Author's response to reviews

Title: Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

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Version: 3 Date: 27 July 2009

Author's response to reviews: see over
27 July 2009

Dear Editor, 

Re: MS: 9896600232720416 - Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

Many thanks for your support with the above manuscript. We found the referees’ comments encouraging and helpful. In the revised version of our manuscript together with our point-by-point replies, you will see that we have made changes to reflect these comments. Should you feel there are other changes needed – please do not hesitate to inform me.

We look forward to an early response.

Yours sincerely,

Hong Yan on behalf of authors

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Response to Reviewer 1

Reviewer's report

Title: Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

Version: 2 Date: 27 May 2009

Reviewer: Gunnar Tschudi Bondevik

Reviewer's report:

My original criticism and additional comments have been answered in the second revision of the manuscript.

A few minor comments:

1. The authors should use the same reference to the altitude correction methods, either "Dirren et al. and Dallman et al. methods" or "Dirren's and Dallman's method". In the current version of the manuscript, the authors use both.

Response: Thanks for the suggestion. We have now used the consistent reference in the revised manuscript.

2. Line 53: Omit "urgently"

Response: Omitted as suggested.

3. Line 91: Add "inhabitants" after million, and replace "was" with "were"

Response: Added as suggested. L104

4. Figure 1 indicates that pregnant women with gestational age 44 weeks are included in the study. Is this due to an approximate last menstrual period?

Response: No. In Figure 1, the x-axis actually means the age group, i.e., 0-7, 8-15, 16-23, 24-31, 32-39, and 40+. We have redrawn this figure.

5. Line 169: Omit "around" and "old"

Response: Omitted as suggested.

Response: Thanks for this point. We’ve rephrased it as: “This hospital-based study conducted in Lhasa showed low Hb concentration among pregnant women.” L238

7. Line 289: Omit "urgent"

Response: Omitted as suggested.

8. Lines 200 & 202: replace "per" with "pre"-adjusted

Response: Thanks for point out. We have revised accordingly. L222

Level of interest: An article whose findings are important to those with closely related research interests

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
Response to Reviewer 2

Title: Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

Version: 2 Date: 2 July 2009

Reviewer: Alistair W Stewart

Reviewer's report:

I have no serious concerns about this manuscript.

Minor Essential Revisions

On line 144 the units for altitude must be described.

Response: Thanks for the suggestion. We have revised as suggested. L160, L162

On line 200 it is inappropriate to say ‘… all appeared to be shifted left …’ as the formulae all require the values to shift left with increasing altitude.

Response: We have changed the text “The three adjusted distribution curves all appeared to be shifted to the left of the pre-adjusted curve (Figure 2)” to ,”The three adjusted distribution curves are plotted in Figure 2” L220

About line 205 there is a sentence using kappa. This is an inappropriate measure in this circumstance. It measures association having adjusted for chance where there is no concept of chance here as there are formulae which deterministically give the adjusted values. The proportion classified as anemic after adjustment gives all the information the reader needs.

Response: Thanks for the comment. We have adopted your suggestion and only reported the proportion classified as anemia after adjustment to assess the consistency among different methods. The proportion is 70.0%, 77.9% and 41.3% for the CDC method, the Dirren et al. method and the Dallman et al. method, respectively. Relevant texts have been revised accordingly. L175-L177, L222-L227

It is not clear what altitude was used in the adjustments. Was it the altitude of the place of residence of the women or the altitude of the hospital (Lhasa)? If the altitude of the hospital was used what effect could the different altitudes where the women lived have on the results?

Response: The altitude of Lhasa (3685 m) was used in the adjustments. L102
All the women in this study lived in Lhasa, and the difference between the highest altitude and lowest altitude in Lhasa is about 100 m. Therefore we did not use individual woman’s altitude in the adjustments.

Line 183 – what is meant by ‘…we adjusted other baseline characteristics to the same levels.’ The wording needs to be changed to make the meaning clear.

Response: The above statement has been removed from the text.

Line 221 – There is a reference to industrialised countries but there is no indication of the altitude of these countries. The details of the countries can be found in the referenced paper but some indication of altitude is needed for the readers of this paper.

Response: Thanks for the above point. We agree that there is no comparability between Tibet region and industrialised countries in terms of altitude and we have therefore removed this part from the text.

Line 242 – it is not clear that the relationship is linear – the word needs to be removed.

Response: Revised as suggested. L260

The English used is quite good but needs tidying up before this paper is published in an English language journal. For example, in the last paragraph before the conclusion (lines 284-294) there are a number of changes needed. First line has the word ‘larger’ but it is not clear what it is larger than. The third line it should read ‘… selection bias of the sample …’. The sentence ‘Lacking of serum indexes …’ needs completely rewriting.

Response: Thanks for the suggestions. We have made following revisions in the revised manuscript:
“This is the first study that described the pattern of Hb concentration and prevalence of anemia among pregnant women living in the highlands of Tibet.” L303
“The hospital-based cross-sectional design and selection bias of the sample in this study may underestimate the real prevalence of anemia and may not provide direct epidemiological inference for causality.” L305
“Lack of measurements of serum indexes such as serum ferritin and transferrin receptor may have limited our understanding of the true prevalence of anemia.” L310

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

'I declare that I have no competing interests'
Response to Reviewer 3

Reviewer's report

Title: Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

Version: 2 Date: 12 June 2009

Reviewer: Michael Höfler

Reviewer's report:

Major Compulsory Revisions

1. The huge method effect in measuring hemoglobin needs substantial and substantive explanation with their empirical and mathematical background. This is essential for assessing whether the authors conclusion that these methods do not work is warranted.

Response: We agree with the reviewer. We used B-Hemoglobin photometer to measure haemoglobin concentration in this study because this device has been proved to have a higher accuracy and reliability compared with standard laboratory methods as shown in the following articles.


   So we would expect that the resulting method effect would be small. Unfortunately, we were unable to assess this effect in this study.

2. In comparing their anemia rates with rates from other parts of China the authors have to adjust for many more (i.e. the essential) risk factors for anemia that could explain the differences if they differ in prevalence between Tibet and other parts of China. Moreover, the adjustment for altitude is implicitly based on extrapolation if altitudes of 4500 are not reached in the places the other data from China come from.

Response: Thanks for the above comments. We are aware of these differences between Tibet and other parts of China, and have elaborated those differences when tried not to make direct comparing anemia rates. L252- L255 The altitude of Lhasa (3685 m) was used in the adjustments. L102
3. Hospitalisation in pregnant women might be different for Tibetan and non-Tibetan women. In that case the differences between them would be biased if the determinants of hospitalisation are not properly adjusted for.

Response: We agree with reviewer’s point here. We did compare the observed characteristics of pregnant women between two ethnic groups and found that the two groups were comparable in terms of these observed characteristics. We have added a sentence in the revision paper. L188 We have also discussed the limitations of this study and stated that the observed difference could be subject to the unobserved heterogeneity. L303

4. No information is provided about response rates and potential selective non-response. Without that information the quality of the sample can not be assessed.

Response: Thanks for the comment. The response rate is 96.0%. Within the 16 (4%) non-response, 2 were later found to be not pregnant, and 14 did not agree to have their Hb levels checked due to fear of pain and religious beliefs. L135- L138

5. Moreover, the authors should compare their laboratory subsample with the total sample to assess whether sampling bias has crept in at the level of providing blood.

Response: We compared the background characteristics of subsample with those of the total sample and found no difference between them. Therefore, we believed that there was very small chance of selected bias crept in at the level of providing blood. However, we have added some sentences showing the result of the comparison. L209

6. p.7: What are "baseline characteristics" if only one assessment was conducted?

Response: This point was also made by Reviewer 2. The text has been removed.

7. p.7: The covariates need to be clearly defined along with how they were measured. For instance, how was "rural" vs. "urban" defined if the sample was only drawn in Lhasa? What is meant with "counterparts"? This would require matching and mentioning which variables were used for matching. Although adjustment in regression models has the same aim this terminology is not valid here.

Response: Thank you for point out this. (1) In this study, we defined “rural” vs “urban” according to registered residence. Lhasa includes 7 communities (urban area) and 4 townships (rural area).
We have rewritten this part as: "The figure shows that Hb decreased continually with the increase in gestational age for both Tibetan and non-Tibetan women and that the Hb for Tibetan women was consistently lower than for non-Tibetan."

"Pregnant women living in rural area had higher hemoglobin level than those in urban area (Coeff=5.66, 95% CI: 1.03, 10.30)."

8. Confidence intervals of means minus their reference value, not null hypothesis tests should be used to compare outcomes with respect to their reference values. The sample might have small power to detect differences if the variation in clinical parameters is high.

Response: As suggested by the referee, we have made some corrections as follows: "One-sample t-test was used to compare mean of an erythrocyte parameter with its reference and the difference between the mean and reference together with its 95% CI for a parameter was also derived."

Minor Essential Revisions

1. p.4: What are the highest and lowest altitudes of Lhasa? Or can variations be neglected?

Response: The altitude of Lhasa (3685 m) was used in the adjustments. All the women in this study lived in Lhasa, and the difference between the highest altitude and lowest altitude in Lhasa is about 100 m. Therefore we did not use individual woman’s altitude in the adjustments.

2. p.7, below: "controlling for altitude" has to be replace with "adjustment for altitude".

Response: Revised as required.

3. p.8: "than non-Tibetans" has to be replaced with "than non-pregnant Tibetans"

Response: We have rewritten this part as: "The table shows that Tibetan pregnant women were more likely to have an anemia than non-Tibetan pregnant women (OR= 0.45, 95% CI: 0.23, 0.88)."

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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
Response to Reviewer 4

Reviewer's report

Title: Hemoglobin levels and anemia evaluation during pregnancy in the highlands of Tibet: a hospital-based study

Version: 2 Date: 15 June 2009

Reviewer: Giorgio Bedogni

Reviewer's report:

General comment

I was asked to review this paper as BMC statistical referee. I read version 2 of the manuscript together with the comments made by three reviewers on December 2008.

Major Compulsory Revisions

None

Minor Essential Revisions

P2 L28-30 Does the relationship which “has not been clearly established” refers to pregnant women or to the whole Tibetan population? This is not clear to me based on the present wording of the sentence.

Response: The relationship refers to the pregnant women living in Tibet. We have rewritten this part as: “the relationship between altitude and Hb concentration in the pregnant women living in Tibet still has not been clearly established.” L27

P2 L36 Please, use “association” instead of “influence” as the study design does not allow to model cause-effect relationships.

Response: Thanks for the point. We have revised accordingly. L35

P3 58 Although there is no doubt that anemia has a negative impact on the immune system, I wonder whether the Authors intend to say that this is the major mechanism by which anemia increases morbidity and mortality.

Response: No. We have rephrased it as: “It damages immune mechanisms and is also associated with increased morbidity.” L69
Response: We have rephrased it as “Understanding the relationship between Hb concentration of pregnant women living in Tibet and altitude will have important implications for correctly estimating the prevalence of anemia at high altitudes.” L81

P4 L98-ff & P5 119-ff Please, explain better how sampling was performed. On which days of the week did you perform sampling? I suppose that this was a kind of systematic sampling, wasn’t it? How many subjects did you sample in a day? Was this number fixed or variable? From your reply to one reviewer, I understand that “random” sampling was performed over 1 year on 1200 pregnant women attending your hospital. Please, report this very important number in the manuscript.

Response: The sampling was done in two steps: (1) We randomly selected 2 days from each week to collect blood sample, which was not a systematic sampling. (2) In each selected day, all the pregnant women who sought antenatal care were selected as study subjects.

We have elaborated this point in the revised version: “...and the number of women attending antenatal care at the Obstetrical Department of the Lhasa People's Hospital is about 1200.” L108

P2 L28-30 You selected 396 women (of whom 16 were excluded from the study). However, you planned to measure erythrocyte constants in only 48% of the study sample (190). How was this sample selected? Moreover, 84 women dropped from the study so that erythrocyte constants were evaluated on 106 women (27% of planned sample size). Please, provide evidence that these 106 women are representative of the whole 396. Otherwise, you cannot be confident that the conclusions about erythrocyte constants can be generalized to the whole sample.

Response: (1) “Selection procedures of the subgroup were done as following: First, all the participants on the selected day were sequentially numbered from 1 to the number for the last participant on that day; Secondly, all the participants with either an odd or even number were chosen depending whether a random number was 1 or 2 (one for odd number and 2 for even number).” L119- L123;

(2) Subsample was selected randomly and we compared the background characteristics of subsample with those of the total sample and found no difference between them, suggesting no selected bias crept in at the level of providing blood. However, we have added some sentences showing the result of the comparison.

L209

P6 L154 How the cut-off of 1800 Yuan was chosen? Please, provide a
conversion to USA $ among parentheses.

Response: In the first manuscript, we categorised annual income as “0-1800”, “1800-6000”, “6000-12000”, “12000-36000” and “36000-”. In the version 1, one reviewer advised us: consider only 2 categories regarding Annual income, < and > 1800 Yuan”, therefore we chose 1800 to be cut-off. We converted RMB to USA $ as suggested. 1800 RMB= $264. L170

P6 156 (and elsewhere) I suppose that you are referring to the fit of the covariates in the multiple linear and multivariable logistic regression models (not in simple linear and univariable logistic regression models). Is this right?

Response: Yes, we are referring to the fit of the covariates in the multiple linear and multivariable logistic regression models.

P6 L162 Please, write “agreement” instead of “consistency”.

Response: Revised accordingly. L176

P7 L170 and elsewhere I strongly suggest to report data as mean and standard deviation as this allows a better assessment of the variability inside your study sample.

Response: Thanks for the suggestion. We have revised as suggested. L189- L193

P7 L177 Please, add standard deviations to figure 1. Because there are just 50 non-Tibetans, their “curve” is clearly less defined than those of Tibetans and the comparison must be due with caution. (Provided that there is enough power you could perform a comparison using an appropriate regression model which take into account the “uncertainty” of the curve of non-Tibetans.)

Response: Thanks for the suggestion. We plotted mean ±1SD in Figure 1 but the figure did not look nice due to the huge variation in SD. We therefore plotted mean ±1SE in the Figure 1 and hope this is acceptable.

P8 L204-ff Did you take into account the multiple-comparison issue when comparing the three separate kappa statistics?

Response: Thanks for the comment. We have used the proportion classified as anemia after adjustment to assess the consistency among different methods as suggested by another reviewer. So there is no multiple-comparison issue. L175-L177, L221-L226

P8 L212 “For each characteristic…”: this phrase is not clear and I suggest to
remove it.

Response: Removed as suggested.

P9 L233 Because you do not provide a reference for your hypothesis, one supposes that this is the first time this hypothesis is done. Is there any data to support your hypothesis (in Tibet or elsewhere)? Biological plausibility is very important in explaining your findings especially because of the potential selection bias.

Response: Yes, we agree with reviewer. As we described: "As per the above adaptations, ...", references were provided in [15-18]. L244-L250

P9 L249 You measured mean corpuscular volume (MCV) and red cell distribution width (RDW) in a subsample of the women (see P4 L98-ff & P5 119-ff) and this may partly explain the oddity of your finding. Please, comment on this point.

Response: Subsample was selected randomly and we compared the background characteristics of subsample with those of the total sample and found no difference between them. Therefore, we believed that there was no selected bias crept in at the level of providing blood. However, we have added some sentences showing the result of the comparison. L209

P10 L260-ff How did you collect symptoms? With a standardized form? How did you operationally defined “hypodynamia”?

Response: In our study, we used a standard questionnaire to collect symptoms information. Participants were interviewed face-to-face by interviewers. Hypodynamia was one of self-report items on questionnaire, and it means lack of physical strength. We have made corresponding revisions in the revised manuscript. L280

P6 L168 17% of study women were non-Tibetans. From the data you report for whole Tibet, I learnt that 96% of Tibet residents are of Tibetan origin. Are there more non-Tibetans in Lhasa than in other places of Tibet? Or does your hospital has an high referral rate for non-Tibetans? Please address this point, because ethnicity is an important covariate in your regression models.

Response: Yes. Lhasa is the capital of Tibet. There are more non-Tibetans in Lhasa than in other parts of Tibet. The hospital has equal referral rate for each ethnicity.

Table 1 I strongly suggest to report descriptive data as mean and standard deviations (see P7 L170).
Response: The suggested topic has been added in Table 1.

Table 1 Report the operational definition of “Tibetans” (i.e. Han, Hui, Menba... etc.) also under Statistical analysis.

Response: We agree with the reviewer, but most of the non-Tibetan were Han, as small number of Hui, Menba and etc. So, no further grouping was made in the analysis.

Table 1 Looking at the Table, gestational age appear to have been modeled as 3 trimesters. 1,2 and 3? I wonder how a log-transformation can improve the fit of a three-level equally-spaced variable. I am also not sure that this variable should be modeled as continuous because it takes just 3 values. Please, explain.

Response: When the log-transformed gestational age (in weeks) was entered model as a continuous variable, it produced the best fit in terms of Akaike Information Criterion (AIC) among different transformations including linear, quadratic, categorized to 3 trimesters.

Table 1 Parity was model as continuous. However, this is a 3-level variable and the last value (i.e. 2+) covers “more possibilities” than previous values (i.e. 0,1). Have you tested parity as categorical variable before using it as continuous?

Response: As we did for gestational age, parity was treated as a continuous variable as it fits the model best in terms of AIC.

Discretionary Revisions

None

Level of interest: An article whose findings are important to those with closely related research interests

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