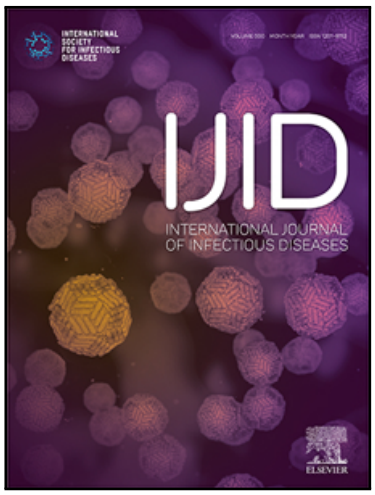
Journal Pre-proof



Associations Between Malaria in Pregnancy and Neonatal Neurological Outcomes

Harriet L.S. Lawford MSc, PhD , Mercy A. Nuamah MD, PhD , Helen G. Liley MBChB, FRACP , Alison Griffin MBiostats , Cecilia E. Lekpor PhD , Felix Botchway PhD , Samuel A. Oppong MBChB, FWACS , Ali Samba MBChB, FWACS , Ebenezer V. Badoe MBChB, FGCP , Sailesh Kumar MBBS, FRANZCOG, DPhil , Anne CC Lee MD, MPH , Richard K. Gyasi MBChB, FWACP , Andrew A. Adjei PhD , Samudragupta Bora PhD

PII: S1201-9712(21)00593-2
DOI: https://doi.org/10.1016/j.ijid.2021.07.037
Reference: IJID 5573

To appear in: *International Journal of Infectious Diseases*

Received date: 3 April 2021
Revised date: 13 July 2021
Accepted date: 14 July 2021

Please cite this article as: Harriet L.S. Lawford MSc, PhD , Mercy A. Nuamah MD, PhD , Helen G. Liley MBChB, FRACP , Alison Griffin MBiostats , Cecilia E. Lekpor PhD , Felix Botchway PhD , Samuel A. Oppong MBChB, FWACS , Ali Samba MBChB, FWACS , Ebenezer V. Badoe MBChB, FGCP , Sailesh Kumar MBBS, FRANZCOG, DPhil , Anne CC Lee MD, MPH , Richard K. Gyasi MBChB, FWACP , Andrew A. Adjei PhD , Samudragupta Bora PhD , Associations Between Malaria in Pregnancy and Neonatal Neurological Outcomes, *International Journal of Infectious Diseases* (2021), doi: https://doi.org/10.1016/j.ijid.2021.07.037

Associations Between Malaria in Pregnancy and Neonatal Neurological Outcomes

Malaria in Pregnancy and Neonatal Neurological Outcomes

Harriet L.S. Lawford[a], MSc, PhD; Mercy A. Nuamah[b], MD, PhD; Helen G. Liley[a], MBChB, FRACP; Alison Griffin[c], MBiostats; Cecilia E. Lekpor[d], PhD; Felix Botchway[e], PhD; Samuel A. Oppong[b], MBChB, FWACS; Ali Samba[b], MBChB, FWACS; Ebenezer V. Badoe[f], MBChB, FGCP; Sailesh Kumar[a], MBBS, FRANZCOG, DPhil; Anne CC Lee[g], MD, MPH; Richard K. Gyasi[d], MBChB, FWACP; Andrew A. Adjei[d], PhD; & Samudragupta Bora[a,*], PhD, samudragupta.bora@mater.uq.edu

[a]Mothers, Babies and Women's Health Program, Mater Research Institute, Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia

[b]Department of Obstetrics and Gynaecology, University of Ghana Medical School, Korle Bu, Accra, Ghana

[c]Statistics Unit, QIMR Berghofer Medical Research Institute, Brisbane, QLD Australia

[d]Department of Pathology, University of Ghana Medical School, Korle Bu, Accra, Ghana

[e]Central Diagnostic Laboratory, Korle Bu Teaching Hospital, Korle Bu, Accra, Ghana

[f]Department of Child Health, University of Ghana Medical School, Korle Bu, Accra, Ghana

[g]Department of Pediatric Newborn Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

[*]Address correspondence to: Mothers, Babies and Women's Health Program, Mater Research Institute, Raymond Terrace, Brisbane, QLD 4101, Australia.

Highlights

1. Prospective study of *in utero* malaria exposure and neonatal neurological function.
2. *In utero* malaria exposure may increase risk of suboptimal reflex in term neonates.
3. Impact of *in utero* malaria exposure on child neurodevelopment must be established.

Abstract

Objective: To compare neurological functioning of neonates born to mothers with and without malaria in pregnancy.

Methods: Pregnant women presenting at Korle Bu Teaching Hospital, Ghana were recruited into this prospective observational study. Malaria exposure was determined by clinically-documented antenatal malaria infection; parasitemia in maternal, placental, or umbilical cord blood; or placental histology. Neurological functioning was assessed using the Hammersmith Neonatal Neurological Examination within 48 hours of birth. Performance was classified as “optimal” or “suboptimal” by subdomain and overall.

Results: Between 21st November 2018 and 10th February 2019, 211 term-born neonates, of whom 27 (13%) were exposed to malaria, were included. In the reflexes subdomain, exposed neonates tended to score lower (adjusted mean difference: -0.34, 95% CI: -0.70–0.03) with increased risk (adjusted risk ratio: 1.63, 95% CI: 1.09–2.44) of suboptimal performance compared to unexposed neonates. There were no significant between-group differences in scores or optimality classification for the remaining subdomains and overall.

Conclusion: Malaria-exposed neonates had similar neurological functioning relative to unexposed neonates, with differences confined to the reflexes subdomain, suggesting potential underlying neurological immaturity or injury. Further studies are needed to confirm these findings and determine the significance of malaria in pregnancy on long-term neurological outcomes.

Keywords

Brain; Infant; Malaria; Neurodevelopment; Sub-Saharan Africa

Abbreviations: HNNE: Hammersmith Neonatal Neurological Examination; IPTp-SP: Intermittent preventative treatment in pregnancy using sulfadoxine-pyrimethamine

Associations Between Malaria in Pregnancy and Neonatal Neurological Outcomes

During pregnancy, naturally acquired immunity to malaria is compromised and pregnant women in endemic regions are at higher risk of malaria infection than their nonpregnant peers (Doolan et al., 2009). It is well-established that malaria in pregnancy is associated with adverse pregnancy outcomes (including miscarriage and stillbirth) (Saito et al., 2020) and maternal and fetal/neonatal complications including malarial anemia, fetal growth restriction, preterm birth, and low birthweight (Rogerson, 2017). Approximately 11 million pregnant women in sub-Saharan Africa were infected with malaria in 2018 resulting in 16% of all low birthweight deliveries in the region (World Health Organization, 2019). While the adverse neurodevelopmental outcomes of children who have suffered from cerebral malaria during childhood have been extensively investigated (Carter et al., 2004, Idro et al., 2010), relatively little is known regarding the impact of malaria in pregnancy on neonatal neurological outcomes. Published reviews have theorized that malaria exposure can impair fetal neurological development and subsequent neurodevelopment (Lawford et al., 2019, McDonald et al., 2013); a number of socioenvironmental and biological pathways are hypothesized to be involved, which we recently summarized in a conceptual framework (Lawford et al., 2019).

Human and animal studies suggest some neurological impact of malaria exposure in pregnancy. Cerebral blood flow redistribution (Arbeille et al., 1998) and faster development in the cingulate gyrus (Rijken et al., 2012) have been documented in fetuses in response to maternal malaria infection, while neurocognitive deficits are evident in the offspring of malaria-infected mice relative to uninfected mice (McDonald et al., 2015). However, only one study to date has reported the neurodevelopmental impact of malaria-exposure among infants. This case-report investigated neurodevelopmental outcomes at 12 and 24 months postpartum in dizygotic twins whose placentas were discordant for parasitemia; the placental malaria-exposed twin demonstrated consistently lower motor, cognitive, and language scores relative to the unexposed twin at both time points (Conroy et al., 2019). However, there was marked discordance in fetal growth with the malaria-exposed twin exhibiting lower birthweight (1,320 g vs.

1,920 g) and head circumference (27 cm vs. 32 cm). As neurodevelopmental disadvantage has previously been reported in the smaller twin of discordant twin pairs regardless of malaria status (Halling et al., 2016), it is unclear whether the neurodevelopmental outcomes reported occurred as a component of the pathophysiology of malaria or was an independent confounder.

To date, no studies have reported neurological functioning of neonates exposed to malaria *in utero*. We conducted a prospective observational study to compare the neurological functioning of neonates ≤48 hours of age born to mothers with and without malaria in pregnancy. We hypothesized that exposure to malaria in pregnancy adversely affects neonatal neurological functioning.

Methods

Sample

The Impact of Malaria in Pregnancy on Infant Neurodevelopment (IMPRINT) study was a prospective observational study conducted at Korle Bu Teaching Hospital in Accra, Ghana. This is the largest tertiary teaching hospital in Ghana and the leading regional referral center, with additional referrals from primary and secondary health facilities in the southern region. It has a catchment population of >3 million in an area of 50 km radius (Adu-Bonsaffoh et al., 2017) and approximately 10,000 live births annually.

Six physicians were recruited and trained to perform study assessments. Pregnant women presenting in the early stages of labor were approached for written informed consent. If granted and a member of the study team was available, neonates that met eligibility criteria were enrolled. Women were not approached if they were <15 years of age, HIV-positive, or had sickle cell disease. A nested sample of singleton neonates was selected for this study by further excluding those who were 1) born preterm or post-term (<37+0 or >42+6, weeks + days gestation), 2) had an Apgar score <7 at 5 minutes, 3) any

recorded admission to the Neonatal Intensive Care Unit, and 4) any recorded diagnosis of congenital anomalies. Ethical approval was obtained from institutional review boards of the University of Ghana and The University of Queensland, Australia.

Malaria Diagnosis

Malaria infection during pregnancy was the primary exposure measured as a binary variable. At Korle Bu Teaching Hospital, pregnant women are routinely tested for malaria at their antenatal visits. If tested positive, women were treated as per the national malaria treatment guidelines for pregnant women. A neonate was classified to be in the "exposed" group if they met one or more of the following conditions: 1) medical records of antenatal malaria infection confirmed by Rapid Diagnostic Test (RDT) or microscopy; 2) positive maternal, placental, or umbilical cord blood samples tested by RDT and/or microscopy; or 3) placental histology. Supplementary File–Appendix 1 further describes how malaria was diagnosed in the "exposed" group.

Neurological Evaluation

The primary outcome was performance on the Hammersmith Neonatal Neurological Examination (HNNE). The HNNE can identify neonates at risk of neurological dysfunction and later neurodevelopmental impairment (Dubowitz et al., 1984, Molteno et al., 1995, Molteno et al., 1999, Setanen et al., 2016, Tuhkanen et al., 2019), and exhibits good sensitivity (88%) to identify significant neuropathology detected by magnetic resonance imaging (Woodward et al., 2004). The HNNE has a total of 34 items stratified into six subdomains: tone, tone patterns, reflexes, movements, abnormal signs/patterns, and orientation and behavior. A scoring system was developed in 1998 based on reference values from a low-risk, term-born sample of 224 British neonates (Dubowitz et al., 1998). This scoring system allows the classification of neonates' performance as "optimal" or "suboptimal" by each subdomain and overall. A score >10th centile of reference values is considered optimal. The HNNE

administration and scoring have been described in detail in the original publication (Dubowitz et al., 1998).

The HNNE was administered to all neonates in the IMPRINT study (irrespective of inclusion in this nested sample) ≤48 hours after birth by trained physicians in the postnatal ward using the standardized assessment proforma (Dubowitz et al., 1998). Details regarding physician training for this study have been described in previous publication (Lawford Harriet LS et al., 2020, Lawford H. L. S. et al., 2020). Examiners were not routinely blinded to gestational age at birth but were blinded to malaria status.

Sociodemographic, Clinical, and Placental Characteristics

Sociodemographic information was collected using a standardized questionnaire administered when participants were not in active labor and following birth. Maternal and neonatal clinical data were extracted from medical records, and the placenta was characterized by examination. Further details are described in Supplementary File–Appendix 1.

Statistical Analysis

Differences in sociodemographic, clinical, and placental characteristics between included and excluded neonates, and malaria-exposed and unexposed neonates were described as mean ± standard deviation (SD), median [interquartile range], or n (%), and were tested using Student's *t*-test or Mann-Whitney U test for continuous data and χ^2 or Fisher's exact tests for categorical data. The association between malaria exposure and mean raw scores for the six HNNE subdomains and overall were assessed using linear regression and standardized effect sizes were reported as Cohen's d values. The association between malaria exposure and the proportion of neonates classified as suboptimal for the HNNE subdomains and overall was assessed using a Poisson regression with robust error variance. Multivariable models were adjusted for covariates determined by our previously published conceptual framework (Lawford et al., 2019), summarized in a qualitative causal model designed using

www.dagitty.net (Supplementary File–Appendix 2). The selected covariates are shown in red (socioeconomic status, education, maternal age, and social risk). There was no adjustment for covariates on the causal pathway (shown in green). Measures of association were expressed as unadjusted and adjusted mean differences and risk ratios. Statistical analysis was conducted using Stata 16.0 (Stata Corp, College Station, TX) and a significance level of .05 was used throughout inferential analysis.

Results

Figure 1 displays the sample recruitment. Between 21st November 2018 and 10th February 2019, a total of 302 mothers and 310 (8 twin births) neonates were recruited. In total, 36/310 neonates met study criteria for exposure to malaria in pregnancy. The HNNE was administered to 296/310 neonates (34/36 exposed to malaria and 262 unexposed) within 48 hours of birth. Of the 14 neonates that were not administered the HNNE, eight were lost to follow-up, five were too unwell, and there was one neonatal death.

After exclusion of 7 exposed and 78 unexposed neonates that did not meet the criteria for this nested sample, the study sample comprised 211 eligible neonates of whom 27 (13%) were exposed to malaria. Demographic and clinical characteristics of included mother-neonate dyads (n=211) and dyads that either did not have the HNNE administered (n=14) or did not meet the inclusion criteria (n=85) are compared in Supplementary File–Appendix 3 and Appendix 4, respectively.

Characteristics of Mother-Neonate Dyads

Table 1 displays sociodemographic, clinical, and placental characteristics of the 211 included mother-neonate dyads by malaria exposure. Compared with mothers of unexposed neonates, significantly more mothers of exposed neonates had no other children (p=.003) and lived in overcrowded dwellings with >1 person per room (p=.03). Mothers of exposed neonates had a smaller average middle-upper arm

circumference compared with mothers of unexposed neonates (p=.03). Significant differences were evident in the timing of first intermittent preventative treatment in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP); while the majority of mothers of exposed and unexposed neonates took their first IPTp-SP dose in the first/second trimester, fewer mothers of exposed neonates took no IPTp-SP but more took their first IPTp-SP in the third trimester relative to mothers of unexposed neonates (p=.03). Mothers of exposed and unexposed neonates did not differ significantly for the remaining sociodemographic or maternal clinical variables, and exposed and unexposed neonates did not differ significantly with regards to clinical or placental characteristics.

Of the 27 mothers who had malaria in pregnancy, 14 of 27 (52%) had active malaria infection at birth (positive RDT and/or blood smear). The timing and type of antimalarial treatment for these cases was not recorded. There were five cases of past-chronic placental infection and one case of active-chronic placental infection. Eleven (41%) mothers had evidence of malaria infection from medical records; of these, two were in the first trimester, two in the second trimester, and three in the third trimester. Timing of infection was not recorded for four infections.

Neurological Functioning of Neonates

Unadjusted and adjusted mean differences in raw scores on the six HNNE subdomains and overall were similar for exposed and unexposed neonates (Table 2). However, in both unadjusted and adjusted models exposed neonates tended to score lower on the reflexes subdomain (adjusted mean difference -0.34, 95% CI: -0.70–0.03)**.**

As shown in Table 3, a large proportion of neonates were considered to be demonstrating “suboptimal” performance [using the original British scoring thresholds (Dubowitz et al., 1998)] by HNNE subdomain: 67% for tone, 67% tone patterns, 37% reflexes, 82% movements, 61% abnormal signs/patterns, 75% orientation and behavior, and 95% overall. In the reflexes subdomain, significantly more neonates

exposed to malaria scored suboptimally than unexposed neonates (55.6% vs. 34.3%; adjusted risk ratio 1.63, 95% CI: 1.09–2.44). There were no significant differences between exposed and unexposed neonates in the risk of suboptimal scores for tone, tone patterns, movements, abnormal signs/patterns, or orientation and behavior. Finally, the association between scoring suboptimally by HNNE subdomain and overall was investigated separately for active (n=14) and past (n=13) malaria infection; however, no significant difference was evident.

Discussion

The objective of this study was to compare neurological functioning of malaria-exposed and unexposed neonates with a widely-used, validated, structured neurological assessment tool. Examining neonates prior to hospital discharge allowed us to assess the impact of malaria without the risk of confounding from subsequent exposure to family socioeconomic adversities and illnesses that may affect studies of outcomes in childhood. Further, assessing neonates within the first 48 hours of life has the advantage of allowing early detection of neurological abnormalities, which can lead to opportunities for targeted intervention.

We found that malaria-exposed neonates ≤48 hours of age had similar total HNNE scores to their unexposed peers. Interestingly, in the reflexes subdomain only, we found a statistically significant higher risk for suboptimal scores (which persisted after adjusting for socioeconomic status, education, maternal age, and social risk), although the mean difference in raw scores was small and did not reach statistical significance. There were no significant associations between malaria exposure and mean raw scores or suboptimal functioning in the tone, tone patterns, movements, abnormal signs/patterns, or orientation and behavior subdomains of the HNNE. Finding a significant difference in only one of the six HNNE subdomains could signify a selective effect on specific neurological function, but also raises the possibility that the finding was due to chance alone since no adjustment of statistical significance was

made for multiple comparisons. These findings may also be a result of the study being underpowered due to our small sample size, thus the study may not be adequately powered to detect patterns of malaria-related abnormality but might support the finding with the reflexes subdomain only. Although all statistical analyses were predetermined according to *a priori* hypotheses, we recognize the limitations on the certainty of the current findings and as such, we emphasize the preliminary nature of our findings and highlight that this study was designed for hypothesis generation.

If there is a true differential impact on primitive reflexes over tone, movements, and behavior, the mechanism and implications are uncertain. HNNE reflexes scores have been strongly associated with motor and cognitive outcomes in preterm-born infants assessed at 32 weeks postmenstrual age (George et al., 2021). Suboptimal reflex subdomain scores have also predicted poor neurodevelopmental outcomes, including lower mental and psychomotor development indices (Molteno et al., 1995, Sanchez et al., 2017) and structural brain abnormalities, including reduced biparietal diameter, increasing severity of cerebral white and gray matter abnormalities, and cerebellar abnormalities (Eeles et al., 2017, George et al., 2018, Sanchez et al., 2017, Woodward et al., 2004). Indeed, a recent study in Brazil reported reduced head circumference in neonates born to malaria-infected mothers (Dombrowski et al., 2017), however there was no intergroup difference in head circumference in our study. We can speculate that exposure to malaria in pregnancy results in adverse neurodevelopmental outcomes and/or subtle alterations in brain development. Unlike changes in gross brain structure, subtle changes would not manifest as differences in HNNE scores across all domains. However, without incorporating neurodevelopmental follow-up of exposed neonates, or including neuroimaging into our study, we cannot determine whether any such brain pathology or long-term neurological adversities exist in malaria-exposed infants.

An alternative explanation for why we found so little difference between malaria-exposed and unexposed neonates is the heterogeneity of malaria exposure in our sample and the lack of dense placental inflammatory response with pigmented monocytes that may be mitigating possible effects of malaria infection. An important pathway identified in our previously published conceptual framework was the role of maternal immune-inflammatory dysfunction and the downstream effects of inflammatory factors and the immune system on fetal brain development (Lawford et al., 2019). However, if there was only clinically mild malaria in our sample with little acute or chronic placental malaria infection, it is unlikely that heightened maternal immuno-inflammatory responses would occur, which would be responsible for impaired fetal brain development and subsequent neonatal neurological functioning. Possibly, replicating this study in a population with denser placental parasitization would find different results. However, this approach presents the serious ethical challenges common to other studies of "natural history" of disease, in that a duty of care would be owed to mothers participating in research to provide them with optimal treatment if malaria is diagnosed early in pregnancy. While there are no major ethical challenges around recruiting women with intense placental inflammation in the labor ward, as in this study, this does increase the challenge of determining the importance of timing of malaria infection on neurological outcomes.

A limitation of this study is that exposure to antimalarial treatment among women with active malaria at birth was not recorded. According to the standard treatment guidelines for malaria in Ghana, pregnant women are administered either artesunate + amodiaquine, artemether + lumefantrine or oral quinine for uncomplicated malaria in the second or third trimester(Ministry of Health & Ghana Health Services, 2014), all of which have a good safety profile. Maternal treatment could have reduced the impact of exposure to malaria on the neonate, biasing our study towards finding no difference between the groups (whereas a study of women without access to treatment might have shown differences).

However, if antimalarial drugs adversely affected the neonates' neurological function, we would have expected this to have exaggerated differences between the malaria-exposed and unexposed groups.

It is important to acknowledge that, despite being the largest study published to date investigating the impact of malaria in pregnancy on neonatal neurological functioning, our study may be underpowered given the small sample size (particularly the sample of neonates exposed to malaria in pregnancy). Given the small sample size (particularly in the malaria-exposed group) and the multiple comparisons in the study, we advise caution in interpreting statistical significance. It is possible that the finding of a difference in neonates meeting the threshold for suboptimal performance in only one of six subdomains is the result of a type 2 error. It is also possible that the increased risk of suboptimal reflexes seen in neonates exposed to malaria could be due to chance (a type 1 error), subtle biases, or unmeasured confounders. The adjusted mean difference between groups for raw scores for the reflexes subdomain was only about a third of a standard deviation and was not statistically significant. The difference we found may or may not be clinically significant and a much larger sample size might find subtle (and yet clinically significant) differences in other subdomains or in total HNNE scores that this study was too small to detect. Ultimately, longitudinal studies are needed to determine the significance of malaria exposure during pregnancy on childhood neurodevelopment, and to distinguish the effects of maternal malaria infection from concomitant comorbid conditions. This will allow an understanding of both the childhood impact of malaria in pregnancy and the specificity and predictive value of neurological assessments at birth in this context.

The HNNE was selected as the most appropriate neurological assessment tool for this study; it assesses neurological functioning at birth, has been widely used both in clinical and research contexts, has excellent (>96%) interrater reliability (Dubowitz et al., 1998) and has high predictive validity to identify structural brain abnormalities and later neurological dysfunction. However, the HNNE has only

277 infrequently been used for research in low- and middle-income countries and has not been validated or
278 standardized in Ghana. Therefore, we are hesitant to interpret Ghanaian neonates as performing
279 "suboptimally" using this (original British) scoring system without more extensive validation of the HNNE
280 in Ghana or follow-up of our sample to determine long-term neurological functioning. Because we are
281 unsure of the reasons why such a high proportion of Ghanaian neonates in the comparison group scored
282 suboptimally we also compared HNNE raw scores, but still found little difference between groups.

283 Based on the original HNNE scoring system established by Dubowitz *et al.* in 1998 (Dubowitz et al.,
284 1998), we would expect that ~10% of our unexposed comparison group would be scoring suboptimally.
285 However, we found a much higher proportion of unexposed neonates scored below the 10th centile
286 when the British scoring system was applied, but we are very uncertain about whether this indicates a
287 much higher baseline of adverse neurological functioning in term-born, malaria-unexposed neonates
288 specific to our study site. We have discussed possible reasons for these findings in both the IMPRINT
289 study (Lawford Harriet LS et al., 2020) and studies conducted in other low- and middle-income countries
290 [Thailand, Myanmar (McGready et al., 2000), Vietnam (Hieu et al., 2006), and Uganda(Hagmann et al.,
291 2015)], which have also reported differences from the original British norms. An important characteristic
292 of the study population that should be noted is the mode of delivery. Overall, 66% of deliveries were by
293 Caesarean section; as discussed in our previous work reasons for this could include the study setting
294 (Korle Bu Teaching Hospital is a tertiary referral hospital), or it could be a reflection of higher
295 socioeconomic status (Lawford Harriet LS et al., 2020). It is important to note that when neonates were
296 stratified by mode of delivery, there was no difference in total HNNE score between neonates delivered
297 by C-section vs. vaginally (25.3±3.7 vs. 25.0±3.7; P=0.52). Therefore, it is unlikely that C-section or the
298 use of postpartum analgesia impacted HNNE scores in this study. Nevertheless, we consider that any
299 confounding or bias in HNNE results in the Ghanaian setting caused by unmeasured comorbidities, test

conditions or conduct, or uncertainties in gestational age estimation should have applied equally to both arms of the current study, not just to the malaria-exposed group.

In conclusion, given the high burden of malaria infection in pregnancy, understanding whether *in utero* exposure to malaria adversely impacts neurological development is important. Our results suggest that a group of term-born neonates exposed to malaria in pregnancy (and whose mothers had generally received treatment) had HNNE scores similar to an unexposed comparison group born in the same hospital. However, we found a higher risk of suboptimal functioning in only the reflexes subdomain, which could be a result of malaria exposure in pregnancy.

Contributors' Statement

The corresponding author, Dr. Samudragupta Bora had full access to all of the study data and is primarily accountable for all aspects of the work, including the decision to submit for publication. The corresponding author, first author, and the statistical advisor, Ms. Alison Griffin verified all the reported data analysis.

Harriet L.S. Lawford conceptualized and designed the study protocol, coordinated data acquisition, performed data analyses, interpreted the results, drafted and revised the initial manuscript, and approved the final manuscript as submitted.

Mercy A. Nuamah designed the study protocol, coordinated and supervised data acquisition, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Helen G. Liley conceptualized the study, supervised data analyses, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Alison Griffin developed the statistical analysis plan, supervised preliminary data analyses, performed data analyses, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Cecilia E. Lekpor designed the study protocol, acquired data, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Felix Botchway designed the study protocol, acquired data, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Samuel A. Oppong supervised the designing of the study protocol, coordinated data acquisition, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Ali Samba supervised the designing of the study protocol, coordinated data acquisition, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Ebenezer V. Badoe supervised the designing of the study protocol, coordinated data acquisition, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Sailesh Kumar conceptualized the study, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Anne CC Lee conceptualized the study, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Richard K. Gyasi designed the study protocol, acquired data, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Andrew A. Adjei supervised the designing of the study protocol, coordinated data acquisition, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Samudragupta Bora acquired funds and resources, conceptualized the study, designed the study protocol, supervised data acquisition and data analyses, interpreted the results, critically reviewed and revised the initial manuscript, and approved the final manuscript as submitted.

Ethical Approval

The study protocol was approved by the Institutional Review Board/Human Research Ethics Committee of the University of Ghana and The University of Queensland, Australia.

Funding

This study was supported by a Mater Foundation Principal Research Fellowship to Dr. Samudragupta Bora and The University of Queensland Research Training Program and Frank Clair scholarships to Ms. Harriet L.S. Lawford. The funding sources had no role in the writing of the manuscript or in the decision to submit it for publication.

mmc1.pdf

Conflict of interest

The authors have no conflict of interest relevant to this study to disclose.

Acknowledgments

We acknowledge the contributions of Dr. Akomah Kennedy, Dr. Godwin A. Awuni, Dr. Newton E. Ofosu, Dr. Oyeronke S. Oyawoye, Dr. Temitope Akinyemi, Dr. Vida Akrasi-Boateng, and Mr. Hanson G. Nuamah at the University of Ghana for assistance with data collection. Most importantly, we would like to express our sincere gratitude to the children and their families who participated in the IMPRINT study.

References

Adu-Bonsaffoh K, Ntumy MY, Obed SA, Seffah JD. Perinatal outcomes of hypertensive disorders in pregnancy at a tertiary hospital in Ghana. BMC pregnancy and childbirth 2017;17(1):388.

Arbeille P, Carles G, Bousquet F, Body G, Lansac J. Fetal cerebral and umbilical artery blood flow changes during pregnancy complicated by malaria. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 1998;17(4):223-9.

Carter JA, Neville BG, White S, Ross AJ, Otieno G, Mturi N, et al. Increased prevalence of epilepsy associated with severe falciparum malaria in children. Epilepsia 2004;45(8):978-81.

Conroy AL, Bangirana P, Muhindo MK, Kakuru A, Jagannathan P, Opoka RO, et al. Case Report: Birth Outcome and Neurodevelopment in Placental Malaria Discordant Twins. The American journal of tropical medicine and hygiene 2019;100(3):552-5.

Dombrowski JG, de Souza RM, Lima FA, Bandeira CL, Murillo O, de Sousa Costa D, et al. Plasmodium falciparum infection during pregnancy impairs fetal head growth: prospective and populational-based retrospective studies. bioRxiv 2017:203059.

Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clinical microbiology reviews 2009;22(1):13-36.

Dubowitz L, Mercuri E, Dubowitz V. An optimality score for the neurologic examination of the term newborn. The Journal of pediatrics 1998;133(3):406-16.

Dubowitz LM, Dubowitz V, Palmer PG, Miller G, Fawer CL, Levene MI. Correlation of neurologic assessment in the preterm newborn infant with outcome at 1 year. The Journal of pediatrics 1984;105(3):452-6.

Eeles AL, Walsh JM, Olsen JE, Cuzzilla R, Thompson DK, Anderson PJ, et al. Continuum of neurobehaviour and its associations with brain MRI in infants born preterm. BMJ paediatrics open 2017;1(1):e000136.

George JM, Colditz PB, Chatfield MD, Fiori S, Pannek K, Fripp J, et al. Early clinical and MRI biomarkers of cognitive and motor outcomes in very preterm born infants. 2021:1-8.

George JM, Fiori S, Fripp J, Pannek K, Guzzetta A, David M, et al. Relationship between very early brain structure and neuromotor, neurological and neurobehavioral function in infants born <31weeks gestational age. Early human development 2018;117:74-82.

Hagmann CF, Chan D, Robertson NJ, Acolet D, Nyombi N, Nakakeeto M, et al. Neonatal neurological examination in well newborn term Ugandan infants. Early human development 2015;91(12):739-49.

Halling C, Malone FD, Breathnach FM, Stewart MC, McAuliffe FM, Morrison JJ, et al. Neuro-developmental outcome of a large cohort of growth discordant twins. European journal of pediatrics 2016;175(3):381-9.

Hieu NT, Gainsborough M, Simpson JA, Thuy NT, Hang NN, Taylor AM, et al. Neurological status of low-risk Vietnamese newborns: a comparison with a British newborn cohort. Journal of health, population, and nutrition 2006;24(1):57-63.

Idro R, Kakooza-Mwesige A, Balyejjussa S, Mirembe G, Mugasha C, Tugumisirize J, et al. Severe neurological sequelae and behaviour problems after cerebral malaria in Ugandan children. BMC research notes 2010;3(1):104.

Lawford HL, Nuamah MA, Liley HG, Lee AC, Kumar S, Adjei AA, et al. Neonatal neurological examination in a resource-limited setting: What defines normal? European Journal of Paediatric Neurology 2020.

Lawford HLS, Lee AC, Kumar S, Liley HG, Bora S. Establishing a conceptual framework of the impact of placental malaria on infant neurodevelopment. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2019;84:54-65.

Lawford HLS, Nuamah MA, Liley HG, Lee AC, Botchway F, Kumar S, et al. Gestational Age-Specific Distribution of the Hammersmith Neonatal Neurological Examination Scores Among Low-Risk Neonates in Ghana. Early human development 2020;152:105133.

McDonald CR, Cahill LS, Ho KT, Yang J, Kim H, Silver KL, et al. Experimental Malaria in Pregnancy Induces Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent Pathway. PLoS pathogens 2015;11(9):e1005140.

McDonald CR, Elphinstone RE, Kain KC. The impact of placental malaria on neurodevelopment of exposed infants: a role for the complement system? Trends in parasitology 2013;29(5):213-9.

McGready R, Simpson J, Panyavudhikrai S, Loo S, Mercuri E, Haataja L, et al. Neonatal neurological testing in resource-poor settings. Annals of tropical paediatrics 2000;20(4):323-36.

Ministry of Health & Ghana Health Services. Guidelines for Case Management of Malaria in Ghana. 3rd Edition ed2014.

Molteno C, Grosz P, Wallace P, Jones M. Neurological examination of the preterm and full-term infant at risk for developmental disabilities using the Dubowitz Neurological Assessment. Early human development 1995;41(3):167-76.

Molteno CD, Thompson MC, Buccimazza SS, Magasiner V, Hann FM. Evaluation of the infant at risk for neurodevelopmental disability. South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde 1999;89(10):1084-7.

Rijken MJ, de Wit MC, Mulder EJH, Kiricharoen S, Karunkonkowit N, Paw T, et al. Effect of malaria in pregnancy on foetal cortical brain development: a longitudinal observational study. Malar J 2012;11:222-.

Rogerson SJ. Management of malaria in pregnancy. The Indian journal of medical research 2017;146(3):328-33.

Saito M, Briand V, Min AM, McGready R. Deleterious effects of malaria in pregnancy on the developing fetus: a review on prevention and treatment with antimalarial drugs. Lancet Child Adolesc Health 2020;4(10):761-74.

Sanchez K, Morgan AT, Slattery JM, Olsen JE, Lee KJ, Anderson PJ, et al. Neuropredictors of oromotor feeding impairment in 12month-old children. Early human development 2017;111:49-55.

Setanen S, Lehtonen L, Parkkola R, Aho K, Haataja L, Group PS. Prediction of neuromotor outcome in infants born preterm at 11 years of age using volumetric neonatal magnetic resonance imaging and neurological examinations. Developmental medicine and child neurology 2016;58(7):721-7.

Tuhkanen H, Pajulo M, Jussila H, Ekholm E. Infants born to women with substance use: Exploring early neurobehavior with the Dubowitz neurological examination. Early human development 2019;130:51-6.

Woodward LJ, Mogridge N, Wells SW, Inder TE. Can neurobehavioral examination predict the presence of cerebral injury in the very low birth weight infant? Journal of developmental and behavioral pediatrics : JDBP 2004;25(5):326-34.

World Health Organization. World malaria report 2019. 2019.

Figure 1: Sample Recruitment

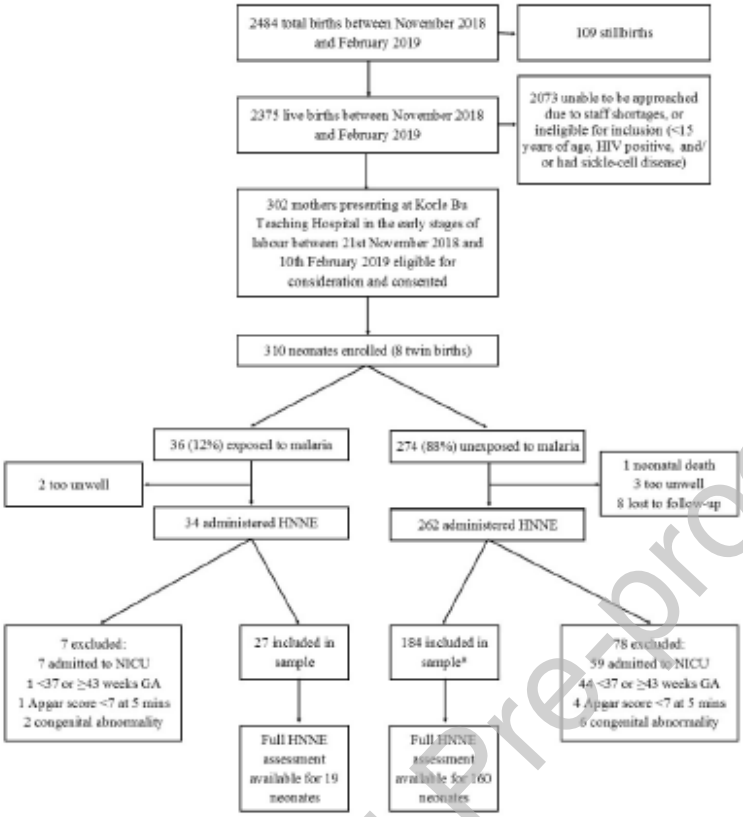


Table 1: Sociodemographic characteristics of neonates assessed using the Hammersmith Neonatal Neurological Examination according to malaria exposure

Characteristics[†]	**All** **[n=211]**	**Malaria-Exposed** **[n=27]**	**Malaria-Unexposed** **[n=184]**	**p**
Maternal Demographics				
Age, years	31.4 ± 6.1	29.8 ± 7.0	31.7 ± 6.0	.14
Literate				

Characteristics[†]	**All [n=211]**	**Malaria-Exposed [n=27]**	**Malaria-Unexposed [n=184]**	**p**
No	46 (22.3)	5 (18.5)	41 (22.9)	.60
Yes	160 (77.7)	22 (81.5)	138 (77.1)	
Education				
None/primary/secondary	137 (66.2)	22 (81.5)	115 (63.9)	.07
Higher	70 (33.8)	5 (18.5)	65 (36.1)	
Amount worked				
None/occasional/seasonal	39 (18.8)	5 (18.5)	34 (18.9)	.96
Full-time	168 (81.2)	22 (81.5)	146 (81.1)	
Wealth quintile				
Poorest [1st–3rd]	100 (47.4)	15 (55.6)	85 (46.2)	.36
Richest [4th–5th]	111 (52.6)	12 (44.4)	99 (53.8)	
Health insurance				
No	6 (2.9)	1 (3.7)	5 (2.8)	.79
Yes	200 (97.1)	26 (96.3)	174 (97.2)	
Other children				
None	42 (20.5)	11 (42.3)	31 (17.3)	·003
≥1	163 (79.5)	15 (57.7)	148 (82.7)	
Overcrowding				
≤1 person per room	51 (24.6)	2 (7.4)	49 (27.2)	.03

Characteristics[†]	**All [n=211]**	**Malaria-Exposed [n=27]**	**Malaria-Unexposed [n=184]**	**p**
>1 person per room	156 (75.4)	25 (92.6)	131 (72.8)	
Social risk				
Low risk (no risk factor)	106 (50.2)	15 (55.6)	91 (49.5)	.55
High risk (≥1 risk factor)	105 (49.8)	12 (44.4)	93 (50.5)	
Maternal Clinical				
Time of first antenatal visit				
Second/third trimester	70 (34.3)	10 (37.0)	60 (33.9)	.75
First trimester	134 (65.7)	17 (63.0)	117 (66.1)	
Gravidity	3.3 ± 1.7	2.7 ± 1.9	3.3 ± 1.7	.09
Middle upper arm circumference, cm	31.3 ± 4.1	29.6 ± 3.6	31.5 ± 4.1	.03
Hemoglobin level, g/dl	10.2 ± 1.5	10.0 ± 2.0	10.3 ± 1.4	.29
Anxiety	2 [0, 4]	2 [0, 6]	2 [0, 4]	.41
Depression	2 [0, 5]	2 [0, 5]	2 [0, 5]	.40
Clinical risk				
Low risk [no risk factor]	152 (72.0)	17 (63.0)	135 (73.4)	.26
High risk [≥1 risk factor]	59 (28.0)	10 (37.0)	49 (26.6)	
Malaria Prevention				
ITN use in pregnancy				
Did not use/no bed net	129 (62.3)	16 (59.3)	113 (62.8)	.72

Characteristics[†]	**All [n=211]**	**Malaria-Exposed [n=27]**	**Malaria-Unexposed [n=184]**	**p**
Used in pregnancy	78 (37.7)	11 (40.7)	67 (37.2)	
Total IPTp-SP doses	2 [1, 3]	2 [2, 3]	2 [1, 3]	.28
Trimester of first IPTp-SP				
No IPTp-SP/not specified	35 (16.7)	1 (3.7)	34 (18.6)	.03
First or second trimester	131 (62.4)	16 (59.3)	115 (62.8)	
Third trimester	44 (21.0)	10 (37.0)	34 (18.6)	
Neonatal Clinical				
Mode of delivery				
Cesarean section	134 (63.8)	14 (51.9)	120 (65.6)	.17
Spontaneous vaginal/ vacuum extraction	76 (36.2)	13 (48.1)	63 (34.4)	
Gestational age, weeks	39 [38.1, 40.2]	39.2 [38.1, 40.4]	39 [38.1, 40.2]	.81
Birthweight, kg	3.2 ± 0.4	3.2 ± 0.4	3.1 ± 0.4	.40
Birthweight Z-score	-0.3 ± 0.9	-0.2 ± 0.9	-0.3 ± 0.9	.44
Low birthweight				
No	201 (95.7)	25 (92.6)	176 (96.2)	.39
Yes	9 (4.3)	2 (7.4)	7 (3.8)	
Apgar score 1 minute	8 [7, 8]	8 [7, 8]	8 [7, 8]	.60
Apgar score 5 minutes	9 [8, 9]	9 [8, 9]	9 [8, 9]	.54
Length, cm	50 [49, 5]	51 [50, 52]	50 [49, 52]	.13

Characteristics[†]	**All [n=211]**	**Malaria-Exposed [n=27]**	**Malaria-Unexposed [n=184]**	**p**
Chest circumference, cm	33 [32, 34]	33 [31, 34]	33 [32, 34]	.18
Head circumference, cm	34 [33, 35]	34 [33, 35]	34 [33, 35]	.62
Ponderal Index	2.6 ± 0.8	2.5 ± 0.3	2.6 ± 0.9	.50
Sex				
Male	108 (51.4)	12 (44.4)	96 (52.5)	.44
Female	102 (48.6)	15 (55.6)	87 (47.5)	
Placental Assessment				
Placental abnormality				
None	50 (23.7)	10 (37.0)	40 (21.7)	.16
1 abnormality	82 (38.9)	7 (25.9)	75 (40.8)	
>1 abnormality	79 (37.4)	10 (37.0)	69 (37.5)	
Cord length, cm	52 [45.5, 60.0]	55.5 [46, 60.5]	51.6 [45.5, 59.2]	.37
Cord diameter, cm	1.2 [1, 1.5]	1.3 [1, 1.5]	1.2 [1, 1.5]	.69
Umbilical coiling index	0.08 ± 0.09	0.07 ± 0.08	0.08 ± 0.09	.86
Placental weight, kg	472.1 ± 100.6	478.8 ± 105.4	471.2 ± 100.3	.73
Placental thickness, cm	1.9 ± 0.4	1.9 ± 0.3	1.8 ± 0.4	.48

[†]Data are number (%), median [interquartile range], or mean ± standard deviation.

Table 2: Unadjusted and adjusted mean differences in raw scores of the Hammersmith Neonatal Neurological Examination subdomain according to malaria exposure

	Malaria-Exposed		Malaria-Unexposed		Mean Difference			Adjusted† Mean Difference		
	N	Mean ± SD	N	Mean ± SD	Mean Difference (95% CI)	p	Cohen's d (95% CI)	Mean difference (95% CI)	p	Cohen's
	27	7.1 ± 2.6	182	7.3 ± 2.2	-0.18 (-1.09, 0.73)	.70	-0.08 (-0.48, 0.33)	-0.00 (-0.93, 0.93)	.10	-0.0 (-0.4
	27	4.2 ± 0.8	184	4.1 ± 0.8	0.13 (-0.18, 0.44)	.41	0.17 (-0.24, 0.57)	0.14 (-0.18, 0.46)	.40	0.18 (-0.
	27	4.6 ± 1.0	178	5.0 ± 0.9	-0.33 (-0.69, 0.03)	.07	-0.37 (-0.78, 0.03)	-0.34 (-0.70, 0.03)	.07	-0.38 (-0
	24	1.9 ± 0.9	179	1.8 ± 0.8	0.06 (-0.30, 0.42)	.75	0.06 (-0.36, 0.49)	0.03 (-0.33, 0.40)	.85	0.04 (-0.
	27	2.4 ± 0.6	181	2.3 ± 0.7	0.06 (-0.21, 0.34)	.66	0.09 (-0.31, 0.50)	0.07 (-0.21, 0.34)	.64	0.10 (-0.
d	20	4.7 ± 1.5	165	4.5 ± 1.6	0.19 (-0.54, 0.93)	.60	0.12 (-0.34, 0.59)	0.28 (-0.47, 1.03)	.46	0.18 (-0.
	19	25.2 ± 3.5	160	25.0 ± 3.8	0.23 (-1.56, 2.02)	.80	0.06 (-0.41, 0.54)	0.46 (-1.35, 2.27)	.62	0.12 (-0.

†Adjusted for socioeconomic status, education, maternal age, and social risk.

Table 3: Risk of suboptimal scores of the Hammersmith Neonatal Neurological Examination subdomain according to malaria exposure

HNNE Subdomain	Neonates with suboptimal scores, n/N (%)			Unadjusted		Adjus
	Malaria-Exposed	Malaria-Unexposed	All	Risk Ratio (95% CI)	p	Risk
Tone	17/27 (63.0)	123/182 (67.6)	140/209 (67.0)	0.93 (0.69, 1.27)	.65	0.90
Tone patterns	16/27 (59.3)	125/184 (67.9)	141/209 (66.8)	0.87 (0.63, 1.21)	.42	0.87
Reflexes	15/27 (55.6)	61/178 (34.3)	76/205 (37.1)	1.62 (1.09, 2.41)	.02	1.63
Movements	18/24 (75.0)	149/179 (83.2)	167/203 (82.3)	0.90 (0.71, 1.15)	.40	0.92

Abnormal signs/patterns	17/27 (63.0)	110/181 (60.8)	127/208 (61.1)	1.03 (0.76, 1.42)	.83	1.03
Orientation and behavior	14/27 (70.0)	125/165 (75.8)	139/185 (75.1)	0.92 (0.68, 1.25)	.61	0.91
Total HNNE score	18/19 (94.7)	152/160 (95.0)	170/179 (95.0)	1.00 (0.89, 1.12)	.96	1.00

471 †Adjusted for socioeconomic status, education, maternal age, and social risk.
472

473

474