m 2) chronic liver disease, celiac dis- ease (as detected by a positive tissue transglutaminase test) previous known hemoglobinopathies, pregnancy and patients with evidence of infection/inflammation (as detected by positive Creactive protein) were excluded from the study **Inclusion criteria** Patients aged from 15 to 60 years, who were diagnosed with iron-deficiency anaemia with exclusion of other disorders, were screened for 25(OH)D levels and those with a level <20 ng/ml were recruited for the study	41	-	15 were randomized into the placebo arm and 15 to vitamin D therapy. **Intervention group** Iron was administered to all the patients (n = 30). On the same day, patients in the intervention arm also received 6 lakh units of Cholecalciferol, i.e. vitamin D 3, intramuscularly (i.m.) **Control group** while patients in the placebo arm received a similar volume of saline (i.m.) as like the intervention group	Change in the levels of 25(OH)D, PTH, Sr Ferritin ad Haemoglobin at the end of 12 weeks **Base line** Hb: 9.7 g/dL 25(OHD):10.3 ng/ml **After 12 weeks** Hb: 11.8 g/dL 25(OHD): 57.7 ng/ml Serum ferritin: 92.3 µg/L
Pandey U *et al.* Period of study: 12 weeks (2015)	Pilot study Country: Varanasi, India Protocol analysis	12 weeks	Randomized = 20 **Inclusion Criteria** pregnant women of age 20-45 years, gestation age 12-22 weeks **Exclusion criteria** non-pregnant or lactating women, age <20 or >45 years, Hb level <8 or >10 g/dl, 25 (OH) D levels > 20 ng/ml, anaemia of any other cause (sickle, thalassemia, aplastic, hyperchromic etc.) and mothers on vitamins/mineral supplements.	21.25	100%	1. Group iron + Vitamin D: tablets containing fixed dose combination of vit D (1000 IU) + ferrous ascorbate (100mg of elemental iron) + folic acid (1 mg) + vit B12 (7.5 mcg) (1 tab/day) for 12 weeks. 2.Group iron alone: tablets containing fixed dose combination of ferrous ascorbate (100 mg of elemental iron) + folic acid (1.1 mg) (1 tablet/day) for 12 weeks. Results	**Primary outcome**: The objective of the study was to evaluate the effect of Vit D and Iron supplementation on Serum Hb level and Vit D level as compared to Iron supplementation. **Secondary outcome**: To evaluate the percentage responders to Iron supplementation when Vit D is added as compared to only Iron supplemented. **Base line** Iron group 25(OHD): 11.31 ng/ml Hb: 9.15 g/dL Vitamin D + iron group 25(OHD): 9.95 ng/ml Hb: 8.56 g/dL **After 12 weeks** Iron group 25(OHD): 11.90 ng/ml Hb: 11.18 g/dL Vitamin D + iron group 25(OHD); 17.38 ng/ml Hb: 10.76 g/dL

Table 2: Summary of findings of comparisons: Vitamin D3 compared with placebo in iron deficient anaemic patients.

Outcomes	Anticipated absolute effects*(95% CI)		№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Placebo	Risk with Vit D			
Haemoglobin	The mean haemoglobin was **12.616** g/dL	The mean haemoglobin in the intervention group was 0.05 g/dL lower (0.39 lower to 0.28 higher)	407 (4 RCTs)	⊕⊕⊕⊕ HIGH	No biases were found in the evaluation of this outcome
Serum Ferritin	The mean serum Ferritin was **64.5** mcg/L	The mean serum Ferritin in the intervention group was 1.7 mcg/L higher (9.12 lower to 12.53 higher)	396 (3 RCTs)	⊕⊕⊕⊕ HIGH[a]	No biases were found in the evaluation of this outcome
Vitamin D	The mean vitamin D was **19.525** nmol/L	The mean vitamin D in the intervention group was 17.28 nmol/L higher (5.64 higher to 28.92 higher)	429 (4 RCTs)	⊕⊕⊕◯ MODERATE[a]	In the study conducted by Hennigar *et al.* the sample size was calculated for evaluating the effect on bone health and not on Fe status.

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **MD:** Mean difference

GRADE Working Group grades of evidence High certainty: We are very confident that the true effect lies close to that of the estimate of the effect **Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different **Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect **Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

Table 3: Risk of bias assessment of the included studies.

	Hennigar SR *et al.* (2016)	
Methods	RCT held in the combat training participants in US army	
Participants	152	
Interventions	Vitamin D and Calcium containing bars	
Outcomes	Hb, Serum Ferritin, Serum Vitamin D	
Bias	**Author's Judgement**	**Support for Judgment**
Random sequence generation (selection bias)	Low Risk	Block randomization by race and sex.
Allocation concealment (selection bias)	Low Risk	Bars were labelled with volunteers ID numbers
Blinding of participants and personnel (performance bias)	Low Risk	Bars were identical in taste and appearance.
Blinding of outcome assessment (detection bias)	Low Risk	Independent individual assessing the outcome
Incomplete outcome data (attrition bias)	Low Risk	All the subjects that were enrolled in the beginning of study were accounted for in the analyses
Selective reporting (reporting bias)	Low Risk	All the relevant parameters were assessed.
Other bias	Low Risk	No other bias were found
	Madar AA et al. **(2016)**	
Methods	RCT	
Participants	251	
Interventions	25mcg and 10mcg Vitamin D	
Outcomes	Serum Ferritin, Hb, Vitamin D	
Bias	**Author's Judgement**	**Support for Judgment**
Random sequence generation (selection bias)	Low Risk	Computer generated block randomization
Allocation concealment (selection bias)	Low Risk	Tablet boxes were numbered according to randomization list by an external pharmacy.
Blinding of participants and personnel (performance bias)	Low Risk	Tablets were similar in color, size and packing
Blinding of outcome assessment (detection bias)	Low Risk	Independent individual assessing the outcome

Continued....

Table 3: Risk of bias assessment of the included studies. (Con)

Bias	Author's Judgement	Support for Judgment
Incomplete outcome data (attrition bias)	Low Risk	The subjects that were dropped out of the study were said to be same as the subjects completed the study and hence they were accounted for.
Selective reporting (reporting bias)	Low Risk	All the relevant parameters were reported
Other bias	Low Risk	No other bias were found
	Sooragonda B *et al.* (2015)	
Methods		RCT
Participants		30
Interventions		6 lakh units of cholecalciferol
Outcomes		Hb, serun Ferritin, serum vtamin D
Bias	**Author's Judgement**	**Support for Judgment**
Random sequence generation (selection bias)	Low Risk	Randomization sequence was generated by computer generated randomized table
Allocation concealment (selection bias)	High Risk	Method not mentioned
Blinding of participants and personnel (performance bias)	High Risk	Method not mentioned
Blinding of outcome assessment (detection bias)	Low Risk	Independent individual assessing the outcome
Incomplete outcome data (attrition bias)	Low Risk	There were no attrition in the number of subjects.
Selective reporting (reporting bias)	High Risk	Serum ferritin levels are not reported at the baseline
Other bias	Low Risk	No other bias were found
	Pandey U *et al.* (2015)	
Methods		RCT pilot study
Participants		20
Interventions		1000IU vit D + 100mg Ferrous ascorbate + 1mg folic acid + 7.5mcg vitB12 tab
Outcomes		average rise in Hb, vit D, % of patients responding to therapy
Bias	**Author's Judgement**	**Support for Judgment**
Random sequence generation (selection bias)	Low Risk	Randomized into groups using a computerized program
Allocation concealment (selection bias)	Unclear Risk	No information on the method used to conceal allocation
Blinding of participants and personnel (performance bias)	Unclear Risk	No data given on type of blinding
Blinding of outcome assessment (detection bias)	Low Risk	Independent individual assessing the outcome
Incomplete outcome data (attrition bias)	High Risk	no data given on the patient who were lost to follow up
Selective reporting (reporting bias)		no other blood parameters except Hb and vit D, were reported
Other bias		No other bias were found

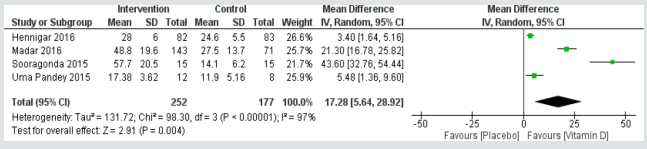

Figure 3a: Forest plot of the change in serum Vitamin D from baseline in the intervention group as compared to the placebo group. Mean difference was used to assess the change from baseline.

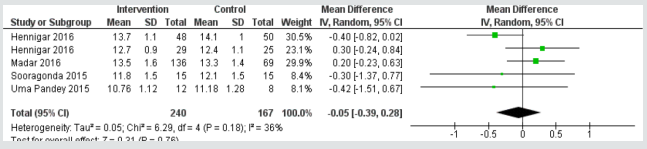

Figure 3b: Forest plot of the change in Hemoglobin from baseline in the intervention group as compared to the placebo group. Mean difference was used to assess the change from baseline.

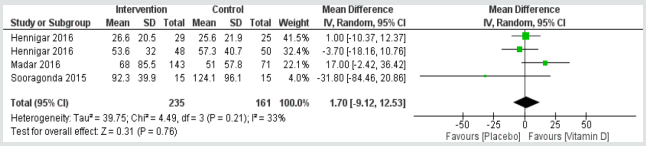

Figure 3c: Forest plot of the change in Serum Ferritin from baseline in the intervention group as compared to the placebo group. Mean difference was used to assess the change from baseline.

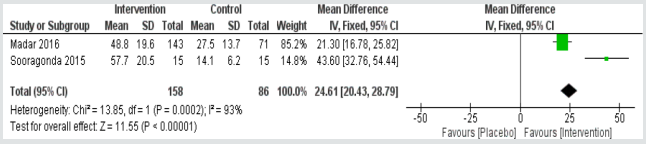

Figure 3d: Subgroup analysis of the change in serum Vitamin D from baseline in the intervention group as compared to the placebo group.

study durations, sample size estimation and the difference in sample sizes. (Figure 3c)

Subgroup analyses

The heterogeneity between the studies ranged for each outcome variables. The forest plot depicting the serum ferritin values with an I^2 of 33% and an I^2 of 36% for hemoglobin. However, the forest plot to evaluate the serum vitamin D values presented an I^2 of 98%. We therefore undertook a subgroup analysis to evaluate any means to avoid heterogeneity. However, the least I^2 value we could present was 93%. This clarifies the outcomes most probably being influenced with the inter study sample size differences, the different doses of the studies and the sample size estimation. (Figure 3d)

Secondary outcomes: Safety

Vitamin D supplementation is generally well tolerated below and any adverse effects are seen above the level of 220nmol/L. The result of a meta-analysis on the safety of vitamin D shows that vitamin D supplementation given for duration of more than 3 years significantly reduces the overall mortality and this effect was enhanced in females. It has been reported that the first sign of vitamin D excess is hypercalcemia, which is manifested as excess urinary excretion of calcium.

Vitamin D versus placebo: sensitivity analysis using Random effects model

Both fixed and random-effects models resulted in similar results for the secondary outcomes. However, since the outcome of this study yields an inconclusive effect and attributing to the high level of heterogeneity in at least one of the studies, there was none any reason to perform a sensitivity analysis.

Vitamin D versus placebo: Funnel plots.

Separate funnel plots for each of the outcomes were plotted (Figure 4a, 4b and 4c). We do not report any publication bias in the study. However, the interpretations of these funnel plots are none justified as the number of the included studies are less.

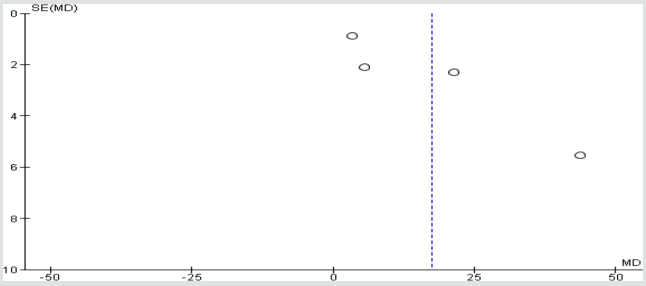

Figure 4a: Funnel plot of the change in serum Vitamin D from baseline in the intervention group as compared to the placebo group.

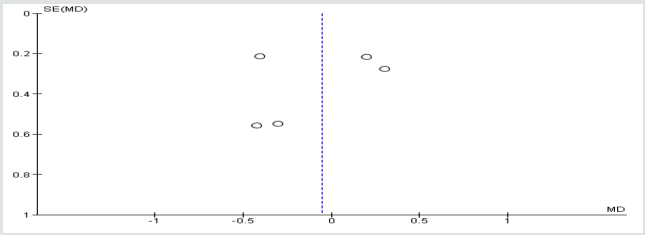

Figure 4b: Funnel plot of the change in Hemoglobin from baseline in the intervention group as compared to the placebo group.

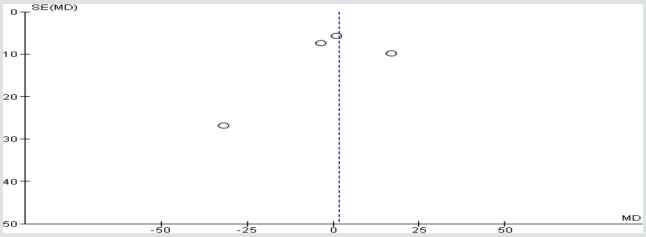

Figure 4c: Funnel plot of the change in Serum Ferritin from baseline in the intervention group as compared to the placebo group.

DISCUSSION

This review evaluates vitamin D supplementation alone or either in combination with Iron or calcium supplements and its effects on iron deficiency parameters such as hemoglobin and serum Ferritin. This review includes four blinded, randomized, placebo-controlled trials of vitamin D supplementation in IDA patients. And these trials contributed to the analysis of changes in hemoglobin and serum ferritin concentrations.

This review compiles all the outcomes and concludes it in this meta-analysis. Though administration of vitamin D supplementation resulted in statistically and clinically significant increase in serum vitamin D (MD: 17.28, 95%CI: 5.64-28.92, I^2:97%), however there were no significant changes in haemoglobin (MD:-0.05, 95%CI: -0.39-0.28, I^2:36%) and serum ferritin (MD: 1.70, 95%CI:-9.12-12.53, I^2:33%) and the results were inconclusive as well.

The studies that were excluded were due to presence of co-morbidities.

For an instance, Ernst JB *et al.*[11] conducted his study in heart failure patients which showed no decrease in anaemia prevalence in patients with vitamin D supplementation. Quite contrary to this, in a 16-week randomized controlled trial conducted by Toxqui L *et al.*[12] the effect of iron versus iron and Vitamin D-fortified milk on improving iron status in 109 iron-deficient women was analysed. Similarly, in another study conducted by Smith *et al.*[13] mechanically ventilated critically ill adults who were administered with 500,000 IU of Vitamin D_3 were associated with increased haemoglobin concentration and decreased serum hepcidin concentration at the end of the study. In the study conducted by Naini AE *et al.*[14] 650,000 IU of vitamin D was administered to the intervention arm showed a significant decrease in the required dose of EPO. There are some evidences that show opposite association as well. There are various trials evaluating the effect of iron supplementation on Vitamin D status.[6,15-19]

This review addresses the effect of Vitamin D deficiency in IDA. This review is strengthened by the fact that four of the studies aims at measuring the primary objectives of this review.

In the study conducted by Sim JJ *et al.*[20] it was found that the subjects with vitamin D deficiency have lower mean hemoglobin levels. Another recent cross sectional study in female athletes evaluating the effect of vitamin D status on iron level and vice-versa report a strong association but due to the limitation of the study design, it could not assess that which nutrient exerts a stronger influence on the other Malczewska-Lenczowska *et al.*[21] Similarly, the study by Pike JW *et al.*[22] concluded on the presence of vitamin D receptors in bone marrow progenitor cells and quite additional to this, the study by Riccio E *et al.*[23] demonstrated the positive effects of calcitriol on erythropoiesis. However, the results of this meta-analysis do not show a casual effect relationship between vitamin D supplementation and IDA.

This review contains evidence from a minimal number of studies. Therefore, the outcomes generated should not be generalized to non-representative patient populations. Furthermore, this attributes to the lack of evidence covering the scope of Vitamin D in IDA patients. This review does not highlight the information regarding the optimum vitamin D doses and circulating 25(OH) D. Only limited data were included from the published manuscripts. Moreover, we are not able to assess the effects of the intervention being modified by any such factors such as lifestyle, comorbidities or the baseline vitamin D concentration. And despite all of these contrasts regarding the external validity, there should be very few reason to question the internal validity of our findings as this review is based on blinded, placebo-controlled trials with minimal bias. Additionally, the effects of vitamin D supplementation for IDA were consistent when expressed in different ways of the fixed verses random effect model. We assessed all the included trials for possible bias. Two studies had high risk of bias in at least one of the bias characteristics. However, as all of these studies contributed high-level evidence for vitamin D supplementation in IDA patients, we regard the evidences for this analysis as high quality. While considering the duration of the studies included, though the evidence can be downgraded in lieu of imprecision or indirectness, the eventual consensus among the authors was that, neither both posed a severe threat to the overall assurance of the result of this meta-analysis to necessitate a down grade. Furthermore, we presented a summary of findings table and assessed any imprecision or indirectness in the studies. And for any arguments, the consensus of the author team was taken as the final decision.

Potential biases in the review process

Our search included several databases for eligible studies using the specified terms and criteria's. However, these strategies also involved unpublished data, which are not included in this review. Though the number of studies included in this review is less, updating the review in the forthcoming terms may clarify the favorable effects of vitamin D supplementation in IDA patients.

CONCLUSION

We did not find a clinically and statistically significant effect of vitamin D supplementation in IDA patients and the meta-analysis yielded an inconclusive effect size. The included trials majorly consist of subjects with mild or moderate Iron deficiency. Apart from these observations, the beneficial effects of Vitamin D supplementation in subjects with comorbidities where the outcomes may be attributed to the unjustifiable numbers of possible pathological and physiological pathways may not be related to Vitamin D administration. Further research is needed to clarify the issues including the pediatrics, pregnant women and subjects with comorbidities. More set of evidence is required for a conclusive meta-analysis before any definitive clinical recommendations are made.

Implications for research

As mentioned, more evidences from clinical trials are required for a conclusive meta-analysis. Furthermore, as mentioned above, the optimum concentrations of vitamin D supplementation and the circulatory 25(OH) D levels are yet not known and needs additional evidence.

CONTRIBUTIONS OF AUTHORS

S Ponnusankar (SP), Roopa BS (RB) and Tsundue T (TT) wrote the protocol. Hema S (SH), Anju Rose (AR), Roopa BS (RB) and Tenzin Tsundue (TT) did database searches for studies. Furthermore the same members evaluated the eligibility of the trials for inclusion, extracted the data and performed the risk of bias assessments. SH and AR drafted the summary of finding table, which was confirmed by RB and TT. SH and TT entered the data into Review Manager 5.3 for statistical analysis, which was checked by RB. RB and TT drafted the manuscript, which was reviewed by SP and all the review authors approved the final version to be published.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DMT1: Divalent Metal Transporter1; **CYP27B1:** Cytochrome P450 Family 27 Subfamily B Member 1; **IDA:** Iron Deficiency Anemia; **SMD:** Standardized Mean Difference; **CIs:** Confidence Intervals; **RCTs:** Randomized Controlled Trials; **EPO:** *Erythropoietin*.

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APPENDIX: Search Terms

Vitamin D

Hydroxy vitamin D
Vitamin D
Ergocalciferol
Calcitriol
cholecalciferol
25 hydroxy vitamin D
Vitamin D deficiency
Hydroxycholecalciferol
Dihydroxycholecalciferol
Calcifediol
Calcidiol
Calciol
Alpha calcediol

Iron Deficiency Anaemia

Iron deficiency anemia
Iron deficiency
TIBC
Haemoglobin
RBC
Mean cell Volume
Mean cell haemoglobin concentration
Hematocrit