Peer Review File

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Review comments

Reviewer A

The title itself is misleading:
"Vitamin D Levels Or Supplementation Play A Role In COVID-19 Outcomes?"
Why is such a negative connotation when more than 95% of the published clinical articles and RCTs (now totaling over 170) using vitamin D-SRAS-CoV-2 is POSITIVE??
This illustrates the inherent bias or lack of awareness of the biology of vitamin D and viruses.

Reply: We thank the reviewer for putting forward this clarification. We would like to clarify at the very first instance that the above manuscript was an invited narrative review with the **title already being pre-selected and pre-specified from the journal's and editors' end**. The authors did not have any role in choosing the title. Hence, if the journal and editors permit, the title may be changed as desired by the reviewer.

Abstract - 2nd paragraph, What is inception mean?

Reply: Thank you for putting forward the clarification. Inception in this regard means the date when PubMed came into existence. Usually, in majority of narrative reviews, systematic reviews and meta-analyses, the literature search begins from the inception of the database being searched so as not to miss out on any relevant literature.

When authors stated that " hypovitaminosis D
42 might be associated with a higher risk of acquiring COVID-19. Similarly, the
43 majority of the literature supports a negative association between 25-hydroxyvitamin
44 D levels and COVID-19 severity", then why do they need more RCTs? It makes no sense and has contradictory statements.
However, "evidence is plagued by a need for sufficient randomized controlled trials" makes no sense and is a misleading conclusion.
There are more than 150 vitamin D/SARS-CoV-2 clinical studies published, including 31 RCTs.
Correctly, the authors stated in the last paragraph of the abstract "that vitamin D supplementation improves clinical outcomes in COVID-19."

Reply: We thank the authors for the insightful comments. We would like to clarify the queries put forward by the reviewer in detail. We would like to explain the queries under 3 broad headings:

Association between vitamin D levels and risk of SARS-CoV-2 infection

The data regarding the association between hypovitaminosis D and increased risk of acquiring COVID-19 is consistent across the existing literature. We have clearly mentioned the same in the manuscript (**page 7, line 165-166**).

Association between vitamin D levels and COVID-19-related health outcomes

In this regard, the literature is inconsistent. We agree that majority of the evidence suggest that hypovitaminosis D is associated with poor COVID-19 outcomes. However, a meta-analysis had included 31 peer-reviewed observational studies failed to show any statistically significant difference between serum 25(OH)D level < 20 ng/ml and COVID-19-related health outcomes, notably, mortality, ICU admission, invasive mechanical or non-invasive ventilation requirement or SARS-CoV-2 positivity status (reference 59) (**page 9, line 209-214**). Such inconsistencies have been attributed to confounding factors that have not been well accounted in majority of the studies. This fact is exemplified in a recently published meta-analysis by Bignardi et al. (reference 61). The authors included 21 observational studies and observed that the overall mortality was significantly higher in patients who were vitamin D deficient as compared to those who were vitamin D sufficient (RR 1.49, 95% CI: 1.15, 1.83, p = 0.000). Subsequently, they performed a subgroup analysis including only studies adjusted for age and at least one more confounding factor (obesity, hypertension, diabetes, chronic kidney disease, or cardiovascular disease) that revealed no association between low vitamin D levels and death (RR 1.82, 95% CI: 0.61, 3.04 for cutoff levels of < 10 or < 12 ng/ml and RR 1.56, 95% CI: 0.80, 2.31 for cutoff levels of < 20 or < 25 ng/ml). In contrast, the analysis including studies not mentioning adjustment for confounders, showed an increased risk of death for low vitamin D levels (RR 1.72, 95% CI: 1.09, 2.36 for cutoff levels of < 10 or < 12 ng/ml and RR 1.48, 95% CI: 1.23, 1.72 for cutoff levels of < 20 or < 25 ng/ml) (**page 9-10, line 222-237**).

Association between vitamin D supplementation and COVID-19 outcomes

Here, the data is extremely dichotomous. Most of the observational studies and non-randomized intervention studies and meta-analysis based on these studies have shown that vitamin D supplementation improves COVID-19 outcomes. However, it would be extremely unwise to arrive at a conclusion based only on observational studies. Hence, in this regard, data from randomized controlled clinical trials (RCTs) are of most importance. We beg to differ from the reviewer in the fact that only 20

RCTs have been conducted looking into the effects of vitamin D supplementation on hardcore clinical outcomes like disease severity, the requirement of hospital/ICU admission, oxygen requirement, need for non-invasive/invasive ventilation, and/or mortality. In addition, there are only 2 additional RCTs have looked into the effect of vitamin D supplementation solely on the rate of SARS-CoV-2 RNA negativity (**clearly mentioned in the manuscript, page 10, line 245-251**). We would like to clarify that 13 out of 20 RCTs *does not show any significant difference in clinical outcomes* between the intervention (vitamin D supplementation) and control/placebo group (**page 11, line 253-255**). Only 3 RCTs showed improvement in clincial outcomes in the intervention arm as compared to the control/placebo group (**page 11, line 255-257**). On the other hand, 4 clinical trials comparing high-dose vs. low-dose vitamin D supplementation have shown improved outcomes in the high-dose group; nevertheless, all these 4 studies did not have a third placebo arm for comparison (**page 11, line 261-264**).

Data from meta-analysis in this regard is also inconsistent. However, most of the recently conducted meta-analyses including only RCTs have shown that vitamin D supplementation may reduce the need for ICU admission but **has no effect on mortality (page 13, line 308-312, line 316-319)**.

What else one needs to know: even with additional 50 RCTs, these authors will come up with the same conclusions - this is not how clinical studies are evaluated, especially with threshold nutrients like Vit D. Available data unequivocally satisfy Hill's criteria of Causality; there is no point conducting more RCTs for this particular purpose.

Reply: We beg to differ in this regard. Firstly, Hill's criteria of causality are mostly applicable for epidemiological studies. Second, as is evident, the standards of epidemiologic evidence offered by Hill are saddled with reservations and exceptions. Hill himself was ambivalent about the utility of these "standards" (he did not use the word *criteria* in the original paper described by him). According to him, "None of my nine viewpoints [criteria] can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as a *sine qua non*." (*DOI: 10.1002/0470011815.b2a03072*). Third, amongst the nine criteria proposed by Hill is 'consistency'. As already narrated, there is marked lack of consistency in the association between vitamin D levels and COVID-19 outcomes. Besides, one more criterion is 'strength' of association. As previously mentioned, the presence of confounding factors may affect the strength of the association between vitamin D levels and COVID-19 outcomes (Bignardi et al., 10.20945/2359-3997000000588). Thus, with due respect, we do not believe that Hill's criteria of causality is enough to define causality between vitamin D levels and COVID-19 outcomes.

With regard to RCTs, the only way to best describe the effects of vitamin D supplementation on COVID-19 outcomes is by RCTs only. Observational studies cannot be used as a yardstick to describe the effects of vitamin D supplementation on COVID-19 outcomes. As we have already mentioned, the data available from majority of the RCTs point against any beneficial effects of vitamin D supplementation on COVID-19, especially with regard to mortality. However, most of the RCTs have limited number of patients, with only one study having more than 500 subjects; the results of the study showing no beneficial effects of bolus cholecalciferol supplementation as compared to placebo (Cannata-Andía et al., 10.1186/s12916-022-02290-8). Thus, indeed, with due respect, more RCTs with large number of subjects are required to elucidate the effects of vitamin D supplementation on COVID-19 outcomes.

Similar repetitions of errors are present within the text.
Section 1.2: Hypovitaminosis D has been proposed .... Not really: The beneficial effects of Vit D have been repeatedly shown by more than 100 clinical studies.
The only FOUR negative RCTs were designed poorly and intended to fail (e.g., Murai et al.).

Reply: We beg to differ from the authors. The reviewer is misled by the fact that there are 100 clinical studies. We have scrutinized the all the hitherto available RCTs (summarized in Table 1) and have clearly shown that out of only 20 available RCTs that have looked into the effects of vitamin D supplementation on hardcore COVID-19 clinical outcomes. Out of 20 RCTs, 13 did not show any beneficial effects of vitamin D (AND NOT JUST FOUR). Only 4 RCTs clinical trials comparing vitamin D vs. placebo showed beneficial effects, however these studies had recruited limited number of participants, ranging from only 42 to 116 patients.

In most sections, authors flip-flopped from benefit to no benefits, and much confused to read.

Reply: As mentioned in the previous sections, the available literature is dubious and accordingly we have mentioned the same in our narrative. We believe that turning a blind eye and highlighting on the positive aspects of vitamin D would be biasness. In this regard, we humbly request the review to go through this recent mini-review by Prof. JP Bilezikian (stalwart in the field of bone and mineral metabolism) published in The Journal of Clinical Endocrinology & Metabolism (https://doi.org/10.1210/clinem/dgac719[SEP]) that exactly echos the views narrated by us in this review. Bilezikian et al. have concluded that "The evidence supporting a beneficial effect of vitamin D treatment in decreasing the risk of COVID-19

complications is conflicting. Conclusive statements regarding the beneficial effect of vitamin D in this context await high-quality, randomized controlled trials."

Authors used Ref #19 to state that vitamin D increases mortality; It was a worthless study that should not have been published. Supplement disease like 800 IU/day is no better than a placebo, and both supplement and un-supplement groups were vitamin D deficient. So, one cannot conclude from such a poorly designed study. Seem used this study to prove the bias of the authors.

Reply: We thank the reviewer for the comment. We agree that reference 19 was a poor quality observational study and hence we have refrained from referring to this study in the revised manuscript.

Similarly, the data/conclusions were misinterpreted from references 33 and 34. There are 28 positive SR/Meta-analyses with strongly positive relationships between vitamin D status and clinical outcomes of COVID-19. These have been conveniently ignored in this manuscript, highlighting the bise of the authors.

Reply: In this regard we can only partly agree with the reviewer. We have already explained about the data available from SRMAs (systematic reviews and meta-analyses) in the previous sections. Few recently published meta-analyses have reported a significant association between low serum 25(OH)D level and an increased risk of mortality, ICU admission, and need for invasive or non-invasive ventilation (references 26,36,58). However, a systematic review and meta-analysis that had included 31 peer-reviewed observational studies failed to show any statistically significant difference between serum 25(OH)D level < 20 ng/ml and COVID-19-related health outcomes, notably, mortality, ICU admission, invasive mechanical or non-invasive ventilation requirement or SARS-CoV-2 positivity status (reference 59). The role of confounding factors in modifying the association between vitamin D status and COVID-19-related health outcomes has been eloquently depicted in the recently published systematic review and meta-analysis by Bignardi *et al*. The authors included 21 observational studies and observed that the overall mortality was significantly higher in patients who were vitamin D deficient as compared to those who were vitamin D sufficient (RR 1.49, 95% CI: 1.15, 1.83, p = 0.000). Subsequently, they performed a subgroup analysis including only studies adjusted for age and at least one more confounding factor (obesity, hypertension, diabetes, chronic kidney disease, or cardiovascular disease) that revealed no association between low vitamin D levels and death (RR 1.82, 95% CI: 0.61, 3.04 for cutoff levels of < 10 or < 12 ng/ml and RR 1.56, 95% CI: 0.80, 2.31 for cutoff levels of < 20 or < 25 ng/ml). In contrast, the analysis including studies not mentioning adjustment for confounders, showed an increased risk of death for low vitamin D

levels (RR 1.72, 95% CI: 1.09, 2.36 for cutoff levels of < 10 or < 12 ng/ml and RR 1.48, 95% CI: 1.23, 1.72 for cutoff levels of < 20 or < 25 ng/ml) (reference 61) (**pages 9-10, line 206-214 and line 222-236**).

It is a poor scientific practice to cherry-pick to support authors' bias, as done in this manuscript. It is hardly possible to make constructive criticisms.

Reply: We do not agree with the reviewer in this regard. We have presented the literature, as it is available without any biasness or cherry picking. The existing data is conflicting and being a narrative review, we have just summarized the available data as it is. Having looked only into the brighter aspects of vitamin D would have been cherry picking instead. We would like reviewer to unbiasedly introspect into the fact that had vitamin D been so effective in the management of COVID-19 and with the widespread availability of vitamin D, the adverse outcomes of COVID-19 would have been markedly curtailed with the supplementation of vitamin D (which we are sure most COVID-19 patients must have received during the course of their disease) and the world would not have encountered 6.9 million COVID-19-related deaths.

Summary:

Although a checklist table is included, there is no indication that the authors have followed or fulfilled the narrative review guideline.

Reply: We thank the reviewer for the suggestion. We have followed the narrative review guidelines as mentioned in the index journal website and have provided the checklist table as suggested by the journal. We have followed the template available on the journal website.

Conclusions are highly biased and do not support the published data.

Reply: We thank the reviewer for the comment. We have concluded our manuscript as per the available evidence, which is conflicting. We conclude by saying, "Available data, primarily based on observational and retrospective studies, suggest an association between hypovitaminosis D and increased risk of acquiring SARS-CoV-2 infection and poor COVID-19 outcomes. Nevertheless, randomized controlled trials have failed to consistently demonstrate that vitamin D supplementation either reduces the incidence of COVID-19 or improves COVID-19-related clinical outcomes, even in subjects with severe vitamin D deficiency. Hence, based on the available evidence, routine vitamin D supplementation to prevent or treat COVID-19 should not be encouraged. Vitamin D supplementation should be continued as per the standard Institute of Medicine (IOM) guidelines." (**page 21, line 502-510**)

Reviewer B

This is one of many papers on the correlation between vitamin D and COVID-19, which does not advance our knowledge as it is presented.
There is a limited number of references and this number should be at least duplicated for proper review. Many important studies were not included, and inconclusive statements decrease an enthusiasm for this presentation. In particular that there are many scientifically sound and better presented papers in the literature on this topic.
To be considered the paper has to present a convincing and good analysis based on many epidemiological studies and supported by an in depth knowledge of vitamin D biology.
Reply: We thank the reviewer for the constructive comments. We have provided a more detailed and in-depth review of the available evidence and have increased the number of references to 124 in the revised manuscript.

Key general points are.
Vast majority of investigators agree that vitamin D deficiency correlates with higher probability of getting COVISD-19, severity of the disease and its poor outcome.
The differences of opinion are to which degree vitamin D supplementation can affect the COVID-19, e.g., at which doses, routes of delivery, and to which patients, vitamin D deficient or vitamin D sufficient. Thia is not properly discussed.
Also I am not sure to which degree the authors understand vitamin D biology.
Specifically the authors in the introduction should mention classical pathway of vitamin D activation through hydroxylation at C25 to produce 25(OH)D3 and further activation by C1 hydroxylation to produce biologically active 1,25(OH)D3 with proper citation. Usually, the vitamin D load is measured by 25(OH)D3, an intermediate of this pathway.
The authors failed or are not aware of newly discovered alternative pathways of vitamin D3 activation by CYP11A1 leading to production of alternative hydroxyderivatives (FASEB J 26, 3901–3915, 2012; J Steroid Biochem Mol Biol 151,25-37, 2015; Sci. Rep. 5, 14875; doi: 10.1038/srep14875 (2015); FASEB J 2022;36(8):e22451. doi: 10.1096/fj.202200578R), which are biologically active (Mol Cell Endocrinol. 2021 Jun 15;530:111238. doi: 10.1016/j.mce.2021.111238; Cell Biochem Biophys 78(2):165-180, 2020).
As relates to COVID-19, vitamin D and lumisterol novel metabolites can inhibit SARS-CoV-2 replication machinery enzymes ((https://doi.org/10.1152/ajpendo.00174.2021); (Nutrients 2022, 14, 4779. https://doi.org/10.3390/nu14224779).
Also, there are different mechanisms of action and routes of delivery as discussed in (Exp Derm 29(9):885-890;. doi: 10.1111/exd.14170), which can explain inconsistencies in the literature.
The above considerations are necessary for a good review.

Reply: We again thank the reviewer for the insightful comments. We have incorporated the aforementioned data with regard to vitamin D metabolism by CYP11A1 and its possible role in COVID-19 and the possibility of beneficial effects of systemic over enteral vitamin D (**page 16-18, line 393-432**). We have also incorporated most of the aforementioned relevant references by Slominski and colleagues (**references 110-115**).