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Impact of *Plasmodium falciparum* malaria infection on serum cortisol, adrenocorticotropic hormone, pregnancy associated plasma protein-A and alpha-fetoprotein in pregnant women at Nnewi

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ABSTRACT

The present study assessed the maternal cortisol, Adrenocorticotropic hormone (ACTH), Pregnancy associated plasma protein-A (PAPP-A) and alpha-fetoprotein (AFP) concentrations in malaria infected pregnant women. A total of 76 (40 apparently healthy pregnant and 36 malaria-infected pregnant) women aged 18-40 years were prospectively recruited. Early morning blood samples (5 ml) were collected from each subject at 1st and 2nd trimesters. 1 ml of whole blood was used for the diagnosis of *P. falciparum* malaria using *malaria Plasmodium falciparum* Rapid Test Device (RTD) and Giemsa stained thick blood smears for microscopic detection of *P. falciparum* parasites while the remaining 4 ml was centrifuged, separated and serum used for estimation of cortisol, ACTH, AFP and PAPP-A using ELISA-based method. The mean cortisol (125.80 ±30.80 ng/ml) and AFP (1.9 ±0.7 MoM) concentrations in malaria-infected pregnant women were significantly ($p<0.05$) higher than those of normal pregnant women (86.70 ±3.30 and 1.5 ±0.7 respectively). Malaria-infected pregnant women had higher percentage of low birth weight babies (27.8%), preeclampsia (11.1%), premature rupture of membrane (11.1%), preterm delivery (30.6%), miscarriages (27.8%) and low APGAR score at one minute (2.8%). This shows the possible impact of malaria infection on pregnancy and birth outcomes. The increased cortisol concentration in malaria infected pregnant women shows that malaria infection in pregnancy increases the stress pregnant women are exposed to but the placental defect associated with increased placental permeability to AFP is not related to the effect of the stress (cortisol) and thus does not influence birth outcomes.

Keywords: *Plasmodium falciparum*, pregnancy, cortisol, maternal serum markers, pregnancy outcome.

INTRODUCTION

Malaria is a serious public health problem in endemic countries such as Nigeria. The most vulnerable are pregnant mothers and their fetus (Schantz-Dum and Nour, 2009; Takem and D'Alessandro, 2013; Ikpa et al., 2014). *Plasmodium falciparum* specie is more prevalent in Nigeria. Severe anemia is the consequence of this infection and is a contributory factor to reduced immunity in pregnancy (Ukibe et al., 2010; Chinedum et al., 2010). This result to common and frequent

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death observed among pregnant mothers in malaria endemic region (Nosten et al., 2007; Campos et al., 2011). Due to reduced immune response, pregnant women are more prone to malaria infection (Ukibe et al., 2010). Therefore, the infection can be in its severe form with more complications in the mother and her fetus than non-pregnant women from the same area (Conroy et al., 2012). Report has shown that oxidative stress occurs in acute malaria infection and as a result, depletes antioxidant levels (Sibmooh et al., 2000). Cortisol under normal condition protects the body from stress by regulating the blood pressure and immune function, through a negative feedback loop mechanism. However, stress could cause the feedback mechanism to malfunction leading to excess production of corticosteroid releasing hormone (CRH) and hence, cortisol, which can enhance inflammation thereby, resulting in various disease conditions (Hilary, 2002; Behrman and Butler, 2007). Studies have reported direct relationship of some maternal biomarkers such as fetal fibronectin (FFN), salivary estriol, serum CRH, PAPP-A, alpha feto-protein with adverse obstetrics outcome (Shah and Baxi, 2016). PAPP-A and Alpha fetoprotein have been shown to be maternal 1st and 2nd trimester screening markers for congenital abnormality as well as accessing pregnancy at the risk of adverse outcomes (Smith et al., 2002; Krantz et al., 2004; Goffinet, 2005). Prevention of spontaneous preterm delivery and adverse pregnancy complication through early screening of these markers during antenatal clinics is urgently required. The present study is therefore, aimed at assessing the impact of malaria infection on some stress hormones, maternal serum biomarker and their relationship with pregnancy outcomes at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out at antennal clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. The analysis of Cortisol, ACTH, AFP and PAPP-A hormones was done at the department of Chemical Pathology (NAUTH), Nnewi.

Subjects

A total of 76 pregnant women aged between 18 and 40 years were recruited at antennal clinic at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. Thirty (36) of them were positive for *P. falciparum* malaria infection while the remaining 40 were apparently healthy pregnant women (control subjects), who were not diagnosed of any disease condition at the time of sample collection. Initial 5 ml of blood was collected from each subject in their 1st trimester by venopuncture technique from the cubital fossa into labeled plain test tubes. The samples were allowed to clot and centrifuged at 5000 rpm for 5 minutes. The serum was transferred into properly labeled plain containers and stored at -4 degree centigrade for the analysis of cortisol, ACTH and PAPP-A. Then, another 5 ml was also collected from each subject at their 2nd trimester for the analysis of alpha feto-protein. The analysis of cortisol, ACTH, AFP and PAPP-A hormones were done using ELISA kit methods.

The anthropometric measurement which included body weight and height of the pregnant women during their 1st booking was obtained from maternal health records file. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). Also the blood pressures at the two points of sample collections were obtained from their records.

Other maternal variables assessed were age, gestation age at delivery, mode of delivery, premature delivery at less than 37 weeks of gestation and maternal complications. The neonatal outcomes assessed included birth weight, gestational age at birth and rate of APGAR scores less than 7 at one and five minutes. APGAR score is an accepted and convenient method for reporting the status of the newborn infant immediately after birth and the response to resuscitation if needed (Li et al., 2013).

Exclusion and inclusion criteria

Only pregnant women with malaria infection at the time of sample collection were included for the study. Apparently healthy

pregnant women were also included and used as the control. Pregnant women with multiple pregnancies were excluded; diabetics, hypertensive, HIV positive pregnant women or those with other chronic systemic infection were excluded. Pregnant women within their 3rd trimester were also excluded from the study. Results of subjects with incomplete data were excluded from the study.

Diagnosis of *P. falciparum* malaria

Whole blood was used for the diagnosis of *P. falciparum* malaria using *Malaria Plasmodium falciparum* Rapid Test Device (Para check, Orchid Biomedical systems, Vena Goa, India) and Giemsa stained thick blood smears for microscopic detection of *P. falciparum* parasites. The principle of the *P. falciparum* antigen detection is based on a rapid chromatographic immunoassay, for the qualitative detection of circulating *P. falciparum* antigen in the whole blood. This method utilizes Gold conjugate to selectively detect *Plasmodium* antigen. The procedure was as described by the manufacturer. Briefly, 10 µl of the whole blood specimen from the participant were transferred into appropriately labeled specimen cassettes containing sample well. Subsequently, 3 drops of buffer supplied by the manufacturer (approximately 120 µl) was added into the sample wells. After 15 minutes the results were read. The test device has inherent quality control that validates the result. The presence of two pink lines at the region of the control and test sample signifies presence of *P. falciparum* malaria infection while the presence of only1pink line in the control region signifies absence of *P. falciparum* malaria.

Determination of cortisol, ACTH, PAPP-A and AFP

Cortisol, ACTH, PAPP-A and AFP were determined using solid phase competitive Enzyme-linked Immunosorbent Assay (ELISA) method as described by PERFECT EASE BIOTECH (Beijing) Co., Ltd.

Ethics approval and consent to participate

The ethical approval for this research was obtained from ethics committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria in accordance with the Helsinki declaration by the World Medical Association (WMA) on the ethics principles for medical research involving human subjects. Informed consent was obtained from the subjects prior to sample collection.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 2.0 was used for statistical analysis. The results were expressed as mean standard deviation and percentages. Comparisons of mean were made using Student's t-test. Pearson correlation analysis was used to establish possible correlation between cortisol on AFP and PAPP-A and also between AFP and PAPP-A on birth outcomes. Results were considered significant at $P \leq 0.05$.

RESULTS

Demographic characteristics of the study population

Table 1 shows that there were no significant ($p>0.05$) differences observed in the Ages, systolic (mmHg) and diastolic (mmHg) blood pressures of malaria-infected pregnant women (28.7 ± 4.5; 105.0 ± 7.1; 63.5 ± 6.3 respectively) compared to those of the apparently healthy pregnant women (28.6 ± 4.5; 104.3 ± 7.5; 63.4 ± 6.2 respectively) ($P>0.05$). However, body mass index of malaria-infected pregnant women was significantly ($p<0.05$) lower than apparently healthy pregnant women.

Serum Cortisol (ng/ml), ACTH (pg/ml), AFP (MoM) and PAPP-A (MoM) concentrations in malaria-infected pregnant and control subjects

The result showed that the mean serum cortisol and AFP concentrations in malaria-infected pregnant women (125.80 ±30.80 and 1.9 ±0.7 respectively) were significantly ($p<0.05$) higher when compared with control (86.70 ±3.30 and 1.5 ±0.7 respectively). However, there was no significant ($p>0.05$) difference in the mean levels of ACTH and PAPP-A when compared in both groups (Table 2).

Relationship between stress hormones and fetal viability hormones in malaria-infected pregnant women

After controlling for age and BMI, there was no observed significant ($p>0.05$) associations between cortisol (ng/ml) and AFP (MoM), cortisol (ng/ml) and PAPP-A (MoM), ACTH (pg/ml) and AFP (MoM) and ACTH (pg/ml) and PAPP-A (MoM) respectively.

Maternal outcomes in malaria-infected pregnant women and control participants

The result shows that malaria-infected pregnant women had (61.1%) normal vaginal delivery, (11.1%) preeclampsia and (11.1%) premature rupture of membrane (PROM) compared with (72.5%) normal vaginal delivery, (2.5%), preeclampsia and (2.5%) PROM observed in normal pregnant women (Table 4).

Infant outcomes in malaria-infected pregnant women and control group

The results shows that malaria infected pregnant women had (27.8%) low birth weight babies, (30.6%) of preterm delivery, (27.8 %) miscarriages, (2.8 %) Apgar score of less than seven at one minute when compared with the apparently healthy pregnant women with (5%) LBW, (12.5%) preterm delivery, (12.5%) miscarriages and (2.5%) Apgar score of less than seven at one minute (Table 5).

Relationships between maternal AFP (MoM) and PAPP-A (MoM) with infant outcome in malaria-infected pregnant women

The result exhibited no significant ($p>0.05$) associations between PAPP-A and Birth weight; PAPP-A and APGAR score at one minute and at five minutes; AFP and birth weight; AFP and APGAR score and one minute and five minutes respectively (Table 6).

Table 1: Demographic characteristics of the study population.

Characteristics	Malaria-infected pregnant women n=(36)	Normal pregnant women n=(40)	P-value
AGE (years)	28.70 ± 4.50	28.60 ± 4.50	0.818
SBP (mmHg)	105.00 ± 7.10	104.30 ± 7.50	0.712
DBP (mmHg)	63.50 ± 6.30	63.40 ± 6.20	0.978
BMI (Kg/m^2)	25.40 ± 1.90	26.80 ± 2.80	0.032

P-value was significant at ($P<0.05$). SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index.

Table 2: Serum Cortisol and ACTH in malaria-infected pregnant and control subjects.

Parameters	Malaria-infected pregnant women N=(36)	Normal pregnant women N=(40)	P-value
Cortisol (ng/ml)	125.80 ±30.80	86.70 ±3.30	0.000
ACTH (ng/ml)	4.60 ±2.30	5.90 ± 0.10	0.516
AFP (MoM)	1.90 ±0.70	1.50 ±0.70	0.027
PAPP-A (MoM)	1.20 ±0.90	1.30 ±0.90	0.851

P-value was significant at ($P<0.05$).

Adrenocorticotrophin hormone (ACTH), AFP- Alpha feto-protein, PAPP-A- pregnancy associated plasma protein-A.

Table 3: Correlation between stress hormones (cortisol (ng/ml), ACTH (ng/ml)) and serum markers AFP (MoM), PAPP-A (MoM) in malaria-infected pregnant women.

Variables	R	P-value
Cortisol vs AFP	-0.069	0.738
Cortisol vs PAPP-A	-0.181	0.377
ACTH vs AFP	-0.247	0.225
ACTH vs PAPP-A	0.082	0.692

Key: Adrenocorticotrophin hormone (ACTH), AFP- Alpha feto-protein, PAPP-A- pregnancy associated plasma protein-A.

Table 4: Maternal outcomes in malaria-infected pregnant women and control participants.

Outcomes	Malaria infected (36)	Normal pregnant women (40)
Ceaserean section	4(11.1%)	6(15%)
Vaginal delivery	22(61.1%)	29(72.5%)
Preclampsia	4(11.1%)	1(2.5%)
Prom	4(11.1%)	1(2.5%)
Prolonged labour	2(5.5%)	3(7.5%)

Table 5: Infant outcomes in malaria-infected pregnant women and control group.

Outcomes	Malaria-infected pregnant (36)	Normal pregnant women (40)
Birth weight		
Low birth weight(<2.5kg)	10(27.8%)	2(5%)
Normal birth weight(2.5-4.0kg)	23(63.9%)	24(60%)
Macrosomia(>4.0kg)	3(8.3%)	14(35%)
Gestational age at birth		
Preterm(<37wks)	11(30.6%)	5(12.5%)
Term(38wks-40wks)	25(69.4%)	34(85%)
Posterm(>40wks)	0(0.0%)	1(2.5%)
Live birth	24(66.7%)	26(83.1%)
Still birth	2(5.6%)	6(15%)
Miscarriage	10(27.8)	5(12.5%)
Apgar score at one minute		
7-10	35(97.2%)	39(97.5%)
<7	1(2.8%)	1(2.5%)
Apgar score at five minute		
7-10	36(100.0%)	40(100.0%)
<7	0(0.0%)	0(0.0%)

Table 6: Correlation between maternal serum markers [AFP (MoM), PAPP-A (MoM)] and infant outcome in malaria-infected pregnant women.

Variables	R	P-value
PAPP-A vs birth weight	0.148	0.240
PAPP-A vs Apgar score at one minute	0.068	0.592
PAPP-A vs Apgar score at five minutes	0.149	0.238
AFP vs birth weight	0.039	0.756
AFP vs Apgar score at one minute	0.092	0.464
AFP vs Apgar score at five minutes	0.193	0.123

DISCUSSION

In the present study, it is apparent that cortisol was significantly increased in malaria infected pregnant women compared with normal pregnant women (control). This shows that malaria posed tremendous stress on pregnant women. This is consistent with other findings (Mastorakos and Ilias, 2003; Bouyou – Akotet et al., 2005). Hilary (2002) reported that infection with *P. falciparum* malaria increases the secretion of hormonal mediators which include cortisol as well as pro-inflammatory cytokines and antimicrobial agents. Previous report showed that the increased serum cortisol in malaria infection could indicate intact hypothalamus/pituitary/adrenal axis. Activation of these axes in malaria might be as a result of release of cytokines and/or stress generated by the disease itself (Wilson et al., 2001). Pregnant women and the fetus are more attractive to mosquitoes making the parasite densities higher in them than in non-pregnant adults (Rogerson et al., 2007; Takem et al., 2013). The authors attributed this to lack of immunity to specific variant surface antigens (VAR2CSA) in the placenta. It has been shown that malaria infection might increase the cortisol concentration and if not treated can lead to adverse pregnancy outcomes (Muehlenbein et al., 2005). Some authors have attributed the sequestration of trophozoite and schizont stages into maternal vascular area of the placenta to high cortisol level and this could alter cortisol metabolism (Beeson et al., 2000). Ibrahim et al. (2011) on the other hand, did not find significant difference in the cortisol level in patients with malaria infection.

In the present study, AFP level in malaria infected pregnant women was significantly higher when compared with normal pregnant women. This might result from sequestration of *P. falciparum* infected erythrocytes in the placenta which can lead to severe placental changes thereby form the basis for the pathogenesis of placental malaria. Report has shown that elevated level of maternal circulating AFP indicates defect placentation and could represent ongoing placental damage and adverse pregnancy outcomes (Rogerson et al., 2007).

There non-significant correlation observed between cortisol and AFP; cortisol and PAPP-A; ACTH and AFP and ACTH and PAPP-A respectively in malaria-infected pregnant women, shows that increase or decrease in stress hormone does not have effect on the fetal viability hormone. This suggests that the increase in the mean level of AFP observed in this study was independent of the concentration of cortisol. This finding was in agreement with previous reports (Yuan et al., 2009).

From this study, malaria infected pregnant women had higher percentage of LBW, preterm delivery, miscarriages, preeclampsia and premature rupture of membrane. Several studies have linked malaria infection and non-communicable diseases in pregnancy to many

adverse maternal and birth outcomes including miscarriage, premature delivery, low birth weight, anaemia, congenital infection, fetal and perinatal death (Menendez et al., 2000; Chinedu et al., 2010; Conroy et al., 2012; Shah and Baxi, 2016). LBW of the infant has been implicated with poor cognitive and neurosensory development of the child (Murphy and Breman, 2001). The miscarriages observed may not be from the increased cortisol concentration. Allthough, the cortisol concentration observed in this study was significantly elevated more than apparently healthy pregnant women; their cortisol level was still within the reference range (70-280ng/ml). However, some previous studies found elevated cortisol which is a stress hormone to be associated with spontaneous abortion (Ezechi et al., 2003, Lalita and Geeta, 2017). Lalita and colleague attributed this complication to great infiltration of *Plasmodium falciparum*-infected red blood cells in the intervillous spaces of placenta.

Furthermore, it was observed that malaria-infected pregnant women had higher percentage of low APGAR score at one minute but had 100 percent of APGAR score at 5 minutes. APGAR score is a quick test performed on a baby at 1 and 5 minutes after birth. The 1-minute score determines how well the baby tolerated the birthing process. The 5-minute score tells the doctor how well the baby is doing outside the mother's womb. It was observed that correlation of maternal apha feto-protein and pregnancy associated plasma protein-A with infant outcomes in malaria-infected pregnant was not significant. This show that the birth weight, APGAR scores at 1 minute and at 5 minutes of the babies was not totally influenced by the circulating levels of the maternal AFP and PAPP-A. This was in contrast with the previous reports (Gentile et al., 2015). PAPP-A is a protease of IGFBP4 which acts as a binding protein for IGF-1 and a powerful inhibitor for IGF-1. IGF-1 plays an important role in regulating fetal growth by controlling glucose and amino acids absorption in trophoblastic cells (Gentile et al., 2015).

Conclusion

Cortisol was significantly increased in malaria infected pregnant women compared with normal pregnant women (control). This shows that malaria posed tremendous stress on pregnant women and could lead to adverse pregnancy outcome. The elevated AFP level in malaria infected pregnant women could result to severe placental changes observed in this study. The non-significant correlation between cortisol and AFP; cortisol and PAPP-A; ACTH and AFP and ACTH and PAPP-A respectively in malaria-infected pregnant women, shows that increase or decrease in stress hormone (cortisol) does not have effect on the fetal viability hormone. The malaria infected pregnant women had higher percentage of LBW, preterm delivery, preeclampsia, miscarriages and PROM showing the possible impact of malaria infection on pregnancy and birth outcomes.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization, CCO and NRU; Methodology, CCO, NRU and AAA; Software, EIO and OAK; Validation, CCO, NRU, SNU; Formal analysis, AAA; Investigation, AAA and NRU; Resources, AAA, EIO and OAK; Data curation, AAA; Writing – Original Draft Preparation, AAA, NRU and CCO; Writing – Review & Editing, AAA, NRU, CCO, SNU, EIO and OAK; Visualization, AAA, EIO and OAK; Supervision, CCO and NRU; Project Administration, AAA.

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