Author’s response to reviews

Title: Significantly elevated foetal haemoglobin levels in individuals with Glucose 6-phosphate dehydrogenase disease and/or sickle cell trait: a cross-sectional study in Cape Coast, Ghana

Authors:

Patrick Adu (patrick.adu@ucc.edu.gh)
RICHARD EPHRAIM (rephraim@ucc.edu.gh)
Essel Bashirudeen (esseldeen@gmail.com)
Edward Adela (delmakeous@rocketmail.com)
Haruna Florence (florenceharua25@gmail.com)

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Author’s response to reviews:

Response to Editor’s comments.

1. The design of the study. It is not clear how the study population was recruited to the study
Response: The study design has been clarified in the section “Participants” (see page lines 95 – 100, page 2 & 3).

2. The statistical analysis does not describe the statistical methodology
Response: Sufficient details have been provided under section “Statistical analysis” (see page 4, lines 121 – 131).

3. The paper requires editions by an expert in English language
Response: The comments have been taken into consideration and manuscript revised accordingly.
4. Some parts of the results section belong to the figure legends.
Response: The details provided in the results section are to give clarity to the results presented in the manuscript. We believe these should remain as their removal might make it difficult for prospective readers of the article once published.

RESPONSES TO REVIEWER’S (ADEMOLA SAMSON ADEWOYIN) COMMENTS

Title:
In the title, ‘elevated % Hb F’, should pass for foetal haemoglobin levels or Haemoglobin F levels (so as to avoid use of abbreviations in the title). Again, I think the location of study should also be captured in the title, something like: Significantly elevated foetal haemoglobin levels in individuals with ……….. In Cape Coast, Ghana.
Response: Title revised accordingly to read “Significantly elevated foetal haemoglobin levels in individuals with Glucose 6-phosphate dehydrogenase disease and/or sickle cell trait: a cross-sectional study Cape Coast, Ghana.” See Lines 1 – 3 (title page)

ABSTRACT:
Comment: Line 4: severer should be replaced with ‘more severe’.
Response: severer has been replace with more severe (see line 26); page 1

Comment: Result section (line6/7): the statements lack continuity, grammatical construction should be revised.
Response: Statement has been revised to read “…although the %Hb F levels was comparable in non-G6PD deficient individuals.” Line 46, abstract page

BACKGROUND:
Comment: Should include a stronger review of established data on foetal Hb proportions in G6PD and sickle cell disorders.
Response:

Comment: However, in paragraph 2: ROS ?? does not fit in to the listed agents.

Response: Statement has been revised to read “Agents causing oxidant stress in G6PD deficient individuals include fava beans and drugs such as aspirin, primaquine and quinine”. See Line 68, page 1.

Comment: Moreover, in paragraph 3, the word (ref) in bracket is out of place and proper citation should be done.

Response: Relevant references 12 & 13 have been cited (see line 72, page 1)

Comment: The meaning of ROS should be spelt out at first use
Response: ROS has been defined on first use (see line 63, page 1)

METHODS:

Comment: study site: I do not really see the place of those demographic details in the design of this study.

Response: Comment taken into account and manuscript revised to read “This cross-sectional study was conducted at the University of Cape Coast hospital in the Central region of Ghana from January to May 2016. The hospital has a bedding capacity of 65, and has an average yearly OPD attendance and admissions of 61,509 2608 respectively. The hospital has OPD, A & E unit, Surgical ward, Medical ward, laboratory department, radiography unit and physiotherapy unit.” See lines 91 – 94.

Comment: Participants: the sampling technique described does not fit for simple random sampling. It is a convenience sampling technique (consecutive participants were recruited). How did you randomize? Did you use any table of random numbers?? Please revise.

Response: Comment taken into consideration and manuscript revised accordingly (see line 96 – 97)
Comment: G6PD screening assay: …as previously described. Where is the description???
Response: Appropriate reference (20) cited (see line 108, page 3)

Comment: Haemoglobin estimation: …following manufacturers protocol: what reference???
Response: Appropriate reference (21) cited (see line 112)

Comment: Haemoglobin electrophoresis: …in accordance with previously published protocols. ??? where? What reference???
Response: Appropriate reference (24) cited (see line 118, page 4)

Statistical analysis:

Comment: No statement was made to capture inferential statistics performed, no mention of the statistical tools used in comparative analysis and how inferences where drawn.
Response: Comments taken into consideration and manuscript revised accordingly (see lines 122 – 130; page 4).

Comment: The second to last statement in this section is tautology.
Response: comment taken into consideration and statement removed.

RESULT/DISCUSSION:

Comment: not well presented. The presentation style is very clumsy and lack clarity. This should be seriously revised please. Table 1: stratification based on gender portends that gender has some influence on Hb F levels in G6PD and sickle cell trait.
Response: Comments taken into consideration, and results section revised accordingly.
Comment: I think that G6PD Status should just simply be presented as defect or no defect. I believe that would give a clearer/stronger level of evidence.

Response: The study used previously published protocol (Cheesbrough, M. 2012) which grades the G6PD status as No defect, partial defect, or full defect based on colour change. We believe this grading is a true reflection of the test results and must be presented as such.

Comment: FIGURE 2D: this figure is not really useful in my assessment. It is an established fact that Hb levels are higher in males due to androgen effect in haemopoiesis. The interest should be why haem F levels are higher in men with G6PD/SCT versus those without. What plausible explanations? How do this findings compare with other studies

Response: Comment taken into consideration and revised accordingly (see line 465, page 18)

CONCLUSION:

Comment: This is no conclusion but rather a representation of results. Conclusion should be rewritten

Response: Comment taken into consideration and manuscript revised accordingly (see line 250 – 255, pages 9 & 10)

REFERENCES:

Comment: Please ensure conformity with Vancouver style, supposed number of authors before et al should be written, other non-conformities should be addressed.

Response: Manuscript revised to address these
Dear AMEL RENAUD BIDIAS (Reviewer)

Responses for reviewer comments for submission BHEM-D-17-00010

1) Title.

Comment: (The title is a representative of the work and includes all the important information, for example study design, setting and population…):

I think the title is very specific and appropriate.

Response: Comment well noted.

2) Background.

Comment: As these red cell pathologies are independently inherited, the potential for co-inheritance may be high especially in sub-Saharan Africa where either of these pathologies has been shown to provide protection against malaria infection [ref].

Response: References (12, 13) provided; see line 72, page 1.

Comment: To provide more specifics you could indicate in the Background which drugs cause G6PD and/or haemoglobin deficiency and how those drugs are represented in your cohort. Provide references for the underline text also.

Response: Appropriate medications and background provided; see line 79 - 84, page 2)
3) Methods.

Hb Electrophoresis

Comment: Please provide references for (Haemoglobin variants in the participants were determined using the cellulose acetate electrophoresis (pH 8.2 – 8.6) in accordance with previously published protocols [ref].

Response: Appropriate reference (24) provided; see line 118, page 4.

Comment: Just to add more depth to the Methods, would it be possible to include more information on the types of participants in this cohort (pregnant women, those taking drugs causing G6PD and/or haemoglobin deficiency… were they included)?

Response: More details of the cohort have been provided; see line 97 – 99, page 2 & 3.

Comment: In the Conclusion you comment on sex being a factor in %Hb F levels. Could you give more detail in the methods about male/female distribution in your cohort?

Response: Male and female distribution has been provided; see line 96, page 2.

Comment: Can you define the different groups that you include in the results (Tables)?

Response: Definitions have been provided; see line 108 -109, page 3 & line 119, page 4.

Comment: Did you used the logistic regression for enabling you to verify the existence or not of risk factors between quantitative variables? Can you provide additional data about what variables are included in the logistic regression?

Response: Logistic regression and correlation analyses have been added; see line 163 – 171, page 6; & line 232 – 239, page 9.

Comment: Since you discuss it in the Results could you discuss the groups you are referring to?

Response: Definitions have been provided; see line 108 -109, page 3 & line 119, page 4.
4) Results.

Comment: Revise your results underline and provide the correct unit that miss (% or else?).
Response: Units have been defined appropriately; see line 134 (page 4); lines 146, 147, 153, 157 (page 5).

Comment: In general the results are very interesting, but could benefit from a little more detail. It is a little unclear if your G6PD status and haemoglobin variants and which groups correlate with which other (if applicable).
Response: Correlation analysis as well as logistic regression analyses have been provided; see line 163 – 171, page 6; & line 232 – 239, page 9.

5) Discussions.

Comment: Does your study involved many countries? I prefer that you compare your results with those observed in countries not in region (WHO, Africa).
Response: It was conducted solely in Ghana. The reason we provided country-specific reference (line 180, page 6) as well as regional-specific reference (line 181, page 6) was to contextualise the study. We believe both references should hold.

Comment: Can you provide more references for these countries? It seems this one is only for Nigeria.
Response: Appropriate references have been provided (see lines, 195 & 196, page 7)

Comment: Can you provide more references for these regions? It seems this one is only for Arabian Peninsula.
Response: Appropriate reference (41) provided (see line 204, page 7).
Comment: Did you worked with children? I don’t think this sentence has a strong impact in discussion. Can you replace it by a study done among adults?

Response: Reference on children deleted and relevant references and studies in adult population cited. (see lines 207 – 216, page 8).

6) Conclusions.

Comment: I think the Conclusion section does a nice job of indicating how your findings should be applied going forward, but since you clearly demonstrate G6PD enzymopathy to be associated with elevated %Hb F levels, maybe more mention could be given about what factors may should be considered in the management of the patients.

How do you think that %Hb F levels would help in the monitoring or treatment of patients with G6PD enzymopathy and/SCT? I think that the conclusions will depend on the results of your logistic regression - if there are covariates that are associated with particular haemoglobin variants (for instance, gender, age, drugs), that would be a major conclusion.

Response: Comment well noted.