

December 16th, 2010

Dear UBCMJ editors and reviewers,

Thank you for your consideration of our manuscript, entitled “Assessment and management of anemia in a population of children living in the Indian Himalayas: A student-led initiative,” for publication.

We have revised our manuscript incorporating the reviewers’ suggestions and comments. The following are responses addressing each reviewer’s concerns and comments. The reviewer’s comments are in *Italics* and our responses are in Normal font.

Please feel free to contact me with any further suggestions or concerns.

Thank you for this opportunity.

Sincerely,

Diala El-Zammar
On behalf of the Global Health Initiative – Spiti Valley

**Reviewer #1**

*This was a great study that not only had scientific curiosity, but also it possessed a humanitarian core to genuinely improve the living conditions in Spiti Valley even involving the local administrations. This is indeed the goal of medicine. The study was of an impressive size and the logistics were indeed advanced. Overall, it was a very interesting paper.*

*I think the figures would benefit from more detailed legends. This is especially true for Figure 2 which is focused on age-based categories but does not mention it in the legend. Or for example in Table.1, the altitude used for adjustment, although I assume is between 3700-4500m, should be mentioned in the Table itself.*

* The figure captions and graph titles have been clarified to reflect the age-based categories in the figures and the altitude was specified.

*Another aspect is that according to Fig.1, the cohort size is increasing over the years and this might affect the study i.e. where did the new students come from? were all of them they treated? Is there a reason why the new students would not be excluded from the study to focus on the original cohort?*

* In the 1st paragraph of the Methods section, see lines 86 to 90.

*The hypothesis of study was clearly mentioned but the second component of it i.e.”though dietary folate and vitamin B12 deficiencies may contribute to the burden of illness” sounded additive. If this was part of the original hypothesis why weren’t vitamin B12 and folate supplementations also included in the study to see their effect in lowering anemia rates even further?*

* Explained in Methods section under the Treatment subheading lines 138-140. Greenhouses were built as a sustainable solution to nutritional deficiencies. The practicality of administering multiple supplements to children was limited, as a good combined supplement could not be found in India. The literature supports iron supplementation the most.

*Lastly, the difference in the outcomes between female and male subpopulations was a major finding in the results section. This was not discussed further in the discussion section. I think such finding warrants a bit more input and possibility for more rigorous interventions for females should be mentioned in the discussion.*

* Paragraph 5 was added in the Discussion to address this issue.

*The article was well organized and written. As mentioned, the legends could be more thorough. With regards to age categories on page 10, it was not very clear why the particular 3 year intervals were chosen. For example for the 7-10 years group, were these 7 years old in 2007 who were followed and were 10 years of age by the end of study or were these all individuals between the ages of 7-10 in each years?*

* Figure legends have been clarified.

*Also including values of RDW for table 3 could benefit analysis of results support the suggested possibilities. High RDW would be supportive of the hypothesis that both Iron deficiency and B12 deficiency were present.*

* See lines 117 to 119 in the Methods section.

*In table 2, including the frequency of anaemia in the diseased states i.e. helminth infected individuals would be informative as if all the helminth infected students were anaemic, the anti-helminth treatment may have had an additional role to the iron supplements in lowering the rate of anemia.*

* Table 2 was modified under the “disease present at screening” section, and only components relevant to anemia were included. The only finding relevant to anemia here was the students’ report of worms in their stools. “Worms” were also discussed in the Results section, in the last paragraph under ‘Health Screens’.

*Lastly, the first paragraph of results on page 8, is not completely clear. Why are the results for males analyzed in the 2007-2009 period while for the females year by year analyses are given. Including year by year p-values and results for the male population may make this paragraph more clear.*

* The male year-to-year p-values were added at the end of this paragraph. ***Suggestions for improvement***

*Even though not essential, it would be nice to mention why the Spiti valley population is unique. Was is chosen because as an example of a high-altitude region or would the high-altitude with the socioeconomic status and ethnic distribution of the population all be significant.*

* The Munsel-ling school’s local NGO contacted UBC Medicine requesting assistance with increasing healthcare awareness at the school. This was clarified in the 1st paragraph of the Methods section.

*On the second paragraph of page 3, it is mentioned “The most common cause of anemia in developing nations is iron deficiency. Other causes of anemia include: 1) other micronutrient deficiencies; 2) infections (malaria, hookworms, schistosomiasis); and 3) inherited conditions such as thalassemia”. I believe there are still several other possible causes of anaemia such as autoimmune conditions and genetic disorders (G6PD deficiency). It would be nice to include all causes or restructure this sentence to make it more correct.*

* We have included another category of causes as “other chronic diseases,” which would capture autoimmune disorders. “Inherited conditions” would encompass G6PD. We wanted to focus on the common causes in developing countries in children.

*On page 12, end of paragraph 1, why was there no treatment implemented in 2009 even though more than 40% are still anaemic, It would be nice to include a brief explanation/limitation.*

* A sentence has been added to explain the reason iron supplementation was not provided in 2009.

*If iron deficiency is a major contributor to the anaemia, why would the majority of cases be normochromic rather then hypochromic. Could this be discussed or addressed in discussion?*

* This question has been addressed in paragraph 2 of the Discussion. In summary, we believe the normocytic normochromic prevalence is mainly due to mixed nutritional deficiencies, with other possible causes being early iron-deficiency and genetic adaptation.

*Last but not least, the first sentence on page 15 mentions, “whereby RBCs become more efficient and thus, require less Hb to transport oxygen to body tissues” is a bit unclear. It would be great to mention what is exactly meant by this and physiological explanation of this theory would be much appreciated by the reader.*

* In the second paragraph of the Discussion, we have eliminated the sentence in question, and reworded the sentence. We have also included two recent papers that found two new gene variants in Tibetans in the Himalayas that may suggest a mechanism for this adaptation (Reference 12 and 13).

***Reviewer #2***

*Represents an interesting study on anemia in a population of children living in the Indian Himalayas. The authors identify and characterize the anemia and attempt to come up with a system to manage it. They identified that the problem may be more complex than simply providing supplements demonstrating an important interplay between medicine and social and economic status. The manuscript represents data collected from three years providing a more detailed description of the population than what was previously known.*

*Abstract: Overall good abstract but it would be useful to define what “Analysis of Hb levels demonstrated a negative skewed distribution” in the second line of the results section of the abstract means. For the conclusion in the abstract providing a sentence on what the findings are first before talking about their significance or reasons for the short comings of the study would have been useful.*

* The abstract Results and Conclusion sections have been modified to address these points.

*Introduction: 3rd line “…Hb concentrations below a recommended threshold value…” It would be helpful if the authors defined these since it will be critical later in the paper. At this point the authors could put in and reference table 1 as a possible solution.*

* Table 1 was added as a reference.

*The 2nd paragraph starting “Residents of the rurally situated Spiti Valley…” there are no references. If there are none then state that they are the author’s personal experience.*

* A reference for this information from “Himachal Tourism” has been referenced.

*Materials and Methods: As a general note there should be no elements of an introduction in the materials and methods section. 1st and 2nd paragraphs contains several sentences of introduction about the school that should be in the introduction to make it more comprehensive and give a better description of the community. This will also focus the materials and methods.*

* This has been adjusted as recommended by the reviewer.

*Under the blood smears section “A greater sample size for females was chosen as there is an established higher prevalence of anemia in females, especially in menstruating age” seems to be referring to a fact and needs a reference.*

* WHO reference has been added.

*The same is true for the nutritional assessment and treatment sections, the WHO guidelines should be referenced.*

* References have been provided.

*For the stats section, everything but the first two sentences should be put into the corresponding tables or figure legends since that is what they are describing.*

* Changes were made to the legends of table 1 and 2 to include the statistical details in the Statistical Analysis section that particularly pertained to these Tables.

*As a general rule of thumb there should not be tables or figures in the materials and methods section. Since there is actual data in the tables they belong in the results section.*

* Tables 1 and 2 were moved into the Results section.

*Results: A good results section has a brief rationale for why you are looking at these specific results in the first place. Most readers will not remember all of the details of the intro nor will they usually read the materials and methods section in detail to understand your train of thought. The results section would benefit from 1-2 sentences of intro/rationale. If the results section is going to be broken up into subheading (ie health screen, blood smears) then it would be useful to make those headings descriptive of what was done or found. An example: Pretreatment health screen identifies low Hb levels and high prevalence of anemia.*

* An introduction/rationale to the Results section was added, and defines our subheadings.

*Stats: In scientific literature you usually report P values as < or > confidence intervals to indicate significance. Example: Male levels of Hb were significantly lower than females (P<0.05). This makes it easier for the reader to understand when things are significant or not if the 95% confidence intervals are consistently used. These P values are also usually on the graphs or in the tables directly comparing the two points of interest and not in the text itself so that there is less interruptions.*

* We have listed the exact p-values and commented where statistically significance exists. We feel this is more informative than providing inequalities.

*In the paragraph about Figure 2 it is confusing where the exact numbers for anemia are coming from in the figures. I am assuming figure 3 but this could be made more clear. It would also help to clarify which year (2007-2009) this data is coming from and whether some of the data is pretreatment or posttreatment (ie include in text as well as figure legends).*

* The figure legends and titles have been adjusted to be more clear. Also, Table 1 was referenced for the percentages mentioned in the paragraph above Figure 2.

*Figure 1: Good. For all of the figure legends there needs to be more description including what the data is, the source of data, a bit of methods, and stats with P values.*

*Figure 2: If left as is, need to add A, B, C, etc to each individual graph so that they can be referenced accordingly in the text. Alternatively I would suggest that this figure is overly complicated and could be condensed into 3 bar graphs with both M and F on the same graph for each age group. Since the authors compare M and F at the same age groups the data should be on the same graph.*

*Figure 3: This graph is complicated with many lines and might benefit from being split up.*

* Figure legends have been revised to include the pertinent methods and results of each figure.
* We have explored different configuration of graphs for Figures 2 and 3, and we feel that our current configuration best captures the results of interest.

*Underneath Table 3 there are footnotes which need to go with the correct table (I think table 1).*

* The footnotes were relocated to Table 1.

*Discussion: Overall good, could be a bit more focused. The authors may want to look at one of the conclusions “Finally, the lack of improvement in post-treatment Hb concentrations supports the hypothesis of a genetic adaptation to chronic hypoxia in high-altitude inhabitants.” This doesn’t really go with the rest of the discussion in the previous paragraphs and is not completely substantiated.*

* See the 4th paragraph of the Discussion (Lines 385-400) for an expanded explanation. Two more recent references were added (References 12 and 13) to provide support by outlining a possible mechanism of the genetic adaptation. Also, references 29-35 are studies that have raised the possibility of this genetic adaptation in high-altitude inhabitants, and our results suggest that this may be a contributing factor to the documented anemia in the Spiti Valley.

*The limitations of the study could be put into the materials and methods and briefly integrated into the discussion so that the paper ends on a clean summary paragraph.*

* The study limitations were moved to the Materials and Methods section and a brief summary of the limitations was added prior to the summary paragraph in the Discussion.

*The authors could talk more about the overall decrease in anemia over the three year study period. While the pre and post-treatment outcome was not as desired the overall decrease may suggest that the GHI multifactorial approach is working. This could possibly be a good discussion point.*

* This topic was expanded further in the Discussion, lines 355-368 and 450-460.

***Reviewer 3***

*Abstract is very clear and concise. I would have liked a little more detail in recommendations for the future .*

* Further elaborated in the Conclusion section of the abstract on lines 45-46.

*Methodology is clear and limitations are stated because of isolation and logistic factors.*

*Please explain correction for high altitude.*

* In Table 1, a reference for WHO and the CDC was provided in the citation of the Table. An explanation briefly outlining the approach we followed for altitude-adjustment was added to the figure citation.

*I believe the multifactorial nature of the anaemia is a new finding.*

*Also please comment on whether the one anti-helminthic treatment would be adequate or whether there was still a possibility of recurring infestation.*

* This was addressed in the Discussion on lines 430-433 and 457-460.

*I think the final comments re multifactorial causes and possible future treatments needs more detail*

*Specifically have you formulated any plans for the ongoing treatment and investigation of the children?*

* Addressed in the final paragraph of our Discussion, lines 461-466.

***Academic Section Editor Comments***

*The study was ambitious in scope and succeeded in gathering extensive longitudinal data. Its purpose and design gives insight into the challenges faced in designing and implementing sustainable interventions abroad.*

*Please consider the following questions and concerns:*

1. *The manuscript lists 19 authors, which is unusual in an academic paper. Authorship credit should be based on: 1) substantial contribution to conception and design, or acquisition of data, or analysis and interpretation of data; and 2) drafting the article or revising it critically for important intellectual content. Please confirm whether each of the listed authors meets these criteria and change your authorship as necessary.*
* The authors’ list has been revised according to the extent of participation in writing the manuscript and field work in collecting and analyzing the data. There remains 15 authors who most actively contributed to this paper over the 3-year study. The list follows in the appropriate order. I was not able to make these changes to the online submission. Please advise us as to how we could do this if it must be modified by us.

El-Zammar D, Yan M, Huang C, Fang D, Petigara F, Bornn L, Ngai T, Brkanovic S, Alexander N, Khangura J, Lubin J, Hendry S, Wallis C, Ford J, Kapoor V

1. *A main finding is decreased prevalence of anemia from 2007-2009. Does this suggest that iron supplementation for only 3 months each summer is sufficient to reduce anemia over 3 years? Were teachers or families instructed to give iron throughout the year? What patient education on nutrition and iron deficiency was conducted, if any?*
* Addressed in 3rd paragraph of Discussion on lines 373-376.
1. *It’s interesting that you suggest that these children of Tibetan descent may naturally have lower Hb levels due to adaptation. If indeed the WHO guidelines don’t’ apply to these children, and they’re mostly asymptomatic, and O2 saturation is normal, is anemia still the biggest concern, or are other nutritional deficiencies more concerning? Perhaps a stronger conclusion on this question can be made.*
* Addressed in the Discussion on lines 422-433 and 450-460.
1. *Please clarify whether the same children where followed over three years*
* This concern was also raised by reviewer #1 and addressed in the 1st paragraph of the Methods section on lines 86-90.
1. *Please note whether there was any measurement of compliance to treatment, and if not, consider noting that in limitations*
* Addressed in the method section on lines 135-136.
1. *Please correct the sentence of your discussion that reads “...and hence, thalassemia cannot be ruled.”*
* The sentence was corrected to read “…and hence, thalassemia cannot be ruled out.”

***Concerns about References:***

*1) Reference 10 is the only citation used to justify effects of chronic high altitude on Hb and O2 delivery, but reference 10 seems to refer to acute high altitude experienced by travelers*

* We updated our literature search via Medline (OVID) on the effects of altitude on the physiology of oxygen transport and found two recent studies (References 12 and 13) published in the last few months that have identified two hypoxia-related genes that may contribute to the mechanism by which exposure to chronic altitude hypoxia may affect Hb and O2 delivery. See lines 71-73.

*2) In the Discussion, there is an error in reference number placement regarding ref 13 and 14.*

* The reference of the study in the urban slum of Pune was corrected.

*3) Reference 14 is used to justify why they didn’t see a post treatment decrease in anemia prevalence over 3 months. However, reference 14 was done in infants age 1-3, and the youngest in this study was 3. Do you feel these results are comparable?*

- References 14 and 21 were added to explain that anemia should have improved more than anticipated over the 8 weeks in older children. Also, references 19 and 22 were added to provide evidence that weekly or biweekly, rather than daily, iron administration may have better intestinal absorption with greater reduction in anemia prevalence.

*4) There is no reference for the nutritional deficiencies experienced by Spiti Valley children in the introduction.*

* WHO reference was added.

*5) Reference 20 – only one of 12 authors listed (as per Vancouver style, please list first six).*

* This is now Reference 28: The citation was corrected to include the first six authors.

*6) References 22 & 24 – These are Review articles, but are cited to support original research – please cite the original research reports.*

* Now References 30 and 32: There are many articles on adaptation to high-altitude. Instead of citing the many papers, these review article best summarize the evidence and it would be easy for readers to obtain the original article references from this review. We have also added references 12 and 13, which are more recent original research papers that demonstrate the genetic adaptation at high altitude.

*7) References 23 & 27 – Greater than six authors listed (as per Vancouver style, please list first six).*

* Now References 31 and 35: The citations were corrected to list the first six authors.