Author’s response to reviews

Title: Vitamin D in children with growth hormone deficiency due to pituitary stalk interruption syndrome

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Reviewer reports:

Luisa De Sanctis (Reviewer 1): Cécile Delecroix et colleagues report a retrospective single-center study aimed to assess the vitamin D status in 50 children with GH deficiency due to pituitary stalk interruption syndrome and the relationship between 25OH vitD and 1,25 (OH) 2D serum levels and patients' clinical features. They found a vitamin D status similar to that reported in national and European studies in healthy children and a positive significant correlations between the GH peak and the 1,25 (OH)2D concentration and with the 1,25 (OH)2D/25OHD ratio.

The manuscript add some new information to the specific population of GH deficient patients; however, it cannot be accepted in its present version and an extensive revision is recommended.

Major changes:

An extensive revision of the manuscript has been performed.

- In the Methods section, it is not so clear why the Authors have chosen to indicate that they have initially considered a larger case series of 86 patients, if they already known that in 50 patients only they had samples availability.
Moreover, I'm wondering if Fanconi and Blackfan-Diamond anemias were the only syndromes that have been excluded. In this case it would be better to indicate them with the "i.e." expression. In Figure 1, the title should define which kind of flow chart is depicted.

We would like to retain the comparison between the included patients and the excluded with unavailable samples to exclude bias. This is indicated in the short paragraph addressing the limitations/strengths of the study. We transferred Table 1 comparing the 2 groups from the Results to the Patients section. “Such as” has been changed to “i.e.”. The title of Figure 1 has been completed.

In the Discussion section the Authors could reduce the part of the comparison of vitamin D status in their GH deficient patients with healthy population. It is too long and probably it could be sufficient to report the comparison with one or two studies in healthy subjects (in particular, the discussion referred to the French study of 326 healthy subjects (ref.11) and the one related to ref.34 could be deleted since they don't add any specific information). Moreover, the Authors are aware that they lack information about vitamin prophylaxis in the considered populations.

As suggested by the reviewer, this part of the Discussion has been shortened, and reference 34 has been removed.

In the discussion they also report that in France vitamin D supplementation is systematically recommended for the first 5 years of life; it is thus surprising to find that the lowest vit D levels are in the group of younger GH deficient patients. The Authors could discuss this finding.

This point has been addressed in the discussion.

On the other hand, the discussion could be enlarged by the comparison with the previously similar reports on vit D status, its correlation with IGF-1 values and the correlation between GH peak and 1,25 (OH)2vitD levels in the GH deficient population.

We have enlarged the discussion on this point and have added a reference (ref 34 of the revised version).

Literature data indicate that the vitamin D status can be influenced by BMI: the Authors could comment their results also for this feature.

A sentence addressing this point has been added to the Discussion.
Another result that could be stressed and discussed is the higher vit 1,25(OH)2D found in multiple HP, by evaluating if the multiple endocrine disruption may play a role.

A sentence addressing this point has been added to the Discussion.

The conclusion is rather far from the discussion, since it considers several aspects, clinically important, not previously discussed.

The conclusion has been modified.

Overall, English style of the manuscript should be revised by a native English speaker.

The English language has been revised a second time; the manuscript has been revised by AJE.

Minor changes:

- Introduction, line 20: "Human can get vitamin D from exposure to sunlight": they Authors should precise "mainly from exposure.." This change was made.

- Discussion: The sentences "As indicated in the introduction section, it was demonstrated, both in experimental studies [36,37], ….and have a low 1,25 (OH) D serum concentration" can be shortened in a single sentence "as GH seems to increase the level of 1,25 (OH)2D, probably indirectly, through the effect of IGF1, it may be hypothesized… low 1,25 (OH) D serum concentration".

This sentence has been modified as suggested by the reviewer.

Gianpaolo De Filippo (Reviewer 2): The paper of Cécile Delecroix et al. deals with the possible relationship between vitamin D status and GH/IGF-1 axis. The first objective is to evaluate the vitamin D status (i.e. 25OHD and 1,25 (OH)2 D circulating levels) in a group of patients with GH deficiency (GHD) belonging to a pituitary stalk interruption syndrome. The secondary one is to investigate the relationship between Vitamin D status and patients characteristics, specially, GH peak after pharmacological stimulation test.
The idea is of interest and strength of the is study the studied population, with a homogeneous diagnosis (stalk interruption syndrome), permitting to avoid considerable selection biases affecting several studies on GH deficiency. However, some concerns need to be clarified.

1. Vitamin D status has been shown to be potentially able to increase IGF-1 circulating levels and a regulatory role of 1,25 (OH)D3 was shown in GH/IFG1 axis gene expression in human epiphyseal chondrocytes; at the state, no evidence of a feedback vitamin D/IGF1 or vitamin D/GH has been proven. In other words, if it is possible that low levels of vitamin D could affect GH secretion and/or IGF-1 levels, no clear evidence exists that in turn IGF-1 levels could affect vitamin D status. The cited studies of Wei and Saggese (references 19 an 20) show an influence of GH therapy (inhibition of 24 hydroxylase?) at pharmacological doses. Thus, the finding that these patients have normal vitamin D status is probably independent from their GHD. The present observation has the value to assess it, but this result should be more discussed and detailed.

The discussion has been modified according to the suggestions of the reviewer.

2. More interestingly, a relationship is observed between vitamin D status and GH peak. This result is on my opinion the real strength of the study, leading to several considerations for the evaluation of GH status in clinical practice, for example, to assess vitamin D status and if a deficiency exists, to correct it before testing.

This possibility (assessing Vit D status before testing GH) is addressed in the Discussion in the revised version (see the Discussion section around the (new) reference 34 of the current version).

To do that, more details should be done on GH stimulation tests. First, the relationship should be evaluated only on mUI/L values and not ng/mL: in effect, the standardization process varied on the time. The entire study group originating from a total of 86 patients has been formed over long time (1982-2016) and the GH standard was not the same. The recombinant DNA derived standard (IS 98/574) replaced the pituitary derived standard IS 80/505 in this period and the correspondence 20 U/l = 6.7 ng/mL reported in the text corresponds to the former. This item should be discussed in the methods section.
We now express the GH peak results in mU/L. This is described in the Methods section. Note that correlation between the GH peak and 1,25OH2D or 1,25/25OHD are still significant but slightly attenuated.

Second, the type of stimulation test should be specified, pointing out the differences in responses (i.e. simple versus coupled pharmacological test), if any.

That type of stimulation test is no longer available.