Article Type: Meta-Analysis

Title: Vitamin D supplementation, COVID-19 & Disease Severity: A meta-analysis

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Introduction:

Evidence suggest, 25-hydroxy vitamin D – a major circulatory metabolite of Vitamin D stimulates production of protective peptide in response to any viral or bacterial infection1. In case of vitamin D deficiency, this mechanism is hampered and makes host susceptible to the variety of infection including respiratory tract infections. Effect of Vitamin D supplementation on acute respiratory tract infection has been established by many randomized controlled trials2,3. It was observed that irrespective of age, sex and study duration, vitamin D supplementation reduces risk of acute respiratory tract infection in all the patients4,5. The benefits were also found to be dose dependent and profound in individuals with deficiency of vitamin D at baseline6 (5).

Novel coronavirus – COVID-19 is a respiratory disease that causes inflammation and irritation in upper and lower respiratory tract. In severe cases, it travels through alveoli and as a response there is respiratory inflammation that can be visualized on chest X-ray or CT image as "Ground-glass opacity"7. These characteristics share similarities with previously reported respiratory infections and hence remedies showing promising effect in management of them were also explored for potential use during COVID-19 infections. Initially, it was observed that the COVID-19 patients with vitamin D deficiency had poorer outcomes with longer stay in the intensive care unit (ICU) and are at higher mortality rates than their counterparts8. Numerous attempts in the form of systematic reviews and meta-analysis to assess the potential role of vitamin D deficiency in COVID-19 infection, severity and mortality9,10,11. However majority of the reviews remained inconclusive and highlighted the need for more primary studies in the form of randomized controlled trials. 9,10,11. Similarly, unlike other respiratory tract diseases, evidence showing effect of supplementation of Oral VitD on improving the outcome of COVID-19 is still limited to few trials with smaller sample size. Current meta-analysis aimed to synthesize cumulative evidences

from the studies reporting the effect of vitamin D supplementation on ICU stay and mortality outcomes in patients suffering from COVID-19 infections.

Methods:

In accordance with the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA), current systematic review and meta-analysis were conducted11. "Cochrane Handbook for Systematic Reviews of Interventions" was followed for planning and conducting the review12,13.

Search strategies and data extraction

For retrieving eligible articles reporting effect of vitamin D supplementation on COVID-19 outcome, MEDLINE (through PubMed and CENTRAL using MeSH Terms), google scholar, and Preprint servers were searched. Search terms related to Vitamin D supplementation and COVID-19 were designed to obtain relevant articles from the databases. The articles published between December 2019 to 17th December 2020 were independently screened by two reviewers. Initially articles were screened using title and abstract, after this eligible articles were evaluated separately by two reviewers using full text. We aimed to include any study that assessed effect of vitamin D supplementation (irrespective of dose and form) on ICU admission and mortality in laboratory-confirmed COVID cases. Discordance between the authors were settled by discussion and any difference of opinion that arose was resolved through mutual consensus. Articles published in English language were included in the study. Other language articles were also included if English summary was available. Included articles were also looked for additional studies through reference list searching and any eligible article found was included in the review. Relevant details from each screened article were extracted in an electronic data collection matrix by two reviewers

independently. After final selection three articles were found eligible for the review, of them two were randomized controlled trials and one was retrospective study. The detailed PRISMA chart regarding search is presented as figure 1.

Risk of bias assessment

The RCTs were assessed for risk of bias using Cochrane tool14 and quality of each trial was studied by all two reviewers and any discrepancy was addressed through re-evaluation and consensus among the authors. Quality score was generated using various criteria – randomization, double-blinding, and dropouts on five methodological domains - random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential threats to validity. Based on the score generated on different aspects the studies were categorized into low risk, moderate risk and high-risk categories. For the observational study "Risk of Bias Assessment tool for Nonrandomized Studies (RoBANS)"15 for the controlled observational studies, as recommended by the Cochrane Handbook for Systematic Reviews of Interventions was used. The study was assessed based on six factors - (1) the selection of participants (2) confounding variables (3) measurements of exposure (4) blinding of outcome assessments (5) incomplete outcome data (6) selective outcome reporting. Based on the factors the study was categorized into either high, unclear or low risk of bias category.

Statistical analysis

Statistical analysis was performed using Review Manager (RevMan) Version 5.3, Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. The software was used for pooling the data and deriving cumulative effect of the intervention on outcome of interest. The

results were specifically assessed for presence of heterogeneity using Q statistics (significant at $p<0.10$). I2 – a quantitative measure of heterogeneity was used to categorize studies into various levels of heterogeneity (high: 75–100%, medium: 50–70%, and low: 0–50%). In case of I2 more than 50% two-tailed values of random-effect model were considered to measure the impact of an intervention, whereas fixed effect model was applied for the cases having I2 less than 50%. Cumulative results showing improvement in ICU need and mortality rates with vitamin D supplementation are presented using forest plots. Publication bias was assessed using both quantitative and qualitative methods. Presence or absence of statically significant bias was concluded from the quantitative results of Egger's and Begg and Mazumdar rank correlation test, whereas visual inspection of bias was undertaken using Funnel plot. Forest plot was used to display the relative treatment effect and its 95% CI for each study.

Results:

The final systematic review and meta-analysis included three studies16-18, two randomized controlled trials and one retrospective case-control study. The detailed characteristics of the study are presented in table 1. The review included details from 532 hospitalized COVID-19 positive patients. Though all the studies used oral supplementation of vitamin D, the duration and dosage of treatment varied. The details are provided in table 1. The methodological quality of included studies was reasonably fair, as all three studies had a low risk of bias.

The cumulative effect of vitamin D supplementation on ICU admission and mortality in hospitalized patients of COVID-19 were assessed using meta-analysis. It was observed that there is a statistically significant ($p<0.0001$) difference between ICU admission rate in patients with vitamin D supplements as compared to patients without the supplementations (odds ratio: 0.36; 95% CI: 0.210 to 0.626; table 2; figure 2). Though all three studies favored the intervention arm

the degree of impact varied among the studies and that resulted in heterogeneity as indicated by higher I2 (82.94%) and deviation from funnel shape (figure 3). Removal of one study Murai et al., has reduced the heterogeneity and indicated significant reduction in the overall ICU needs with vitamin D supplementation. However, the studies were free from any significant publication bias as assessed by Egger's and Begg's test. (table 3)

Meta-analysis of morality proportion in both the groups were assessed and compared. It was found that vitamin D supplements has no effect on mortality as compared to placebo treatment/usual care (odds ratio: 0.93; 95% CI: 0.413 to 2.113; p=0.87; table 4; figure 3). The findings were consistent with no heterogeneity as indicated by I2 (21.71%; p=0.27). Even the funnel plot showed a satisfactory distribution of the studies (figure 4). Similarly, Egger's and Begg's tests showed absence of any significant publication bias (p>0.05).

Discussion:

To the best of our knowledge this is the first meta-analysis that synthesized cumulative evidences assessing impact of vitamin D supplementation on intensive care needs and mortality in hospitalized COVID-19 patients. It was observed that as compared to conventional care, vitamin D reduces severity of the disease; however, the results regarding improving mortality statistics could not reach to a statistically significant conclusion.

The first study conducted by Castillo et al.16 randomized 76-consecutive hospitalized COVID-19 patients in intervention to control group in ratio of 2:1 in Spain. The patients in the intervention arm received soft capsules of calcifediol (0.532 mg) on the day of admission through oral route and continued with the oral calcifediol (0.266 mg) on day 3, 7, and then weekly until discharge or ICU admission. Whereas the patients in the control arm followed the standard treatment protocol

with combination of hydroxychloroquine (400 mg every 12 h on the first day, and 200 mg every 12 h for the following 5 days), azithromycin (500 mg orally for 5 days). Though baseline characteristics of patients in both the arms were matched, controls were more hypertensive (57.69% vs 24.19%; p=0.002). To adjust the effect of the confounders such as hypertension and diabetes, authors applied multivariate logistic regression analysis and found that the lower probability of ICU admission in intervention still remained significant [odds ratio=0.03 (95 % CI: 0.003− 0.25)] as compared to control. Similarly, the mortality rates were also lower in patients treated with calciferol, however it could not reach to a statistically significant level possibly due to extremely lower number of patients with adverse outcome. However, authors also acknowledged potential confounding effect of obesity and pre-existing deficiency of vitamin D as limitation of study. They recommended need of more extensive research with appropriately matched arms.

A double-blind, randomized, placebo-controlled trial in Brazil conducted by Murai et al.,17 showed an effect of a single dose of 200,000 IU of vitamin D3 supplementation to hospital stay in severely ill COVID-19 patients. During the trial, 240 patients were equally randomized either in vitamin D supplementation or placebo arm. The baseline demographic and clinical characteristics were comparable between both the arms. Though the supplementation was found to be safe and it improved serum 25-hydroxyvitamin D levels, it did not translate into any clinical benefits to the patient in the form of reduced hospital stay, the requirement of ICU support or mortality rate. Hence the authors recommended against the use of vitamin D as adjuvant therapy in hospitalized COVID patients. Though it was found that requirement of oxygen therapy was low in patients treated with vitamin D as compared to placebo group (65.5% vs 85.9%; p=0.008). Removal of this

study from overall analysis resulted in lowering of heterogeneity in cumulative findings of meta-analysis and yielded beneficiary effect on ICU needs with vitamin D.

Hernandez et al.,18 retrospectively assessed the role of vitamin D supplementation on 216 hospitalized COVID-19 positive patients in Spain. It was observed that 19 patients who were on vitamin D supplementation, had drastically low requirement of ICU care as compared to their counterparts (5.3% vs 25.4%), however similar to other studies there was no difference in mortality (10.5% vs 10.4%) between both the groups. The study also compared the serum levels of 25-hydroxyvitamin D in hospitalized COVID patients and compared it with the population-based controls of similar age and sex. It was found the patients had significantly low levels of serum 25-hydroxyvitamin D as compared to population based-controls even in the presence of main confounding factors. However, the observational nature of the study was accepted as one of the important limitations. One important finding emerged from the study was that unlike other reports there was no relationship between serum 25-hydroxyvitamin D levels and the parameters of COVID-19 severity, such as ICU admission, the need for mechanical ventilation, or mortality. This might be due to smaller number of events in the groups. However, it also highlights the need to assess Vit D supplementation's effect in a prospective manner using the randomized controlled trial study design.

SHADE study19, a randomized controlled trial assessed effect of high-dose vitamin D supplementation (60 000 IU of cholecalciferol - oral nano-liquid droplets) on 21-days recovery in COVID-19 patients. The authors found that greater proportion of vitamin D-deficient individuals with SARS-CoV-2 infection turned COVID-19 negative with a significant decrease in fibrinogen on high-dose cholecalciferol supplementation. However, they did not assess the role of

supplementation on ICU requirement and mortality and hence were not included in the current meta-analysis.

Despite this heterogeneity among the studies, cumulative findings of the meta-analysis favored vitamin D supplementation for reduction of COVID-19 severity. However, more trials are required to substantiate the findings on other outcomes, especially community based and in-hospital trials should also be conducted in developing countries to assess potential of vitamin D supplementation in reducing hospitalization-, ICU- and ventilation needs and mortality rates.

Limitations:

The quality of meta-analysis is directly proportionate to the quality of available secondary literature. As this meta-analysis is based on very early outcome reports exploring impact of vitamin D on various outcome indicators of COVID-19, it suffers from some inherent limitations as follows: 1) Number of trial available right now provides insufficient information regarding effect of various doses and appropriate duration of therapy on the outcome of interest. All three studies included in the meta-analysis used vitamin D supplementation for different duration and at different dosage. Evaluation of the impact using a standard protocol needs to be explored to understand exact effect of the intervention on various indicators 2) The studies are also underpowered to assess the impact of supplementation in patients having severe deficiency of vitamin D at baseline. More robust randomised controlled trials with sufficient sample power are needed to obtain detailed understanding of these limitations 3) Similarly, the baseline characteristics of the population enrolled in the studies were heterogeneous with respect to other comorbid conditions. Due to limited number of available evidences, assessment of vitamin D effect in these individual groups was not possible.

Conclusion:

The findings of current meta-analysis suggest a potential role of vitamin D in reducing COVID-19 severity. However additional evidences with larger sample size and prospective study designs are needed to substantiate it further.

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Table 1: Characteristics of the studies included in the meta-analysis

Sr No	Author	Country	Study type	Interventi-on (N)	Control (N)	Intervention	Control	Intervention details and duration	Outcome	ICU Admission in intervention arm (%)	ICU Admission in control arm (%)	Mortality in intervention arm (%)	Mortality in control	Mortality in control arm (%)	Risk of bias
1	Castillo et al., 2020	Spain	Parallel pilot randomized open label, double-masked clinical trial	50	26	Oral calcifediol	Usual care	Oral capsule (0.532mg) at the day of admission; 0.266mg on day 3 and 6; then weekly until discharge or ICU stay	ICU admission; Death	2	50	0	2	7.7	Low
2	Murai et al., 2020	Brazil	Multicenter, double-blind, randomized, placebo-controlled trial	120	120	Oral Vitamin D	Placebo	Single 11 oral dose of 200,000 IU of vitamin D3	Hospital length of stay, Death, admission to ICU, mechanical ventilation requirement, serum level of Vitamin D and biomarkers	15.83	20.83	6.67	6	5	Low

3	Hernández et al., 2020	Spain	Retrospective case–control study	19	197	Oral Vitamin D	Usual care	3 Months (Dose details are not available)	Admission to the intensive care unit, requirements for mechanical ventilation, or mortality	5.263	25.38	10.53	20	10.15	Low

Table 2: Meta-analysis summary for vitamin D supplementation and ICU requirement

Study	Intervention	Controls	Odds ratio	95% CI	z	P	Weight (%)	
							Fixed	Random
Castillo et al., 2020	1/50	13/26	0.0204	0.00244 to 0.171			8.02	29.86
Murai et al., 2020	19/120	25/120	0.715	0.370 to 1.382			83.28	39.64
Hernández et al., 2020	1/19	50/197	0.163	0.0213 to 1.255			8.70	30.51
Total (fixed effects)	21/189	88/343	0.363	0.210 to 0.626	-3.645	<0.001	100.00	100.00
Total (random effects)	21/189	88/343	0.158	0.0171 to 1.452	-1.631	0.103	100.00	100.00

Table 3: Publication bias analysis

ICU admission and Vitamin D supplementation	
Egger's test	
Intercept	-3.4604
95% CI	-21.9819 to 15.0610
Significance level	P = 0.2538
Begg’s test	
Kendall's Tau	-1.0000
Significance level	P = 0.1172
Mortality and Vitamin D supplementation	

Egger's test	
Intercept	-2.4958
95% CI	-9.4871 to 4.4954
Significance level	P = 0.1381
Begg’s test	
Kendall's Tau	-1.0000
Significance level	P = 0.1172

Table 4: Meta-analysis summary for vitamin D supplementation and mortality

Study	Intervention	Controls	Odds ratio	95% CI	z	P	Weight (%)	
							Fixed	Random
Castillo et al., 2020	0/50	2/26	0.0970	0.00448 to 2.100			7.72	10.56
Murai et al., 2020	8/120	6/120	1.357	0.456 to 4.037			61.38	55.00
Hernández et al., 2020	2/19	20/197	1.041	0.224 to 4.839			30.90	34.44
Total (fixed effects)	10/189	28/343	0.934	0.413 to 2.113	-0.164	0.869	100.00	100.00
Total (random effects)	10/189	28/343	0.938	0.332 to 2.651	-0.122	0.903	100.00	100.00

Figure 1:

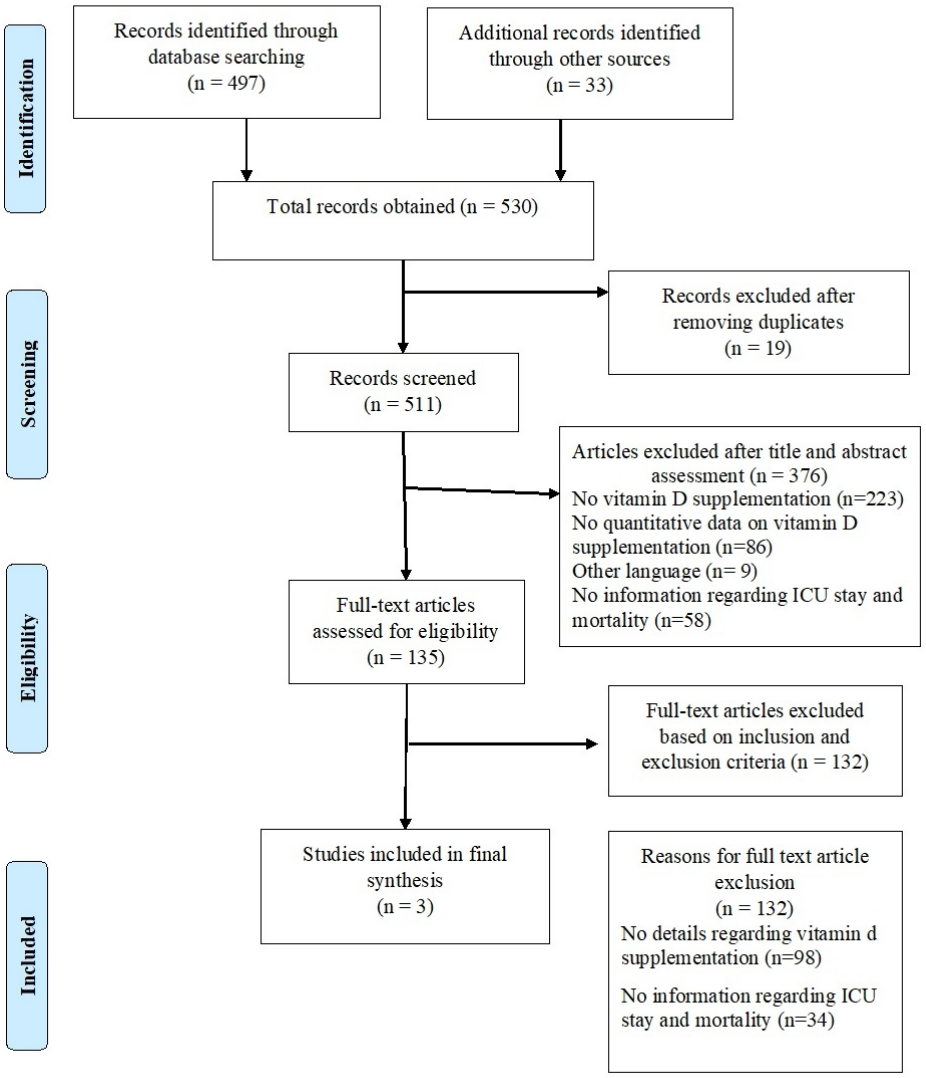

Figure 1: PRISMA

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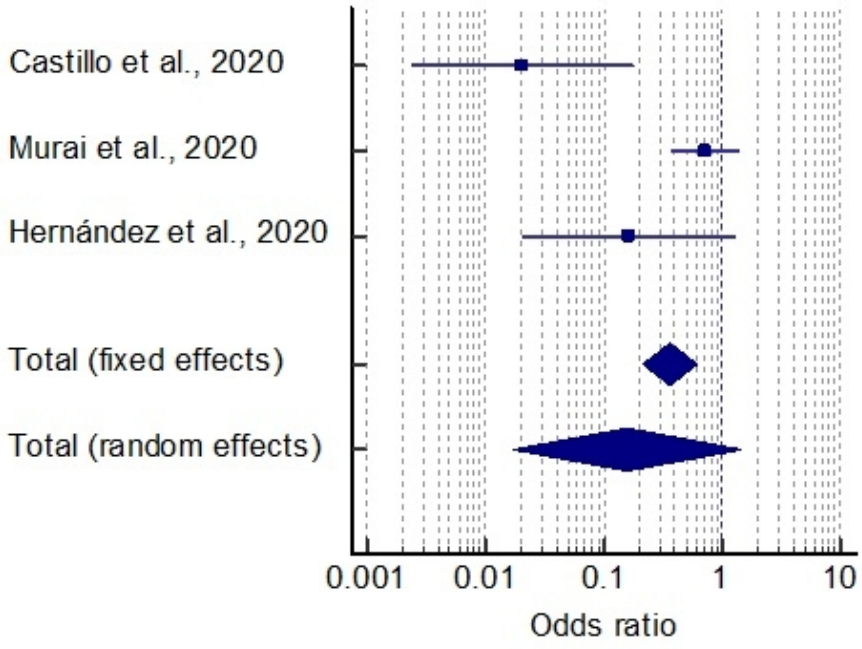

Figure 2: Forest plot for vitamin D supplementation on ICU stay

22x18mm (600 x 600 DPI)

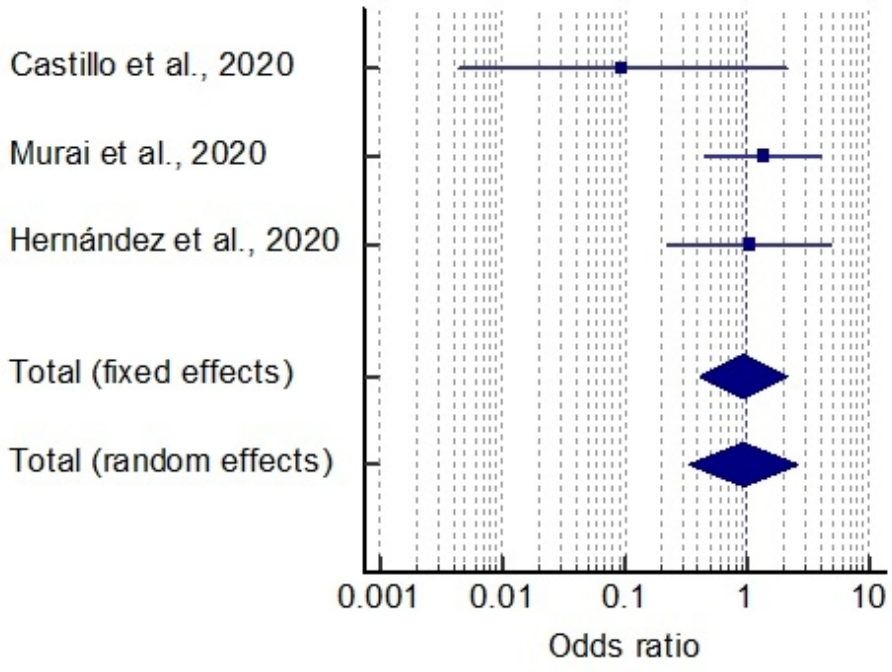

Figure 3: Forest plot for vitamin D supplementation on Mortality

21x15mm (600 x 600 DPI)

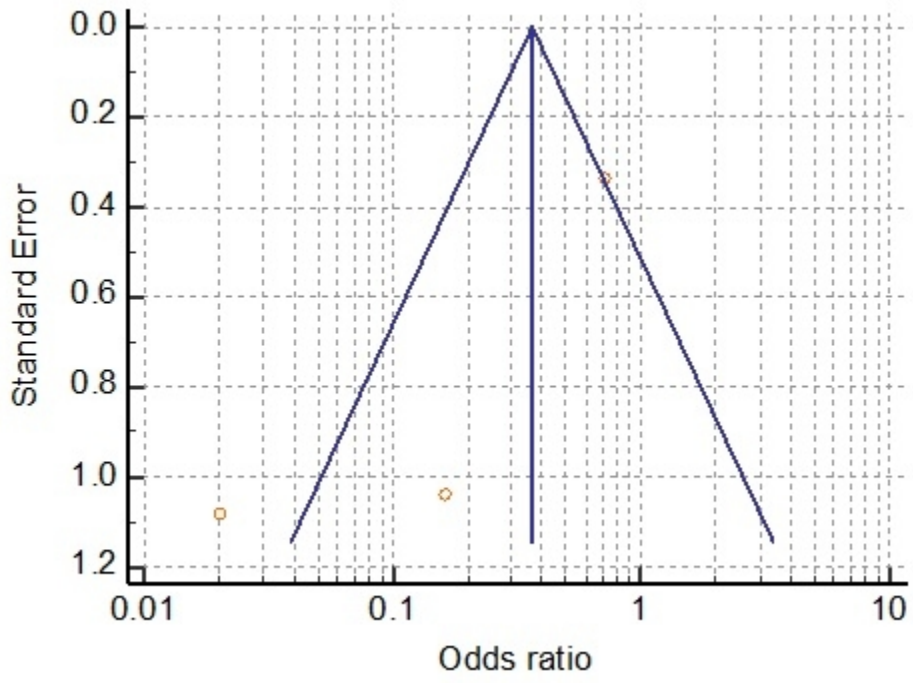

Figure 4: Funnel plot for vitamin D supplementation on ICU stay

20x14mm (600 x 600 DPI)

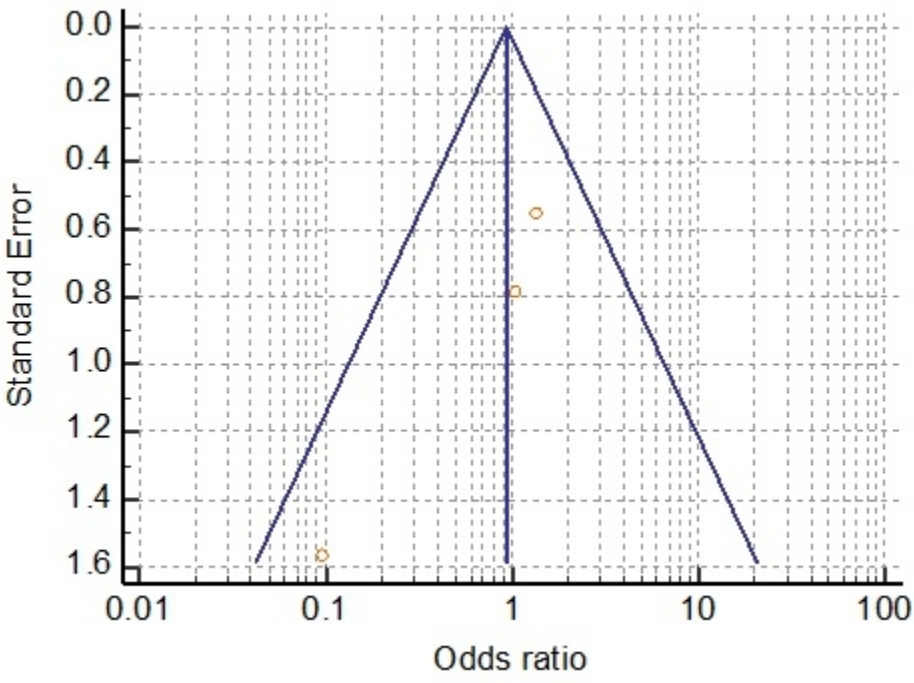

Figure 5: Funnel plot for vitamin D supplementation on mortality

20x15mm (600 x 600 DPI)