

REVIEWER 4: Comments and Suggestions for Authors

Authors study the co-occurrence of the G6PD deficiency, sickle cell trait, anemia and various infections among pregnant women in Ghana. Number of participants is pleasantly high. This study is important for physicians, attracting the attention for the therapy by antimalarial drugs capable to elicit the additional hemolysis (anemia) and providing the ideas for targeted antimalarial treatment of G6PDd and SCD pregnant woman. Additionally, this study provides new insights into infection, anemia, G6PDd and SCD co-occurrence in Ghana.

Minor critics:

1. One of the main result in this paper is anemia frequency, maybe it's worth including anemia in the title?

Response: We appreciate the reviewer's efforts to improve our manuscript. Although anaemia is one of the study's primary findings, we still want to keep the title because it captures the study's main focus.

2. Authors' affiliation: for the authors from Tamale, the country, Ghana, is missing.

Response: Ghana has been included to the affiliations (see lines 6 – 10)

3. Abstract: define ANC acronym when first time used.

Response: This correction has been effected (see line 23)

4. Introduction, line 45: The citation [4] for G6PDd world carriers is from 2009. Hope, more recent data are available. The same for SCT, line 74, the citation [16] is from 2010.

Response: As much as possible we have cited the most updated literature per our search.

5. Figure 1. In actual figure size/resolution, the name of the cities in the main map are poorly readable. Please, fix it.

Response: This has been fixed (line 103)

6. Methods, line 137, G6PD test chapter. Please, indicate, in which step of erythrocyte washing the white blood cells were removed to insure G6PD detection only in erythrocytes.

Response: The method as described in the manuscript (following the protocol described in ref [26]) does not involve erythrocyte washing. However, in order to do away with white blood cells, we used pack blood (as described in the manuscript we used increased PCV of 0.4 (40%)) which corresponds to an increased red cell count (see lines 141 – 144).

7. Methods, line 137, G6PD test chapter. The test for G6PD deficiency is performed (as follows from text) by eye distinguish between red, light brown and deep brown colour of the tube. This “ancient” method for colour detection is used in the literature until today, thus roughly could be accepted in this paper. In the future the authors could introduce the spectrometric quantitative evaluation.

Response: This method was employed in the current investigation because it is the method most frequently used to diagnose G6PDd in the study areas. We acknowledge that there is a chance that human error contributed to the results, but there were enough controls in place to reduce this risk, and three independent laboratory technicians all agreed on the results. Additionally, to reduce this risk of misclassification, the study classified G6PDd individuals to include both full defect (homozygous G6PD deficient) and partial defect (heterozygous G6PD deficient) (see lines 164 – 165). We agree with the reviewer’s recommendation to apply spectrometric quantitative evaluation in subsequent studies in our study areas.

7. Methods, line 137, G6PD test chapter. Both G6PD homozygotes and heterozygotes are included in statistics as 8473 G6PDd persons? Please, indicate it clearly.

Response: This has been clearly stated in the methodology section (lines 152 – 163). That notwithstanding we have insert the sentence “G6PDd individual in this study include both full defect (homozygous G6PD deficient) and partial defect (heterozygous G6PD deficient)” in lines 164 – 165 to bring more clarity.

8. Methods, line 168, please, spell out the full term of HRP and LDH acronyms.

Response: These acronyms have been spelt out as suggested by the reviewer (see lines 172 – 173)

9. Methods, lines 174, 178 and 183. Which type of primary human samples (blood?) were used for HBV, HIV and Treponema identification?

Response: Whole blood was used while oral fluid was used for confirmatory detection of HIV (See lines 180, 185 – 188)

10. Results, line 236. The Table S2 reports the important information about anemia percentage in G6PDd, probably worse to use this Table in the main text, non-supplemental?

Response: We thank the reviewer for this critical comment. Once the prevalence of anaemia has been stated in pros in the main text, we would like to keep the table as supplementary data.

11. Discussion, line 266. The initial phrase “Anemia is associated with pregnancy...” is overstatement, better to add the clarification “...in some conditions” or similar.

Response: This statement mainly talks about an association but not causality, and we have supported the statement with relevant references.

13. References, line 423, the [16] authors are listed as: “Tinley, K.E., A.M. Loughlin, A. Jepson, E.D. Barnett, and hygiene”, is it typo?

Response: This was a mistake, thus all references have been formatted and any duplicate references have also been deleted.