Reviewer's report

Title: Vitamin D3 and Gargling for the Prevention of Upper Respiratory Tract Infections: A Randomized Controlled Trial

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Reviewer: Peter Bergman

Reviewer's report:

The study by Goodall et al describes a randomised controlled trial on vitamin D (vitD) and gargling (G) as preventive measures against respiratory tract infections (RTIs). The study design was a 2x2 factorial trial with 4 treatment arms: vitD+G, placebo+G, vitD+ no G, Placebo + no G. Six hundred University students were recruited and given 10,000 IU vitamin D or placebo per week for 8 weeks. The primary endpoint was the incidence of clinical upper respiratory tract infection (URTI), as measured by an electronic survey. One of the secondary endpoints was laboratory confirmed viral infection as measured by self-collected nasal swabs and subsequent PCR-analyses for respiratory viruses. There was no significant effect on the primary endpoint, although the point estimate was a risk ratio of 0.79 (CI 0.61-1.03, p=0.09). Notably, allocation to vitamin D treatment was associated with a significantly lower risk of laboratory confirmed URTI (RR 0.54, CI 0.34-0.84, p=0.007). Gargling did not appear to have any effect on the primary or secondary endpoints.

The manuscript is well written and largely follows the CONSORT guidelines for reporting of randomized and placebo-controlled interventional trials. However, I have a number of concerns that should be explained and clarified.

MAJOR COMMENTS

1. Background. Very short background, should be expanded and also included some of the more recent RCTs on vitamin D and RTI. In particular, the studies by Bergman et al (BMJ Open, 2012) and by Rees et al (CID, 2013) should also be discussed here. In fact, REFs 11-18 are not correctly commented upon. The sentence “many of these trials were post-hoc analyses // other were limited by small sample size // and relatively low dose” it not correct. The studies referred to are very heterogeneous also with regard to study population, length of the study and dosing schedule. This could be clarified better. Moreover, it is not clear to me what the authors actually mean when they state that “more rigorously designed trials” are needed? Please, explain what kind of studies that are warranted and expand this para.

2. Methods/Study design: How did the study team deal with consent from legal guardians from participants below the legal age in Ontario? (18 or 21 for clinical study consent in Ontario?).

3. Methods/Study design: were only those on vitamin D above 1000 IU/day excluded? In this case, how many were taking vitamin D and which doses were
used? This could be of great importance for the interpretation of the study. Rees et al (CID, 2013) had the same acceptance for vitamin D supplements and encountered some problems (also null effect). Please, refer to Rees et al and expand this very important point in the discussion. In fact, if the placebo-group were diluted with vitamin D takers, it could very well explain the null effect of the current study.

4. Discussion, page 11: “further meta-analysis // should be conducted”. Please, explain how additional meta-analyses could move this field forward. In fact, there is a strong need for additional interventional studies, just like the current study by Goodall et al.

5. Discussion. It is somewhat odd to draw conclusions and to reason along lines based on non-significant results, such as “the vitamin D group appeared to experience more severe symptoms”. I would not focus on this non-significant finding although it is an interesting topic if it could be shown to hold true (remove?).

6. The study does not evaluate the vitamin D levels, not at baseline, not during the study or not at the end of the study. This is a major flaw of the study, since it is becoming increasingly clear that healthy individuals with a sufficient vitamin D status does not benefit from extra vitamin D. This is particularly well described in the study by Murdoch et al. In contrast, the studies by Camargo et al and Bergman et al, where there is vitamin D deficiency and a non-healthy population, show small (23-50%) but statistically significant effects. It is quite possible that vitamin D-supplementation could be even more beneficial in a VitD-deficient subgroup in the current study. However, since no such information is available we cannot know. Please, comment this further in the discussion section.

7. Discussion. Page 12. The discussion on dosing and that “larger and less frequent doses may be an effective alternative” is not fully correct and updated with regard to RTIs. It could well be true for other indications apart from infections, such as bone health. However, Bergman et al discuss this point in detail (Bergman et al, PLoS One, 2013) and argues along the opposite line, i.e. that a daily dosing schedule is preferable to a bolus schedule. Please comment.

8. Conclusion. I do not agree on the final sentence in this manuscript. In fact, additional meta-analyses would not be informative. The field is in great need of larger interventional studies in different study populations.

9. Table 1: Between 14-26.7% was taking vitamin D according to this table. What doses? How did this affect the study outcome and why was this allowed in the study?

MINOR COMMENTS

1. Abstract. The intervention should be mentioned here, ie the dose and time of vitamin D.


3. Abstract. It should be defined that URTI only includes viral infections and that bacterial infections is not included in the definition.
4. Please explain the rationale for performing a study where gargling and vitamin D is combined.

5. Methods/Study design: Did the participants ever pass by a hospital outpatient clinic? Were there any contacts with doctors involved prior to inclusion? Please specify.

6. Method/intervention: please describe where the vitamin D and placebo came from, which company, country? Were there any financial connections between the study team and the company? Please specify and explain.

7. Method/assessment: please describe the survey, what questions were asked?

8. Since seven consecutive swabs were collected, the results from this interesting material would be relevant to disclose. How was the kinetics here? Which criteria were used to define a positive swab, one positive day / 7 or 7/7. Please expand.

9. Method/assessment: it would be extremely interesting to report also the viral findings from those not having symptoms. It is well known that the presence of a virus does not always connect to clinical symptoms. A ‘Devil’s Advocate’ could argue that vitamin D affects the general well-being in yet undescribed ways and that symptoms and the presence of viruses are not connected. The design of 7 consecutive samples from symptomatic individuals could thus inflate vitamin D-mediated effects on the general health. A thorough description of viral findings also from asymptomatic persons could help to clarify these matters. Likewise, it is interesting to note that effect on symptoms (1’ endpoint) was not significant but viral findings (2’ endpoint) and viral load (which are based on symptoms, a priori) were highly significant. How do the authors explain this? This could be discussed more in detail.

10. Methods/statistics: please describe which statistical analyses were predefined and those that were chosen after the study was completed.

11. Methods/statistics: How was the result from the ‘per-protocol’ analysis?

12. Discussion: page 10. The sentence “previous trials in adult populations have not yielded statistically significant results” is not correct, since the study by Bergman et al provided data on modest, but statistically significant effects. If the sentence above include the word “healthy” it is correct, since the study by Bergman et al included patients with primary immune deficiency and frequent RTIs. Please comment and correct.

13. Likewise, the sentence “consistent with previous studies in adults…” is erroneous in line with the statement above.

14. Discussion/Result: it would be informative to have information on other viruses, apart from rhino/entero-viruses.

15. Again, I miss information on the viral findings.

To conclude, I congratulate the authors to a very interesting study, which definitely moves the field forward. In fact, it clearly points to a role for vitamin D as a preventive measure against respiratory viruses, which could be relevant for...
specific populations. The study follows CONSORT guidelines, is fairly large, well conducted and properly reported. One main problem is the lack of vitamin D-levels of study participants and also the acceptance of vitamin D doses up to 1000 IU/day in both placebo- and vitamin D groups. In fact, 14-26.7% were taking vitamin D across groups (table 1), a fact that could have a major impact of the study outcome and also for interpretation of the results.

I also find it odd that the authors, having performed a first-class and high-quality interventional trial, conclude that the field needs “more systematic reviews and meta-analyses”. In fact, more original data, like the results provided here, will be much more instrumental and important for the field than additional reviews and meta-data.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests'