



PRENATAL EXPOSURE TO *PLASMODIUM FALCIPARUM* LOWERS HUMORAL RESPONSES DURING CLINICAL MALARIA EPISODES

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ABSTRACT

Prenatal exposure to Plasmodium falciparum infection affects the development of fetal immune cells in utero by inducing immunotolerance or immunosensitization of fetal immune cells to P.falciparum antigens. This study aimed at determining the effect of prenatal exposure to P.falciparum on specific humoral responses, with a focus on immunoglobulin M (IgM) and total IgG against blood stage Plasmodium falciparum merozoite surface protein 1 (PfMSP1-19) and Plasmodium falciparum merozoite surface protein2 (PfMSP2) during clinical malaria episodes in the first 24 months of life. A birth cohort study was conducted in Rufiji district, between January 2013 and June 2017. Infants (n=215) were recruited after delivery from mothers who were diagnosed of placental malaria (pm+) and without placental malaria (pm-). Total IgG against PfMSP2 in peripheral blood of exposed and unexposed infants were 49.4 (95% CI 43.5-55.4) and 64.6 (95% CI 61.1-68.2) antibody units respectively, and the difference was statistically significant (P<0.01). The levels of IgM against PfMSP2 in exposed and unexposed infants during clinical malaria episodes were 47.9 (42.8-52.9) and 58.8 (56.1-61.6) respectively, and the difference was statistically significant (P<0.01). Prenatal exposure to Plasmodium falciparum modulates humoral immune response to specific PfMSP1-19 and PfMSP2 with characteristically low total IgG and IgM in infants aged 0-2 years during clinical malaria episodes

Keywords: Humoral, *P.falciparum*, in utero, prenatal, episodes

INTRODUCTION

Malaria caused by *Plasmodium falciparum* is a leading cause of mortality and morbidity especially among pregnant mothers and children under five years of age (Osier et al., 2008; Riley et al., 1992). Infants who are born to mothers with placental malaria have been reported to have increased susceptibility to malaria infection, (Aribodor et al., 2009; Asante et al., 2013; Awine et al., 2016;



Bardaji et al., 2011; Borgella et al., 2013; Bouaziz et al., 2018), which has been associated with prenatal exposure to blood stage *P.falciparum* antigens (Malhotra et al., 2009).

The placental intervillous sequestration of *P.falciparum* infected erythrocytes is mediated by the VAR2CSA protein, which binds to placental chondroitin sulfate A and leads to fetal exposure to the *P.falciparum* blood stage antigens (Broen, Brustoski, Engelmann, & Luty, 2007; Dent et al., 2006; King et al., 2002; Sylvester et al., 2016). Early exposure to the parasite antigen has been shown to induce immunotolerance or immunosensitization of fetal immune cells to the same parasite antigen, therefore early exposure to *P. falciparum* antigens is likely to modulate the newborn's malaria immunity during natural challenges of malaria infection early in life.

Immunoglobulins have been demonstrated to play an important role in acquired immunity to *Plasmodium falciparum* malaria and have been demonstrated to target *P.falciparum* merozoites to reduce or prevent blood-stage replication and the development of malaria (McCallum et al., 2017; Richards et al., 2013). Although it has been demonstrated that prenatal exposure to *P.falciparum* increases infant susceptibility to malaria infection, the knowledge on humoral response to blood stage *P.falciparum* antigens; during clinical malaria episodes in infants prenatally exposed to *P.falciparum* infection remains limited. This study was designed to assess the effect of prenatal exposure to *P.falciparum* infection on humoral immune responses, with a focus on IgM and total IgG responses during clinical malaria episodes. The effect of other factors including; season of birth, gravidity, birth weight and age of the mother at delivery, considered likely to influence the immunoglobulin responses were also assessed.

RESEARCH METHODOLOGY

Study area

Details of the study area have been reported elsewhere (Sylvester et al., 2016). Briefly, four health facilities in Rufiji District, coast Region in Tanzania were involved in this study. The area is low-lying, below 500 m above sea level located within the flood plain of Rufiji River. The area experiences a long rainy season between February and May and a shorter, less intense one from October to December. The area was conducive for this study as it is epidemiologically defined as a hyper endemic district for malaria

Study design, and research participants

Details for the study design, study population, inclusion criteria and recruitment procedures for the research participants were described elsewhere (Sylvester et al., 2016). Briefly, the study was a prospective birth cohort follow up study, which included 215 infants who were monitored for clinical malaria episodes from birth to 24 months.

Procedures for diagnosis of placental malaria

Detailed procedures for the placental tissue collection, storage and processing have been reported elsewhere (Sylvester et al., 2016). The diagnosis of placental malaria was performed as previously reported (Parekh, Davison, Gamboa, Hernandez, & Branch, 2010). Briefly, the placental tissue specimen were stored in 10% neutral buffered formalin and the tissues were processed overnight on automatic tissue processor, then sectioned on a microtome and stained by Haematoxylin and Eosin. The stained sections were examined using a light compound microscope and results were reported as positive (For presence of *P. falciparum* in placental tissues) or negative for absence of



P.falciparum infection in the placental tissues.

ELISA for determination of IgM and Total IgG against recombinant PfMSP1-19 and PfMSP2 in cord and peripheral blood

Recombinant P.fMSP1-19 and PfMSP2 (from European malaria reagent repository, UK) were used in the assays to determine the IgM and total IgG levels in cord blood and peripheral blood drawn from infants during clinical malaria episodes. The recombinant antigens were diluted to a concentration of 0.5 µg/ml in coating buffer (0.1 M carbonate buffer composed of Sodium carbonate and Sodium bicarbonate at a concentration of 1.5g and 2.93g in 1000ml at a PH of 9.6. The blocking buffer was composed of Phosphate buffer saline (PBS) with 0.1% Bovine serum albumin and 0.05% tween 20. The washing buffer composed of phosphate buffer saline with 0.05% tween 20. The coating process involved pipetting 100 µl of diluted antigen to the wells and incubated overnight then aspirated. The plate wells (Nunc) were washed three times with washing buffer. Then blocking buffer was pipette into the wells at a higher volume (300 microlitres). The blocking buffer was incubated for 24 hours at room temperature in darkness. Then the blocking buffer was aspirated and wells were washed three times and dried by allowing them to sit on a bench for 24 hours and protected from light.

The ELISA was performed as previously described (Metzger et al., 2003). Briefly, 100 µl of serum diluted to 1:1000 was added to each of the duplicate wells and incubated overnight at 4°C; the plates were washed then horseradish peroxidase conjugated goat anti-human IgM or IgG antibodies diluted at 1:6000 (Mabtech, Sweden) was added to the wells and incubated for three hours at room temperature. The test was developed with a substrate, Tetramethylbenzidine at 4°C for 10 minutes and reactions were stopped using 20 µl of 2 M H₂SO₄ per well. The optical density (OD) was measured at 450 nm. Control samples were used, and tested sera were defined positive at a cut-off point of mean + 3SD of negative control groups born and living in Sweden. Optical density values were converted to arbitrary units using standards fitted on each plate.

Determination of clinical malaria episodes

The detailed description of procedures for determination of clinical malaria episodes were reported elsewhere (Sylvester et al., 2016). Briefly, the recruited infants were followed up at community and facility level. The scheduled visits were conducted at three months interval and whenever infants had signs of illness. The clinical signs were recorded on questionnaires. The blood smears were collected for determination of malaria infection using light microscopy. Clinical malaria was defined as fever ($\geq 37.5^{\circ}\text{C}$) with any level of microscopically determined parasitaemia. A new clinical malaria infection was recorded if it occurred after a period of 14 days or beyond from the previous treated clinical malaria episode. Infants who were diagnosed of clinical malaria infections or any other illness were treated within the routine system of the health facilities.

Data analysis

Double entry of data was performed and cleaned data were analyzed using IBM SPSS version 20 the mean levels of total IgG and IgM responses during clinical malaria episodes were assessed using independent samples t test. The effect of prenatal exposure to *P.falciparum* and adjustment for other factors were assessed using multiple linear regression model. The significance of difference was judged at $P < 0.05$ and confidence level of 95%.



RESULTS

Socio-demographic characteristics of the study population

The detailed socio-demographic characteristics of the research participants were reported elsewhere (Sylvester et al., 2016). Briefly, out of 215 infants who were recruited in this study, 50 of them were born to pm+ mothers and 165 infants were born to pm- mothers. There were no significant difference in the socio demographic characteristics of the recruited infants, except the birth weights of infants born to mothers with placental malaria were significantly lower compared to the birth weights of infants born to mothers without placental malaria ($P < 0.05$).

Clinical malaria episodes and detection of IgM in cord blood sera

Results for the clinical malaria episodes were described earlier (Sylvester et al., 2016). All recruited infants were assessed for presence of IgM specific for the recombinant PfMSP1-19 and PfMSP2 antigens in their cord blood. The IgM was detected in 10% ($n=5$) of the infants who were born to pm+ mothers (exposed-sensitized) while the remaining 90% ($n=45$) of the infants who were born to pm+ mothers had no detectable IgM (exposed-non sensitized) against the antigens. All infants who were born to pm- mothers had no detectable IgM in their cord blood sera (defined as unexposed).

Immunoglobulins levels in cord blood and peripheral blood

The Mean infant peripheral blood total IgG against *P.falciparum* PfMSP1-19 in exposed and unexposed infants during clinical malaria episodes were 44.9 (38.8-47.9) and 53.5 (49.1-57.8) arbitrary units respectively and the difference was significant ($P = 0.02$). Total IgG against PfMSP2 in infants peripheral blood in exposed and unexposed infants were 49.4 (95% CI 43.5-55.4) and 64.6 (95% CI 61.1-68.2) arbitrary units respectively, and the difference was significant ($P < 0.01$). The levels of IgM against PfMSP1-19 in exposed and unexposed infants during clinical malaria episodes were 49.5 (42.5-54.4) and 60.5 (56.5-64.6) arbitrary units respectively and the difference was significant ($P = 0.003$). The levels of IgM against PfMSP2 in exposed and unexposed infants during clinical malaria episodes were 47.9 (42.8-52.9) and 58.8 (56.1-61.6) arbitrary units respectively and the difference was significant ($P < 0.01$) Table 1.

Levels of IgG in cord blood against PfMSP2 in exposed and unexposed infants were 45.6 (95% CI 39.9-48.3,) and 53.09 (95% CI: 49.1-57.1), arbitrary units respectively and the difference was significant ($p = 0.029$). The cord levels of total IgG against PfMSP1-19 in exposed and unexposed infants were 39.25 (95% CI 36.3-42.2) and 58.8 (95% CI: 54.01-63.75) arbitrary units respectively and the difference was significant ($P < 0.01$).

Prenatal exposure to *P.falciparum* was significantly associated with IgM and total IgG responses against recombinant PfMSP1-19 and PfMSP2. The other factors (gravidity, age of the mother, season of birth, infant birth weight) considered in a multivariate model did not significantly associate with the immunoglobulin responses of IgM and IgG against PfMSP1-19 and PfMSP2 (Table 2 and Table 3).



Table 1: Mean levels of Immunoglobulin responses in cord blood and peripheral blood

Immunoglobulins	Infants born to pm+ mothers Mean: antibody Units (95% CI)	Infants born to pm- mothers Mean: antibody Units (95% CI)	Statistical P value
CBIgG PfMSP2	45.6 (39.9-48.3)	53.09 (95% CI: 49.1-57.1)	0.029
CBIgG PfMSPsp1-19	39.25 (36.3-42.2)	58.8 (54.01-63.75)	<0.01
IPBIgG PfMSP2	49.4 (43.5-55.4)	64.6 (61.1-68.2)	<0.01
IPBIgG PfMSP1-19	44.9 (38.8-47.9)	53.5 (49.1-57.8)	0.02
IPBIgM PfMSP1-19	49.5 (42.5-54.4)	60.5 (56.5-64.6)	0.003
IPBIgM PfMSP2	47.9 (42.8-52.9)	58.8 (56.1-61.6)	<0.01

Key: The abbreviations; CBIgGPfMSP2 denotes total Immunoglobulin G against the *P.falciparum* merozoite surface protein 2 in cord blood; CBIgGMSP1-19 denotes total immunoglobulin G against *P.falciparum* merozoite surface protein 1-19; IPB IgG PfMSP2 represents Immunoglobulin G against *Plasmodium falciparum* merozoite surface protein 2 in infant peripheral blood; IPBIgGPfMSP1-19 represents Immunoglobulin G against *Plasmodium falciparum* merozoite surface protein 1-19 in infant peripheral blood; IPBIgMPfMSP1-19 represents Immunoglobulin M against *Plasmodium falciparum* merozoite surface protein 1-19 in infant peripheral blood; IPB IgM PfMSP2 represents Immunoglobulin M against *Plasmodium falciparum* merozoite surface protein 2 in infant peripheral blood. The p value was established using independent samples t test at 95% confidence level.

The effect of prenatal exposure to *P. falciparum* on levels of IgM and IgG during clinical malaria episodes.

The effect of prenatal exposure to *P.falciparum* was adjusted for possible confounders of gravidity, season of birth, age of the mother at delivery and birth weight of infants. After adjusting for the confounders, prenatal exposure to *P. falciparum* was an outstanding factor which significantly associated with the levels of IgM and IgG against the recombinant antigens, PfMSP1-19 and PfMSP2, ($p < 0.05$), during clinical malaria episodes. The other factors did not significantly predict the levels of Immunoglobulins during the clinical malaria episodes in a multivariate model.

Table 2: Effects of prenatal exposure, gravidity, season of birth, birth weight and age of mother on IgG response against PfMSP1-19 and PfMSP2 during clinical malaria episodes: Multivariate linear regression model (MLRM)

Predictors	Unadjusted coefficient 95% CI	Statistic P value	Adjusted coefficient 95% CI	Statistic P value
<i>PfMSP2</i>				
Exposure	-15.1(-21.5- - 8.8)	<0.01	-13.8(-20.4- -7.2)	<0.01
gravidity	7.4(0.5-14.3)	0.06	5.1(-1.1-11.3)	0.07
Season of birth	9.2 (0.2-18.1)	0.05	9.8(2.2-17.4)	0.08
Birth weight	7.1(-2.1- 16.1)	0.13	0.3(-7.8-8.4)	0.18
Age of mother	2.5(-1.5-6.5)	0.12	2.9(0.4-6.6)	0.19
<i>PfMSP1-19</i>				



Exposure	-8.6(-15.8- -1.38)	<0.01	-7.5(-15.6-0.6)	<0.01
gravity	3.9(-3.3-11.1)	0.284	2.1(-5.54-9.7)	0.59
Season of birth	1.1(-8.3-10.5)	0.04	1.47(-7.8-10.8)	0.06
Birth weight	5.6(-3.7-14.9)	0.229	2.3(-7.6-12.2)	0.65
Age of mother	0.9(-3.2-4.9)	0.313	0.9(-3.2-5.1)	0.35

Key: The abbreviations; PfMSP1-19 represents the *P.falciparum* merozoite surface protein 1 from domain 19 Kda. PfMSP2 represents merozoite surface protein2.

Table 3 Effects of prenatal exposure, gravity, season of birth, birth weight and age of mother on IgM response against PfMSP1-19 and PfMSP2 during clinical malaria episodes

Predictors	Unadjusted coefficient (95% CI)	Statistic P value	Adjusted coefficient (95% CI)	Statistic P value
<i>PfMSP1-19</i>				
Pf exposure	-11.1(-18.4- -3.8)	0.01	-7.5(-14.7- -0.2)	0.02
gravity	11.1(4.1-18.1)	0.06	9.3(2.5-16.1)	0.07
Season of birth	12.3(2.9-21.6)	0.05	13.3(4.9-21.6)	0.06
Birth weight	8.8(-0.8-18.3)	0.07	4.9(-3.9-13.7)	0.27
Age of mother	-0.5(-4.8-3.7)	0.83	(-3.6-3.8)	0.09
<i>PfMSP2</i>				
Exposure	-10.9(-16.1- -5.8)	<0.01	-10.1(-15.5-4.6)	<0.01
gravity	3.3(-2.2-8.8)	0.23	0.7(-4.3-5.8)	0.77
Season	8.1(1.2-15.9)	0.06	8.5(2.4-14.7)	0.08
Birth weight	7.2(0.09-3.3)	0.05	3.3(-3.2-9.9)	0.31
Age of mother	0.23(-2.2-3.3)	0.08	0.07(-2.7-2.8)	0.96

Key: The abbreviations PfMSP1-19 represents the *P. falciparum* merozoite surface protein 1 from domain 19 Kda. MSPp2 represents merozoite surface protein2

DISCUSSION

Results of this study have shown that prenatal exposure to *P. falciparum* significantly affects the transfer of maternal antibodies to the fetus as previously demonstrated (Dechavanne, Cottrell, Garcia, & Migot-Nabias, 2015) and subsequently modulates the humoral immune response with respect to total IgG and IgM against the recombinant blood stage *P.falciparum* antigens, during clinical malaria episodes in the first two years of life.

Previously, the isotypes of IgG and IgM were demonstrated to be protective against malaria infection (Branch et al., 1998; Stanistic et al., 2015). The protective effect of IgG isotype has been demonstrated to act as an opsonin through Crystallizable Fragment (FC) receptors on phagocytic cells, the process which enhances the intracellular elimination of parasites. The immunoglobulin G has also been demonstrated, to immobilize pathogens and neutralize them apart from activating the classical pathway of complement leading to membrane attack complex (MAC) which combats



pathogens and curbs progression of disease(Hill, Schofield, & Wilson, 2017).

The observed low IgM and IgG responses in infants born to placental malaria positive mothers in this study, indicates that in utero exposure to blood stage *P.falciparum* antigens subsequently affects the humoral immune response to *P.falciparum* antigens. The results of this study imply that infants born to mothers with placental malaria may be more vulnerable to natural challenges with *P.falciparum*, as their immune response to similar parasite antigen is curtailed leading to low humoral responses. The present findings are corroborated by earlier studies which have demonstrated that, in utero exposure to *P. falciparum* affects fetal development of T and B lymphocytes (Malhotra et al., 2009). This effect seems to persist to early childhood, leading to low humoral immunity responsiveness against similar parasite antigens.

The current observations are further supported by previous findings which demonstrated that prenatal exposure to *P. falciparum* affects the acquisition of PfMSP1-19 invasion inhibitory antibodies (Dent et al., 2006). The failure of acquisition of the inhibitory antibodies may render the host to uncontrolled parasite invasion and increased vulnerability to *P.falciparum* infection and clinical disease. Other factors which were likely to affect the antibody responses during clinical malaria episodes were also considered. However, the association of gravidity, birth weight, age of mother at delivery and season of birth were not statistically significant in a multivariate analysis. It is therefore important to focus on the mitigation of malaria during pregnancy in order to protect the fetus from in utero exposure to the parasite antigens and subsequently the infant from natural challenges of malaria parasites.

Despite use of insecticide treated bednets (ITNS), residual sprays, intermittent preventive treatment (IPTp-SP) and other measures, pregnant mothers are still vulnerable to malaria infection during pregnancy. This implies that there is a need to revisiting the existing policies towards prevention of malaria during pregnancy.

Prospectively, the strategies designed to obtain a potent vaccine against malaria need to consider the effect of prenatal exposure to *P. falciparum* on antibody production.

CONCLUSION

Prenatal exposure to *Plasmodium falciparum* modulates humoral immune response in subsequent infant parasite challenges leading to low total IgG and IgM responses specific to PfMSP1-19 and PfMSP2 in infants aged up to 2 years during clinical malaria episodes.

Acknowledgements

Swedish International Development Cooperation (Sida) funded this project. We acknowledge the availability of mothers and infants in the study area and also appreciate the assistance of nurses, laboratory technicians and medical doctors at the health facilities.



REFERENCES

- Aribodor, D. N., Nwaorgu, O. C., Eneanya, C. I., Okoli, I., Pukkila-Worley, R., & Etaga, H. O. (2009). Association of low birth weight and placental malarial infection in Nigeria. *J Infect Dev Ctries*, 3(8), 620-623.
- Asante, K. P., Owusu-Agyei, S., Cairns, M., Dodoo, D., Boamah, E. A., Gyasi, R., Chandramohan, D. (2013). Placental malaria and the risk of malaria in infants in a high malaria transmission area in Ghana: a prospective cohort study. *J Infect Dis*, 208(9), 1504-1513. doi:10.1093/infdis/jit366
- Awine, T., Belko, M. M., Oduro, A. R., Oyakhirome, S., Tagbor, H., Chandramohan, D., . . . Williams, J. E. (2016). The risk of malaria in Ghanaian infants born to women managed in pregnancy with intermittent screening and treatment for malaria or intermittent preventive treatment with sulfadoxine/pyrimethamine. *Malar J*, 15, 46. doi:10.1186/s12936-016-1094-z
- Bardaji, A., Sigauque, B., Sanz, S., Maixenchs, M., Ordi, J., Aponte, J. J., . . . Menendez, C. (2011). Impact of malaria at the end of pregnancy on infant mortality and morbidity. *J Infect Dis*, 203(5), 691-699. doi:10.1093/infdis/jiq049
- Borgella, S., Fievet, N., Huynh, B. T., Ibitokou, S., Hounguevou, G., Affedjou, J., . . . Deloron, P. (2013). Impact of pregnancy-associated malaria on infant malaria infection in southern Benin. *PLoS One*, 8(11), e80624. doi:10.1371/journal.pone.0080624
- Bouaziz, O., Courtin, D., Cottrell, G., Milet, J., Nuel, G., & Garcia, A. (2018). Is Placental Malaria a Long-term Risk Factor for Mild Malaria Attack in Infancy? Revisiting a Paradigm. *Clin Infect Dis*, 66(6), 930-935. doi:10.1093/cid/cix899
- Branch, O. H., Udhayakumar, V., Hightower, A. W., Oloo, A. J., Hawley, W. A., Nahlen, B. L., . . . Lal, A. A. (1998). A longitudinal investigation of IgG and IgM antibody responses to the merozoite surface protein-1 19-kiloDalton domain of *Plasmodium falciparum* in pregnant women and infants: associations with febrile illness, parasitemia, and anemia. *Am J Trop Med Hyg*, 58(2), 211-219.
- Broen, K., Brustoski, K., Engelmann, I., & Luty, A. J. (2007). Placental *Plasmodium falciparum* infection: causes and consequences of in utero sensitization to parasite antigens. *Mol Biochem Parasitol*, 151(1), 1-8. doi:S0166-6851(06)00286-6 [pii]10.1016/j.molbiopara.2006.10.001 [doi]
- Dechavanne, C., Cottrell, G., Garcia, A., & Migot-Nabias, F. (2015). Placental Malaria: Decreased Transfer of Maternal Antibodies Directed to *Plasmodium falciparum* and Impact on the of Febrile Infections in Infants. *PLoS One*, 10(12), e0145464. doi:10.1371/journal.pone.0145464
- Dent, A., Malhotra, I., Mungai, P., Muchiri, E., Crabb, B. S., Kazura, J. W., & King, C. L. (2006).



Prenatal malaria immune experience affects acquisition of *Plasmodium falciparum* surface protein-1 invasion inhibitory antibodies during infancy. *J Immunol*, 177(10), 7139-7145. doi:177/10/7139 [pii]

Hill, D. L., Schofield, L., & Wilson, D. W. (2017). IgG opsonization of merozoites: multiple immune mechanisms for malaria vaccine development. *Int J Parasitol*, 47(10-11), 585-595. doi:S0020-7519(17)30201-1 [pii]10.1016/j.ijpara.2017.05.004 [doi]

King, C. L., Malhotra, I., Wamachi, A., Kioko, J., Mungai, P., Wahab, S. A., . . . Kazura, J. W. (2002). Acquired immune responses to *Plasmodium falciparum* merozoite surface protein-1 in the human fetus. *J Immunol*, 168(1), 356-364.

Malhotra, I., Dent, A., Mungai, P., Wamachi, A., Ouma, J. H., Narum, D. L., . . . King, C. L. (2009). Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. *PLoS Med*, 6(7), e1000116. doi:10.1371/journal.pmed.1000116 [doi]

McCallum, F. J., Persson, K. E., Fowkes, F. J., Reiling, L., Mugenyi, C. K., Richards, J. S., . . . Beeson, J. G. (2017). Differing rates of antibody acquisition to merozoite antigens in malaria: implications for immunity and surveillance. *J Leukoc Biol*, 101(4), 913-925. doi:10.1189/jlb.5MA0716-294R

Metzger, W. G., Okeno, D. M., Cavanagh, D. R., Robinson, J. V., Bojang, K. A., Weiss, H. A., . . . Conway, D. J. (2003). Serum IgG3 to the *Plasmodium falciparum* merozoite surface protein 2 is strongly associated with a reduced prospective risk of malaria. *Parasite Immunol*, 25(6), 307-312. doi:636 [pii]

Osier, F. H., Fegan, G., Polley, S. D., Murungi, L., Verra, F., Tetteh, K. K., . . . Marsh, K. (2008). Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria. *Infect Immun*, 76(5), 2240-2248. doi:IAI.01585-07 [pii]10.1128/IAI.01585-07 [doi]

Parekh, F. K., Davison, B. B., Gamboa, D., Hernandez, J., & Branch, O. H. (2010). Placental histopathologic changes associated with subclinical malaria infection and its impact on the fetal environment. *Am J Trop Med Hyg*, 83(5), 973-980. doi:10.4269/ajtmh.2010.09-0445

Richards, J. S., Arumugam, T. U., Reiling, L., Healer, J., Hodder, A. N., Fowkes, F. J., . . . Beeson, J. G. (2013). Identification and prioritization of merozoite antigens as targets of protective human immunity to *Plasmodium falciparum* malaria for vaccine and biomarker development. *J Immunol*, 191(2), 795-809. doi:jimmunol.1300778 [pii]10.4049/jimmunol.1300778 [doi]

Riley, E. M., Allen, S. J., Wheeler, J. G., Blackman, M. J., Bennett, S., Takacs, B., . . . Greenwood, B. M. (1992). Naturally acquired cellular and humoral immune responses to the major merozoite surface antigen (PfMSP1) of *Plasmodium falciparum* are associated with reduced



malaria morbidity. *Parasite Immunol*, 14(3), 321-337.

- Stanisic, D. I., Fowkes, F. J., Koinari, M., Javati, S., Lin, E., Kiniboro, B., . . . Beeson, J. G. Acquisition of antibodies against *Plasmodium falciparum* merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response. *Infect Immun*, 83(2), 646-660. doi:IAI.02398-14 [pii]10.1128/IAI.02398-14 [doi]
- Stanisic, D. I., Fowkes, F. J., Koinari, M., Javati, S., Lin, E., Kiniboro, B., . . . Beeson, J. G. (2015). Acquisition of antibodies against *Plasmodium falciparum* merozoites and malaria immunity in young children and the influence of age, force of infection, and magnitude of response. *Infect Immun*, 83(2), 646-660. doi:10.1128/iai.02398-14
- Sylvester, B., Gasarasi, D. B., Aboud, S., Tarimo, D., Massawe, S., Mpembeni, R., & Swedberg, G. (2016). Prenatal exposure to *Plasmodium falciparum* increases frequency and shortens time from birth to first clinical malaria episodes during the first two years of life: prospective birth cohort study. *Malar J*, 15(1), 379. doi:10.1186/s12936-016-1417-0