PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prenatal malaria exposure and risk of adverse birth outcomes: a prospective cohort study of pregnant women in the Northern Region of Ghana
AUTHORS	Hussein, Hawawu; Shamsipour, Mansour; Yunesian, Masud; Hassanvand, Mohammad Sadegh; Agordoh, Percival Delali; Seidu, Mashoud; Fotouhi, Akbar

VERSION 1 – REVIEW

REVIEWER	Gonçalves, Lígia Antunes Instituto Gulbenkian de Ciencia
REVIEW RETURNED	22-Dec-2021

GENERAL COMMENTS	In the manuscript "Prenatal malaria exposure and the risk of adverse birth outcomes: a cohort of pregnant women in from Northern Region of Ghana", Hussein and colleagues analyzed data from pregnant women and their newborns living in the Northern region of Ghana, exploring prenatal malaria and the risk of poor birth outcomes. This prospective cohort study enrolled pregnant at their 28th week of gestational age and followed them until delivery, between July 2018 and May 2019. The authors found that prenatal malaria increases the risk of low birth weight and preterm birth. The novelty of the manuscript relates to the sample size and study setting in Ghana. The manuscript would benefit from improved formatting to convey a couple of paragraphs in a more precise format, refine some of the manuscript's ideas, and additional analysis. For these reasons, considerable modifications should be made before the publication. Specific comments: 1. The title should include the type of cohort developed, prospective; 2. Introduction section: • the correct designation for the preventive treatment during pregnancy is "intermittent preventive treatment of malaria in pregnancy," and its correct abbreviation is IPTp. • It is correct on page 5, line 5, "research conducted in a war hospital"? • It would improve if the abbreviations IPT3 and IPT5 were explained. 3. The methods section needs significant improvements; despite the analyzed data being "drawn" from a previously published study cohort, its focus was substantially different from this manuscript. • The study setting should be more detailed, namely, the characterization of the region as for malaria endemicity, annual parasite incidence, and if there are differences according to the dry or rainy season. • The study procedure should indicate how the gestational age

	was ascertained. • When, in terms of gestational age, and how many times the RDT was performed? • The authors need to explain the rationale behind the criteria to establish anemia whenever Hb < 9 g/dL. According to the WHO guidelines, during pregnancy, anemia is defined when Hb < 11 g/dL. Several studies show that Hb < 11 g/dL, mainly Hb ≤ 10 g/dL, is associated with low birth weight and preterm birth. 4. Regarding the results section, further information is needed to support the authors' conclusions: • The authors show that 9.5% of the pregnant had malaria during pregnancy. Since no description of when and how many times the RDT test was performed, it is difficult to conclude that the infection occurred during the third trimester. Therefore, the authors cannot conclude that "maternal malaria within the third trimester of pregnancy may be a determinant of LBW and preterm birth" unless all women at enrollment tested negative. • What type of specimen was used in the RDT test, peripheral blood or placental blood? • More detailed information about the women with malaria during pregnancy should be presented: how many episodes of malaria during pregnancy, if they were under IPTp, and which treatment was performed. • Also, 6.4% of women tested positive for sickle cell, 50% with the Hb SS genotype. It is not clear if these women tested positive for malaria and how these data were handled in the analysis. It is expected that newborns from sickle cell women have low birth weight. • How were other maternal infections (TORCHs) ruled out or controlled in the analysis, as these may impair fetal development? Also, there is no mention of women with smoking or alcohol habits; both have consequences on fetal development. • Regarding the analysis of the risk of perinatal mortality, the authors wrote that malaria poses a risk (page 11 line 23); though, their analysis (table 5) shows no risk of perinatal mortality in women with malaria during pregnancy (RRa 1.02, 95%CI 0.26-4.01, p-value 0.983). Please explain this conclusion. • Please explain, for each analysis, the rationale to choose some confounders and not others to adjust the relative risk as it is not clear. • Tables need to be proofread. Table 1 has a repetition of malaria data, parity must be under medical history, and whenever abbreviations are used, these must be stated in tables footnotes. 5. Discussion: Page 12, lines 5-12 should be re-written as that information is duplicated. Replace "placental site malaria" with "placental malaria". Also, malaria diagnosis based only on RDT testing is a limitation of the study.

REVIEWER	Teo, Andrew The University of Melbourne
REVIEW RETURNED	29-Jan-2022

GENERAL COMMENTS	Summary: Hussein and colleagues looked into the impact of placental malaria on birth outcomes, including low birth weight, preterm and perinatal deaths, in northern region of Ghana. They found that PM in the third trimester had increased risk of LBW and preterm, commonly observed in PM. Overall, findings were expected, might be useful for local use, but not novel. A bit more effort to proof read and a more focus text would be helpful.

	Major: The introduction is extremely lengthy with no aims or purposes, its repetitively. I suggest that you to provide reader a more concise introduction and remove unnecessary information such as – the first paragraph of the introduction. Consider to explain why MiP is an important health issue, how MiP affects infant and maternal outcomes. Mention what is special about MiP in the third trimester to better suit your study. Malaria parasite – this subheading is incorrect. RDT was used to determine whether participants had parasitaemia, the ensuing description is unnecessary. Consider to remove or to replace with more concise information. Haemoglobin estimation – why is this necessary when nothing was mentioned in the subsequent text? I do not think log binomial regression model is appropriate. This reports relative risk, you reported odds ratio, it should be a logistic regression model. What were the individual covariates added? It would be good to include to provide better understanding for readers. “Some adjusted confounders included…” firstly covariate and confounders cannot be used interchangeably, this statement suggests you have a lot of other variables added into the model there were not listed, any reason why not to list? Why is SP usage not included? Lastly, PM in pregnancy, the most important variable was not included in the adjusted model? Ethics should be mention in the methods Table 1, malaria was reported twice Results section: This is poorly written with unnecessary information. Perhaps structure into subheadings. What is the purpose of reporting education, employment, rich etc? the overall aim is to looked into PM and birth outcomes. Consider listing things at are associated with PM, eg gravidity, numbers of PM positive etc. Table 5, caesarean section had higher risk of mortality, this was not reported, any reason why this was excluded? Table 6, I do not understand the purpose of this test. Should be made clear early on why RR was used instead of OR. I believe OR in this case is more appropriate. Discussion The discussion needs to be more focused on the aims of this study, which is to explore, PM in the third trimester and poor birth outcomes. The main findings is PM increased the risk of poor birth outcomes, it would be appropriate to discuss why and what are the possible mechanisms behind this. It is not helpful to cite studies on vivax, comparing of different techniques etc. Minor:

	Malaria is a disease, not an infection, thus malaria infection is incorrect. This should be updated throughout the manuscript. “They further had 1.93 times (1.11-63.41)” – is the 95CI correct? It seems relatively huge. Mixture of British and American spelling, this needs to be sorted out. Typos in Figure 1, do check Specific: “Briefly, for this study….” Remove “for this study” “… all pregnant women given SP, how many courses?” good to include or even provide what is the take up rate during pregnancy. “The principal investigator monitored…” this is unnecessary, the PI cannot be in the four settings simultaneously or to supervise every single tests.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Lígia Antunes Gonçalves, Instituto Gulbenkian de Ciencia
Comments to the Author:

Reviewer 1: Comments #1:

In the manuscript "Prenatal malaria exposure and the risk of adverse birth outcomes: a cohort of pregnant women in from Northern Region of Ghana", Hussein and colleagues analyzed data from pregnant women and their newborns living in the Northern region of Ghana, exploring prenatal malaria and the risk of poor birth outcomes. This prospective cohort study enrolled pregnant at their 28th week of gestational age and followed them until delivery, between July 2018 and May 2019. The authors found that prenatal malaria increases the risk of low birth weight and preterm birth. The novelty of the manuscript relates to the sample size and study setting in Ghana. The manuscript would benefit from improved formatting to convey a couple of paragraphs in a more precise format, refine some of the manuscript's ideas, and additional analysis. For these reasons, considerable modifications should be made before the publication.

Specific **comments # 1:**
1. The title should include the type of cohort developed, prospective;

Author’s Response.
Title revised as per Reviewer’s recommendation in the Title page.

“Prenatal malaria exposure and risk of adverse birth outcomes: a prospective cohort study of pregnant women in the Northern Region of Ghana”.

Comment #2

2. Introduction section:

• the correct designation for the preventive treatment during pregnancy is "intermittent preventive treatment of malaria in pregnancy," and its correct abbreviation is IPTp.

Author's Response

Thank you, upon review of the paragraph 2, we found that paragraph to be irrelevant to the study and have deleted that part.

Comment #3

• It is correct on page 5, line 5, "research conducted in a war hospital"?

Author's Response

It has been corrected in the manuscript to read,

"Regarding treatment, research conducted in a War Memorial Hospital in the Upper East Region found that children born to mothers on artemether–lumefantrine (ISTp-AL) had a lower risk of malaria than those delivered to mothers on sulfadoxine/pyrimethamine (IPTp-SP."

Comment #4

• It would improve if the abbreviations IPT3 and IPT5 were explained.

Authors Response

Abbreviations IPT3 and IPT5 have been explained as recommended in the text below and in the introduction part of the manuscript

"In Navrongo, uptake of Intermittent Preventive Treatment of malaria in pregnancy using Sulfadoxine-Pyrimethamine (IPT3) i.e., uptake of three doses was 76 percent, while (IPT5) 5 doses uptake was 16 percent, with women who received at least three doses having better health outcomes. (6)"

3. The methods section needs significant improvements; despite the analyzed data being "drawn" from a previously published study cohort, its focus was substantially different from this manuscript.

Authors Response

More explanation provided below

The present this study was designed as part of the parent cohort study to assess how different cooking fuel type affected pregnancy outcomes and baby respiratory problems, this was the primary research question (8, 9), the current study data was drawn out the original study to answer a secondary research question on prenatal malaria exposure and the risk of adverse birth outcomes.

Additionally, the study was designed to recruit third trimester pregnant women, who were primary cooks, non-smokers and carried singleton pregnancies. The process begun in July 2018 and ended by May 2019. The main study was planned for three phases of data collections. At the beginning of the study, women were screened and recruited. In phase 1 during the third trimester, baseline data (demographic, medical history, exposure data for the primary objective (fuel type), and secondary objective (malaria) were collected. Phase 3 saw the collection of data for birth outcomes during delivery at labour various wards. Babies were the followed up to collect respiratory outcomes data.

• The study setting should be more detailed, namely, the characterization of the region as for malaria endemicity, annual parasite incidence, and if there are differences according to the dry or rainy season.

Author's Response

Additional information has been added to the setting in the manuscript as explained below

Setting.

Data for this sub study was drawn from a prospective cohort study that took place in four hospitals in Ghana's Northern region. Three hospitals are located in Tamale, the Northern Regional Capital and also the fourth largest city in Ghana., the fourth hospital is bordered by Tamale to the West: both are within the Guinea Savannah belt. The P. falciparum peripheral parasitemia prevalence Northern Savanna Zone ranged between 26% and 13.4% from 2013 to 2019 respectively.

- The study procedure should indicate how the gestational age was ascertained.

Author's Response
This has been added to the study procedure in the manuscript, see below

"In all hospitals, gestational age was ascertained through an ultrasound, therefore our study relied on midwives validated gestational age."

- When, in terms of gestational age, and how many times the RDT was performed?

Author's Response

RDT was performed from 28 weeks onwards to few hours before delivery.

- The authors need to explain the rationale behind the criteria to establish anemia whenever Hb < 9 g/dL. According to the WHO guidelines, during pregnancy, anemia is defined when Hb < 11 g/dL. Several studies show that Hb < 11 g/dL, mainly Hb ≤ 10 g/dL, is associated with low birth weight and preterm birth.

Author's Response

The 9 g/dl was initially used because most women in our part of Ghana, based on anecdotal evidence commonly come to hospital with at least 9 g/dl and in most cases have normal delivery without complications or adverse events on the mother and child. However, based on the reviewer's recommendation, Hb has been recategorized as recommended by the reviewer using the WHO recommendation. It was still not significant when added to all the three models, hence dropped in the multivariate analysis.

4. Regarding the results section, further information is needed to support the authors' conclusions:
- The authors show that 9.5% of the pregnant had malaria during pregnancy. Since no description of when and how many times the RDT test was performed, it is difficult to conclude that the infection occurred during the third trimester. Therefore, the authors cannot conclude that "maternal malaria within the third trimester of pregnancy may be a determinant of LBW and preterm birth" unless all women at enrollment tested negative.

Author's Response
Admittedly, we included third trimester because most women in our part of Ghana visited ANC may not visit ANC or may attend from the later part of second trimester (Nachinab GT et al 2019), hence we conveniently selected those in trimester who at least did RDT at any period before delivery, this may bias our study, since we cannot account for malaria before third trimester. This has been captured as limitation in the penultimate paragraph of the discussion.
The conclusion has also been revised to reflect this below.

"In conclusion, maternal malaria within the third trimester of pregnancy may be a major contributor"

- What type of specimen was used in the RDT test, peripheral blood or placental blood?

Author's Response
We took peripheral blood, which is stated in the page 6 of the manuscript.

- More detailed information about the women with malaria during pregnancy should be presented: how many episodes of malaria during pregnancy, if they were under IPTp, and which treatment was performed.

Author's Response

Additional information added to table 1, about 88% were under IPTp

- Also, 6.4% of women tested positive for sickle cell, 50% with the Hb SS genotype. It is not clear if these women tested positive for malaria and how these data were handled in the analysis.

Author's Response
Yes, indeed each of them tested for malaria. Yes, 14 of the Hb SS genotype tested positive, this was considered in each of the univariate models, but was not significant hence was dropped in the Log binomial regression model.

It is expected that newborns from sickle cell women have low birth weight.

- How were other maternal infections (TORCHs) ruled out or controlled in the analysis, as these may impair fetal development? Also, there is no mention of women with smoking or alcohol habits; both have consequences on fetal development.

Author's Response

We did not have information on (TORCHs) and this may be shortcoming and hence could not be controlled. However, medical history of pregnant women including heart disease, respiratory disease, TB and HIV. Less than 10 women had each of those condition, and so when included in the univariate log binomial regression, they were non-significant and hence were dropped. Pregnant smoking women were excluded in the study, also less 5 women drunk alcohol and was not significant in the study.

- Regarding the analysis of the risk of perinatal mortality, the authors wrote that malaria poses a risk (page 11 line 23); though, their analysis (table 5) shows no risk of perinatal mortality in women with malaria during pregnancy (RRa 1.02, 95%CI 0.26-4.01, p-value 0.983). Please explain this conclusion.

Author's Response
True, thank for pointing that out, it was an honest mistake. It has been corrected below and, in the manuscript,

"This study found prenatal malaria to be significantly related with preterm birth and LBW after adjusting for parity, maternal age, G6PD, SES, neonatal admissions at birth, and non-significantly associated with perinatal mortality after adjusting for caesarian section."

• Please explain, for each analysis, the rationale to choose some confounders and not others to adjust the relative risk as it is not clear.

Author's Response
Further explanation has been added below and revised in the manuscript

For each models, we set out to adjust potential confounders such as maternal age at birth, Neonatal admissions at birth, mode of delivery, marital status, parity, G6PD, genotype, anemia, Socio Economic Status, drinking of alcohol, and respiratory conditions, and initially added toogistic regression with significance set 0.05%. Those with significant relation with outcome were retained in the model, non-significant ones were dropped.

Therefore genotype, anemia, respiratory condition and drinking of alcohol were all dropped during the initial univariate analysis, and that how some were found in the final models while others were not. Maternal age was however non-significant, but was retained in the models, given its relevance as a confounder and its association with the studied adverse birth outcomes. (Ogawa K et al, 2017)

• Tables need to be proofread. Table 1 has a repetition of malaria data, parity must be under medical history, and whenever abbreviations are used, these must be stated in tables footnotes.

Author's Response
Tables has been proofread and parity moved to medical history, and abbreviation stated in table footnotes

5. Discussion: Page 12, lines 5-12 should be re-written as that information is duplicated. Replace "placental site malaria" with "placental malaria". Also, malaria diagnosis based only on RDT testing is a limitation of the study.

Author's Response
Duplication deleted and "placental malaria" has been replaced with "placental site malaria" as recommended.

Yes, indeed is a limitation of the study, and is captured in discussion part in page 13.

Reviewer 2

Comment 1.

The introduction is extremely lengthy with no aims or purposes, its repetitively. I suggest that you to provide reader a more concise introduction and remove unnecessary information such as the first paragraph of the introduction. Consider to explain why MiP is an important health issue, how MiP affects infant and maternal outcomes. Mention what is special about MiP in the third trimester to better suit your study.

Author's Response.

First Paragraph deleted as recommended, and additional explanation added as recommended in paragraph 1 and 2 in page 4.

Comment 2
Malaria parasite – this subheading is incorrect. RDT was used to determine whether participants had parasitaemia, the ensuing description is unnecessary. Consider to remove or to replace with more concise information.
Author's Response
Sub heading revised to read "RDT malaria Diagnosis" and preceding description revised based on reviewers' suggestions.
Comment 3
Haemoglobin estimation – why is this necessary when nothing was mentioned in the subsequent text?

Author's Response
Hemoglobin estimation was used to determine anemia, which was used to assess confounding effect in a univariate analysis but was found to be non-significant and hence was dropped in the multiple log binomial regression model. Anemia is now mentioned as part of confounders that were non-significant and dopped in the statistical analysis section.
Comment 4
I do not think log binomial regression model is appropriate. This reports relative risk, you reported odds ratio, it should be a logistic regression model. What were the individual covariates added? It would be good to include to provide better understanding for readers. "Some adjusted confounders included…" firstly covariate and confounders cannot be used interchangeably, this statement suggests you have a lot of other variables added into the model there were not listed, any reason why not to list? Why is SP usage not included? Lastly, PM in pregnancy, the most important variable was not included in the adjusted model?
Author's Response
The choice of RR risk because there was malaria exposure before outcome, and in order to be sure the result is in the same direction, Table 6 indicates a sensitivity analysis using logistics regression that was done and found similar results. Nevertheless, the analysis has been done using logistic regression as recommended
Potential confounders (maternal age at birth, Neonatal admissions at birth, mode of delivery, marital status, parity, g6pd, genotype, anemia, Socio Economic Status, drinking of alcohol, and tuberculosis) were initially added to log binomial regression with significance set 0.05%, for each of the models, confounders with significance were retained in the model, non-significant ones were dropped. Maternal age was however non-significant, but was retained in the models, given the its relevance as a confounder and its association with the studied adverse birth outcomes
The SP variable was collected and was realized that almost all the women were put under IPTp, except for those who were G6PD deficient, it was not significant in univariate model so we dropped. This limitation could over or under estimate the odds of exposure, i.e., malaria
"The question was whether the pregnant woman had taken at least one SP, that is a yes or no question"
Comment 5
Ethics should be mention in the methods
Authors Response
Based on the publication format I reviewed, ethics is positioned under discussions.

Comment 6
Table 1, malaria was reported twice
Author's Response
I have checked and Table1 appeared only once, except it is longer and took more than one page.

Results section:
Comment 7

This is poorly written with unnecessary information. Perhaps structure into subheadings. What is the purpose of reporting education, employment, rich etc? the overall aim is to looked into PM and birth outcomes. Consider listing things at are associated with PM, eg gravidity, numbers of PM positive etc.

Author's Response

Revised to include only relevant information especially for Table 1.

Comment 8

Table 5, caesarean section had higher risk of mortality, this was not reported, any reason why this was excluded?

Author's Response

No reason, it was an omission, it has been included in the results, thanks for the intervention

Comment 9

Table 6, I do not understand the purpose of this test. Should be made clear early on why RR was used instead of OR. I believe OR in this case is more appropriate.

Author's Response

Analysis was done using both logistic Regression and log binomial regressions to see if it will produce similar results. Therefore, we presented the logistic regression for preterm and malaria. And it did produce similar results in the same positive direction.

Discussion

Comment 10

The discussion needs to be more focused on the aims of this study, which is to explore, PM in the third trimester and poor birth outcomes. The main findings is PM increased the risk of poor birth outcomes, it would be appropriate to discuss why and what are the possible mechanisms behind this. It is not helpful to cite studies on vivax, comparing of different techniques etc.

Author's Response

The p. Vivax citation has been deleted and more literature regarding trimester PM added. Regarding the mechanism behind the poor birth outcomes, it has been discussed in page 13 part of the discussion. Different techniques were just emphasized their similarities to result whilst explaining mechanism behind poor birth outcomes. Below are some literatures to that effect

Third trimester malaria increased risk of preterm birth by five times and low birth weight by 2.8 times. (Nkwabong, 2020), resonated with our study.

Moreover, the effect of malaria exposure on fetal growth was observed during third trimester of pregnancy regardless of period the exposure and has been blamed for poor birth outcomes. Reason being that the pathway that connects mother to the child during pregnancy may influence the survival of the fetus at birth or even beyond, since the placenta supplies nutrients to the baby through the umbilical cord. Thus, Ouédraogo and colleagues found a significant association between umbilical cord parasitemia level and maternal peripheral blood parasitemia.(28) Also, malaria in pregnancy may have been induced excessive stimulation and dysregulated hemoglobin-scavenging system; and bioavailability of nitric oxide and L-arginine which may be associated poor vascular development and adverse birth outcomes. Although we used RDT with peripheral blood, our findings were consistent with the majority of studies using placental site malaria.(26,27) This could be because peripheral blood infections could promote parasite sequestration in the placenta and activate antibody-antigen immune responses, which can cause complications during delivery.(26,27).

Comment 11

Minor:

Malaria is a disease, not an infection, thus malaria infection is incorrect. This should be updated throughout the manuscript.

Author's Response

Correction effected throughout the manuscript.

Comment 12

"They further had 1.93 times (1.11-63.41)" – is the 95CI correct? It seems relatively huge.

Author's Response
Thank you. It was incorrect, correction effected
Comment 13
"Briefly, for this study…." Remove "for this study"
'for this study' has been removed
all pregnant women given SP, how many courses?" good to include or even provide what is the take up rate during pregnancy.
Author's Response
The SP variable was collected and was realized that almost all the women were put under IPTp, except for those who were G6PD deficient, it was not significant in univariate model so we dropped. This limitation could over or under estimate the odds of exposure, i.e., malaria.
"The question was whether the pregnant woman had taken at least one SP, that is a yes or no question"
This is added to Table 1

VERSION 2 – REVIEW

REVIEWER	Gonçalves, Lígia Antunes Instituto Gulbenkian de Ciencia
REVIEW RETURNED	25-Apr-2022

GENERAL COMMENTS	The authors have adequately addressed most of the comments that improved the quality of the manuscript; nevertheless, some points still need to be clarified before publication to raise the quality of the manuscript: 1. The manuscript needs to be proofread and edited; re-wording a couple of paragraphs could refine some of the ideas presented in the manuscript, mainly in the Discussion section. 2. Abbreviations must be spelled out. In the Introduction section: "ISTp" and "IPTp" are not spelled out whenever were introduced. 3. Results section: a. The authors detailed the study setting as requested, but it is still unclear whether the region has differences in malaria parasite transmission according to the dry or rainy season. This is particularly important, as depending on each women third trimester, it could be a bias for Plasmodium infections. Moreover, it is unclear if the prevalence indicated refers to pregnant women or the general population, as the text in "Response to Comments" differs from "Revised manuscript marked". b. From the text added to the Methods section, "This increased our initial sample size from 1472 published in (15) to 1776…" it is not clear how many women were followed in this study. This should be clear in the Methods section. c. In the "Response to Comments" the authors wrote that only 88% were under IPTp, but in the Methods section, they wrote that all, except those with G6PD deficiency, were under IPTp. This is not clear as only 4.7 were G6PD deficient. d. Although the authors have now introduced the information regarding RDT screening, it still lacks the information on which time points during the 3rd trimester the women were tested and how many times. Also, were all negative when enrolled in the study? 4. Results section: a. According to WHO guidelines, it is only considered severe anemic when hemoglobin is under 7 g/dL (2nd paragraph).

	b. In the sentence "Lastly, pregnant women with malaria had 1.02 times non-significant odds [CI: 95% (0.26 – 4.01)…" it could mislead us into understanding the existence of differences that are non-statistical, which does not apply. c. In Table 3, which model was used, binomial regression as in the title or logistic regression? 5. Discussion section: a. It is long and verbose. The text needs to be re-written. c. It was asked by me to replace "placenta site malaria" with "placenta malaria", as the two were used interchangeably. The authors replaced "placenta malaria" with "placenta site malaria". Placental malaria is defined as the presence/accumulation of Plasmodium-infected red blood cells in the placental. Therefore, the correct term is “placental malaria”.

REVIEWER	Gonçalves, Lígia Antunes Instituto Gulbenkian de Ciencia
REVIEW RETURNED	25-Apr-2022

GENERAL COMMENTS	The authors have adequately addressed most of the comments that improved the quality of the manuscript; nevertheless, some points still need to be clarified before publication to raise the quality of the manuscript: 1. The manuscript needs to be proofread and edited; re-wording a couple of paragraphs could refine some of the ideas presented in the manuscript, mainly in the Discussion section. 2. Abbreviations must be spelled out. In the Introduction section: "ISTp" and "IPTp" are not spelled out whenever were introduced. 3. Results section: a. The authors detailed the study setting as requested, but it is still unclear whether the region has differences in malaria parasite transmission according to the dry or rainy season. This is particularly important, as depending on each women third trimester, it could be a bias for Plasmodium infections. Moreover, it is unclear if the prevalence indicated refers to pregnant women or the general population, as the text in "Response to Comments" differs from "Revised manuscript marked". b. From the text added to the Methods section, "This increased our initial sample size from 1472 published in (15) to 1776…" it is not clear how many women were followed in this study. This should be clear in the Methods section. c. In the "Response to Comments" the authors wrote that only 88% were under IPTp, but in the Methods section, they wrote that all, except those with G6PD deficiency, were under IPTp. This is not clear as only 4.7 were G6PD deficient. d. Although the authors have now introduced the information regarding RDT screening, it still lacks the information on which time points during the 3rd trimester the women were tested and how many times. Also, were all negative when enrolled in the study? 4. Results section: a. According to WHO guidelines, it is only considered severe anemic when hemoglobin is under 7 g/dL (2nd paragraph). b. In the sentence "Lastly, pregnant women with malaria had 1.02 times non-significant odds [CI: 95% (0.26 – 4.01)…" it could mislead us into understanding the existence of differences that are non-statistical, which does not apply. c. In Table 3, which model was used, binomial regression as in the title or logistic regression? 5. Discussion section:

	a. It is long and verbose. The text needs to be re-written. c. It was asked by me to replace "placenta site malaria" with "placenta malaria", as the two were used interchangeably. The authors replaced "placenta malaria" with "placenta site malaria". Placental malaria is defined as the presence/accumulation of Plasmodium-infected red blood cells in the placental. Therefore, the correct term is “placental malaria”.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Dr. Andrew Teo, The University of Melbourne

Comments by the Author:

Comments 1

*Typo -Malaria cases increased by half a million in Ghana in 2018 compared to the year before.(5)Regarding treatment , a

Response # 1

Corrected and revised in the manuscript

Comments #2

*Spell out IST and IPT in first instance

Response # 2

Corrected as recommended below

“intermittent screening and treatment of malaria in pregnancy (ISTp) and intermittent preventive treatment of malaria in pregnancy (IPTp)”

Comment # 3

*Check on formats

Response # 1

Formatted as recommended

Reviewer: 1

Dr. Lígia Antunes Gonçalves, Instituto Gulbenkian de Ciencia

Comments by the Author:

The authors have adequately addressed most of the comments that improved the quality of the manuscript; nevertheless, some points still need to be clarified before publication to raise the quality of the manuscript:

Comment #1

1. The manuscript needs to be proofread and edited; re-wording a couple of paragraphs could refine some of the ideas presented in the manuscript, mainly in the Discussion section.

Response # 1

The manuscript has been proofread and parts of the discussion rewritten

Comment # 2

2. Abbreviations must be spelled out. In the Introduction section: "ISTp" and "IPTp" are not spelled out whenever they were introduced.

Response # 2

Corrected as recommended below

“intermittent screening and treatment of malaria in pregnancy (ISTp) and intermittent preventive treatment of malaria in pregnancy (IPTp)”

3. Results section:

Comment # 3

a. The authors detailed the study setting as requested, but it is still unclear whether the region has differences in malaria parasite transmission according to the dry or rainy season. This is particularly important, as depending on each women third trimester, it could be a bias for Plasmodium infection. Moreover, it is unclear if the prevalence indicated refers to pregnant women or the general population, as the text in "Response to Comments" differs from "Revised manuscript marked".

Response # 3

There is a little seasonal variation within the northern region and hence we do not think it can bias the study as far as Plasmodium infections are concerned. Also, the prevalence indicated refers to pregnant women, it was inadvertently missing in the response to the comments section. The one in the revised version suffices and indicated below and revised in the method section.

“These areas are located within the Guinea Savannah belt,(11) with little seasonal variations in prevalence such as Oheneba-Dornyo and collegues found the prevalence of malaria to be positively correlated with rainfall with almost borderline significance (12) Again, P. falciparum peripheral parasitemia prevalence in pregnant women in Northern Savanna Zone ranged between 26% and 13.4% from 2013 to 2019, respectively. (12)”

Comment #4

b. From the text added to the Methods section, "This increased our initial sample size from 1472 published in (15) to 1776…" it is not clear how many women were followed in this study. This should be clear in the Methods section.

Response #4

We have amended the manuscript as indicated below

“This increased our initial sample size from 1472 published in (15) to 1776, consequently, we followed up 1323 pregnant women in this study, more details can be found in. (13)”

Comment # 5

c. In the "Response to Comments" the authors wrote that only 88% were under IPTp, but in the Methods section, they wrote that all, except those with G6PD deficiency, were under IPTp. This is not clear as only 4.7 were G6PD deficient.

Response #5

Thank you for this intervention, the manuscript has been corrected and indicated below

“Only 88% received at least one sulfadoxine / pyrimethamine (IPTp-SP)”

Comment # 6

d. Although the authors have now introduced the information regarding RDT screening, it still lacks information on which time points during the 3rd trimester the women were tested and how many times. Moreover, were all negative when enrolled in the study?

Response #6

Unfortunately, we relied on the RDT test conducted by the health facilities, in our part of the world, the women decide when to come to the clinic during the third trimester, and so it was difficult to collect the data at a specified time points for each woman. Each woman, however did the RDT test during the third trimester, but they usually frequent the ANC center few weeks to delivery or at delivery.

Comment # 7

4. Results section:

a. According to WHO guidelines, it is only considered severe anemic when hemoglobin is under 7 g/dL (2nd paragraph).

Response #7

Thank you, the correction has been revised in the manuscript to read

“About 47.9% of the women were anaemic with haemoglobin levels of less than 11 g/dl within their third trimester of pregnancy”

Comment # 8

b. In the sentence "Lastly, pregnant women with malaria had 1.02 times non-significant odds [CI: 95% (0.26 – 4.01) …" it could mislead us into understanding the existence of differences that are non-statistical, which does not apply.

Response #8

Thank you, it has been amended as indicated below and in the revised manuscript

Lastly, with the odds of 1.02 [CI: 95% (0.26 – 4.01), there was no significant difference between pregnant women with malaria and those without malaria) for perinatal mortality after adjusting for cesarean section.

Comment # 9

c. In Table 3, which model was used, binomial regression as in the title or logistic regression?

Response #9

It was, Logistic regression, the title has been corrected in the revised manuscript and below

"Table 3: Logistic regression of preterm and malaria"

5. Discussion section:

Comment # 10

a. It is long and verbose. The text needs to be re-written.

Response #10

Parts of discussion rewritten and unnecessary sentences removed

Comment # 11

c. It was asked by me to replace "placenta site malaria" with "placenta malaria", as the two were used interchangeably. The authors replaced "placenta malaria" with "placenta site malaria". Placental

malaria is defined as the presence/accumulation of Plasmodium-infected red blood cells in the placental. Therefore, the correct term is “placental malaria”.

Response #11

“Placental site malaria” is been replaced with “placental malaria” in pages 11, 12 and 13 in the revised manuscript

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COI statements:

Reviewer: 2

Competing interests of Reviewer: NO competing interests

Reviewer: 1

Competing interests of Reviewer: No competing interests.

VERSION 3 – REVIEW

REVIEWER	Gonçalves, Lígia Antunes Instituto Gulbenkian de Ciencia
REVIEW RETURNED	27-Jun-2022

GENERAL COMMENTS	The manuscript "Prenatal malaria exposure and the risk of adverse birth outcomes: a cohort of pregnant women in from Northern Region of Ghana" by Hussein and colleagues is an interesting work that may shed some light on malaria's impact on pregnant women and their newborns living in the Northern region of Ghana. I would like to state that the authors have adequately addressed most of the comments, and I am satisfied with the quality of the revised manuscript. Nevertheless, understanding the constraints of the region, the methods section should state that the RDT test was performed during the third trimester whenever possible.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1
Dr. Lígia Antunes Gonçalves, Instituto Gulbenkian de Ciencia

Comments to the Author:
The manuscript "Prenatal malaria exposure and the risk of adverse birth outcomes: a cohort of pregnant women in from Northern Region of Ghana" by Hussein and colleagues is an interesting work that may shed some light on malaria's impact on pregnant women and their newborns living in the Northern region of Ghana. I would like to state that the authors have adequately addressed most of the comments, and I am satisfied with the quality of the revised manuscript. Nevertheless, understanding the constraints of the region, the methods section should state that the RDT test was performed during the third trimester whenever possible.

Response to comment #1
We agree with reviewer and amended the RDT malaria diagnosis subheading in method section to read

“RDT test was performed during the third trimester whenever possible to determine whether participants had parasitemia in peripheral blood”

Reviewer: 1
Competing interests of Reviewer: No competing interests.