**Reviewer's report**

**Title:** Vitamin D status in early childhood is not associated with cognitive development and linear growth at 6-9 years of age in North Indian Children: a cohort study

**Version:** 0  **Date:** 12 Dec 2019

**Reviewer:** Sebastiano Lava

**Reviewer's report:**

Chowdhury and colleagues performed a follow-up study of children, who participated in a trial investigating the role of Vitamin B12 and Folate supplementation among children 6-30 months of age. Since at baseline also Vitamin D levels were measured, they were able to contact >700 children, at the age of 6-9 years, and to assess their neurodevelopment with several, validated assessment tools and to analyze its possible association with the baseline vitamin D level. The Authors did not detect any significant association between vitamin D status (at baseline) and neurodevelopment (at follow-up), nor between vitamin D status (at baseline) and growth (at follow-up).

The study theme is interesting and relevant, both from a clinical and a public health perspective. Furthermore, focusing on Indian children, the important, global issue of children health in low-income countries is also partially addressed.

The study design is adequate and the study appears to have been well performed. The study has some relevant strengths (big sample size, relatively long follow-up period). The main limitation of this study, however, relates to the choice of the analysis strategy. In fact, looking at the distribution of the vitamin D levels in the included population, most children (>78%) presented vitamin D levels <20ng/mL, i.e. hypovitaminosis D. This implies that, also in the control group, several children had sub-normal vitamin D levels (i.e. <20ng/mL or <50nmol/L). Exactely this point may explain why the authors failed to detect any significant difference, while comparing <10ng/mL vs >10ng/mL. Because of this analysis choice, it is not possible to conclude whether your study indicates that vitamin D has no effect on neurodevelopment at school age among Indian children or whether your analysis lacked a "powerful" comparator group (small effect size). In my opinion, you might find a solution of this problem by comparing "deficient" vs "insufficient" vs "normal" vitamin D children? Or, alternatively, by comparing hypovitaminosis D (<20ng/mL) vs sufficient vitamin D levels (>20ng/mL). (I acknowledge that, according to the Vitamin D distribution, you have few children with normal vitamin D levels, but you still have >20% with vitamin D >20ng/mL, which should allow you to perform some relevant analysis.).
A further limitation relates to the laboratory technique used to measure vitamin D levels, as correctly acknowledged by the Authors in their Discussion. Although vitamin D should nowadays ideally be assessed by tandem mass spectrometry, several studies on vitamin D used electro-chemiluminescence binding assays. And our current knowledge on vitamin D (and its possible implications with various biological outcomes, including neurodevelopment) mainly bases on these studies. Therefore, this does not represent, in my eyes, a major limitation.

The manuscript is too long and should be reduced by at least 15% of its current length (particularly the Introduction and the Discussion). The English quality is insufficient and should be improved.

Some specific comments are provided below.

Page 3: "placebo-controlled trial where in 1000 North Indian children 6-30 months of age were supplemented with B-vitamins or a placebo daily for 6 months" --&gt; "placebo-controlled trial among in 1000 North Indian children 6-30 months of age"

Page 4: do not use abbreviations in the Key words list.

Page 5, line 4: internationally accepted reference values are available. Please refer to them for reporting the prevalence of Vitamin D deficiency in India (if different publications use different cutoffs, please recalculate the prevalences according to the internationally accepted values, e.g.: Braegger C et al. J Pediatr Gastroenterol Nutr. 2013 Jun;56(6):692-701. ; or Grossman Z et al. Eur J Pediatr. 2017 Jun;176(6):829-831. ; or your current reference #32).

The Introduction is fine, but too long. It should be shortened by about 50% (max. 1 page, ideally less).
Page 8, line 90: this is, in fact, a limitation of your study, as you correctly state in your Discussion. Vitamin D should ideally be assessed by tandem mass spectrometry. However, several studies on Vitamin D used this technique, so that this is, in my eyes, a relative limitation.

Page 9, lines 104-106: Why categorized and not as a continuous variable (as it is)?

Pages 9-10, Lines 116-117 ("Children's height-for-age z-score (HAZ) at follow up was calculated based on WHO growth standards."): are the WHO growth charts representative of the Indian population?

Lones 127-134: improve the English of this lines.

Methods

I guess that, since you did not detect any significant difference in the univariate analysis, you did not perform the planned multivariate analysis. Please either state this explicitly in the Results, or delete the corresponding sentence in the Methods.

Page 12, Lines 163-165: did you perform the generalized additive models analysis, even if you did not find any significant difference in the first, univariate analysis? Why?

Page 12, Lines 177-179: please correct the grammar of this sentence.

Page 13, Lines 203-204: please delete "and that the public health relevance of vitamin D deficiency may be limited." (You did not investigate the public health relevance of vitamin D deficiency. Furthermore, vitamin D deficiency might well have other reasons for being of public health relevance, besides neurodevelopment.)
Your study focused on Vitamin D. Although many factors might influence neurodevelopment, your paper is not a systematic review on Neurodevelopment, but an original study focusing on the possible role of Vitamin D on Neurodevelopment.

Page 15, Lines 237-239 ("An 80% successful inclusion of children from the baseline trial after approximately 6 to 8 years also represents strength to the study."): please correct the grammar of this sentence.

Page 15, lines 242-243 ("Type II errors can be due to low sample size"): although in global terms you are right, I do not agree. I think that a pediatric study with >700 patients cannot be considered a "low sample size study". Indeed, the high number of included children is, in my opinion, the main strength of your study.

Page 15, lines 249-250: please rewrite this sentence in better English.

Discussion: please briefly address the current situation on Vitamin D intake among children in India. What about diet (what do Indian children normally eat? Globally and subgroups, for example according to social status and geographical area)? Do (some) Indian children receive a Vitamin D supplementation? If yes, which children, in which age period, which dose?

Table 1: Please also see global comment above. Does it make sense to divise deficient vs "non-deficient"? Should you not compare "deficient" vs "insufficient" vs "normal"? (I realize that, according to the Vitamin D distribution, you have few children with normal vitamin D levels, but exactly this point may explain that you failed to detect any significant difference: also your control group is mainly composed of "low-vitamin D status children", namely in the insufficient range).

Fig. 2: the quality of the reproduction is insufficient, I cannot evaluate neither the Figure nor its corresponding legend.
Level of interest
Please indicate how interesting you found the manuscript:

An article of importance in its field

Quality of written English
Please indicate the quality of language in the manuscript:

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