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*Full Length Research Paper*

# Naturally acquired antibody to DBL5 and ID1-ID2a dynamics in primigravid women during postpartum in a rural setting of Burkina Faso

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**Pregnancy is challenging for women, as their immune system is required not only to protect their body against pathogens, but also to develop an immune tolerance for the foetus growth *in utero*. However, after delivery little is known about the immunological basis of women susceptibility to malaria infection. The aim of this study was to assess the antibody profiles against VAR2CSA recombinant fragments at delivery and to evaluate their dynamics at 1 and 3 months post-delivery. Sera levels of anti-VAR2CSA antibodies were measured by enzyme linked immunosorbent assay using sera samples collected from primiparous and nulliparous women, using DBL5 and ID1-ID2a domains. Pregnancy background information showed that 51.5% of primiparous women experienced at least one malaria episode during their pregnancy. Placental malaria was diagnosed in 23.8% of women at delivery. Women infected during pregnancy showed higher levels of VAR2CSA-specific antibodies. However, the proportion of seropositive individuals decreased during the follow up period. Malaria infection during pregnancy contributes to establish the specific humoral immunity to placental malaria antigens in women living in endemic areas. The naturally acquired specific antibodies are not boosted by postpartum infections; but rather declines overtime.**

**Keywords:** Antibody, DBL5, ID1-ID2a, postpartum, malaria.

## Background

Women living in malaria endemic areas show an increased susceptibility to malaria during the second and third trimesters of their pregnancy and also in the early postpartum period (Diagne *et al.* 2000).The immunological basis of the susceptibility of women to malaria during pregnancy has been of interest in several studies in the past decades. On the contrary, malaria in the postpartum remains controversial and less investigated topic. When there are available, most of the data on postpartum malaria derived from studies not initially designed to answer the questions related to the postpartum events. Consequently, there is a large variability in study designs and subsequently in reported outcomes (Boel *et al.* 2012). While the evidence of spontaneous postpartum clearance of *Plasmodium falciparum* after delivery is reported in some

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studies (Bottero *et al.* 2011; Nguyen-Dinh *et al.* 1988), several others indicated that women are still at greater risk of malaria infections (Diagne *et al.* 2000; Ramharter *et al.* 2005b; Serra-Casas *et al.* 2011). The postpartum is considered as a period of recovery from physiological and immunological changes occurring during pregnancy (Clark & Croitoru 2001; Mayor *et al.* 2012; Singh & Perfect 2007), suggesting a re-adjustment of women's immune system. This re-adjustment might maintain the women susceptibility to new malaria infections and/or allow parasites to persist in the women body during the early postpartum.

Up to date, the dynamic of immune responses and the mechanisms whereby antimalarial immunity may recover during postpartum remain a relatively under-investigated topic. Therefore, characterizing the various changes of the maternal immune system response to malaria infection during pregnancy, and how long these changes last after delivery might provide information of interest in the control of malaria. A variant surface antigen encoded by VAR2CSA was identified on the surface of infected erythrocytes as a potential vaccine candidate for preventing pregnancy malaria (Fried & Duffy 2015). Two domains of the VAR2CSA showed interesting profiles in inducing immune responses in rat models, namely DBL5 and ID1-ID2a (Clausen *et al.* 2012); and are now expected to enter human clinical studies in the near future. We report here an assessment of the antibody profiles against DBL5 and ID1-ID2a at delivery, 1 and 3 months post-delivery in a seasonal malaria transmission setting of Burkina Faso.

## METHODS

### Study site

This study was carried out within the catchment area of Nanoro Health and Demographic Surveillance System (HDSS), which is located at 85 km from Ouagadougou, the capital city of Burkina Faso. Malaria transmission is seasonal and overlaps with the rainy season (June-October). Malaria accounts for almost half (48.6%) of all outpatient consultations from June to December while the prevalence of placental malaria (any category, including past infection) was recently estimated to34.6% (Natama *et al.* 2017).

### Study design and sample collection

This was a cohort study designed to assess the antibody response to the two VAR2CSA domains during the postpartum in a rural setting of Burkina Faso. The study was conducted from May 2015 to April 2016 in order to cover both low and high malaria transmission seasons. The study was embedded into a larger clinical trial investigating the efficacy of a new preventive strategy, namely community scheduled screening and treatment of pregnant women using community health workers against malaria in pregnancy as compared to IPTp (COSMIC) (Scott *et al.* 2014).

Potential primigravidas were identified within the control group of the COSMIC study (IPTp-SP alone as recommended by the national policy). A written informed consent was obtained from those willing to be enrolled and followed up during 3 months post-delivery.

Information related to the pregnancy background (including the number of IPTp-SP doses, history of malaria episodes) were obtained from the main study database. During each visit, women were systematically screened for malaria infection using the *Pf*HRP2 rapid diagnostic test (RDT SD-Bioline) according to the manufacturer's instructions. Any detected malaria episode was treated according to the national guidelines. Haemoglobin was measured at each scheduled visit using a HemoCue® machine (Hb 301, Sweden).

A sample of peripheral blood (ten milliliters) was collected in heparin tubes from each participant at delivery, and another one at three months post-delivery. Blood samples were processed within 4 hours after sampling and the sera were stored at -80°C until use. In addition, a sample of placental biopsy (2cm x 2cm x 1cm) was collected into 10% neutral buffered formalin and later embedded in paraffin wax for histological analysis.

In order to evaluate the primiparous' immunological profile after delivery, a control group of fifteen nulliparous women living in the HDSS catchment area and meeting the following criteria was randomly enrolled: non-infected by malaria (as determined by RDT and microscopy during the screening) and non-pregnant during the screening (as confirmed by the immunological test for pregnancy). Serum samples obtained from this control group were used as non-pregnant controls in this immunological assessment.

### Microscopy

We collected blood slides for microscopy examination using finger pricks. For nulliparous, blood slides were collected during a cross sectional survey in the HDSS catchment area. For primiparous, slides were collected at delivery,1 and 3 months post-delivery. Slides were stained with Giemsa 3% for 45–60 minutes and red by two independent microscopists who were blinded to the RDT results. Parasite density was calculated against 200 leukocytes, or against 500 leukocytes if the count was<10 parasites/200 leukocytes. Slides were considered negative if no parasites were seen after examination of 100 high power fields. In case of discrepancies between the two readers, the opinion of a third independent reader was required.

**Antigens**

The antigens (DBL5 and ID1-ID2a) were produced and offered by Professor AliSalanti (University of Copenhagen, Faculty of Health Sciences). The DBL5 domain (50 kD) from FCR3 was produced in insect cells purified according to the HIS tag purification procedure, while the ID1-ID2a fragment (75 kD) also from FCR3 was produced in *E. coli* purified according to IEX protocol. Both HIS tag purification and IEX method were followed by size exclusion procedure, as described by Clausen *et al*. (Clausen *et al.* 2012).

**Antibody levels measurement by ELISA**

For the serological analysis, optimal concentrations of each protein were coated in 96-well microtitration plates. Different subtypes of specific IgG were measured by ELISA following the method described by Guitard *et al.* (Guitard *et al.* 2008). Briefly, the plates were coated with 1 µg/mL of either DBL5-domain or ID1-ID2a fragment of VAR2CSA overnight and then incubated with 100 µL of human plasma at 1:100 dilution. The secondary antibody used for the total IgG measures was a horse radish peroxidase-conjugated anti-human IgG (Life Technology, A24470). Between each step, the plates were washed with PBS-Tween™ 20 buffer at 0.5%. For the IgG subclasses quantification, we used purified mouse monoclonal antibodies against human IgG1 and IgG3 (Life Technology, A10648, MH1732). All reagents were used at predetermined optimal dilution (IgG: 1:10, 000;IgG1: 1: 2,000 and IgG3: 1: 5,000 respectively). A positive control sample and a negative one were used. The positive control consisted of a pooled serum samples from hyper-immune multigravid women living in Nanoro while the negative one consisted of a pooled serum samples from Caucasian donors. The optical density (OD) was obtained by subtracting the average OD of duplicate wells from that of the corresponding blankwells, using ELISA-Reader Multiskan$^{TM}$ GO's software. Seropositivity was defined for each of the domains as the level (in OD) above the mean + 2 standard deviations (SD) obtained from 15 control plasma samples collected in the nulliparous.

**Placental malaria**

Slides from the placenta biopsies obtained from the maternal-facing side of the umbilic cord were stained with he-matoxylin-eosin (Scott *et al.* 2014) and read by trained microscopists. Placental infection was classified as follows: (1) Acute infection (parasites present, malaria pigment absent), (2) chronic infection (parasites and malaria pigment present), (3) past infection (no parasites but pigment present) and (4) no infection (both parasites and malaria pigment absent) (Bulmer *et al.* 1993).

**Statistical analysis**

We used Stata Software: Release 15 (College Station, TX) for data summary and analyses. The distributions of continuous variables were assessed for normality by using the Shapiro-Wilks W test. Log transformations were applied when appropriate (Altman & Bland 2009). Spearman's rho test was used to assess associations between continuous variables. The comparison of antibody levels in infected and non-infected primiparous at delivery, 1 and 3 months post-delivery was analysed using the Unpaired Mann Whitney U test. Values of $p < 0.05$ were considered as statistically significant.

**RESULTS**

**Characteristics of the study population**

We enrolled 33 primiparous women in the study. Among them, 17 women (51.5%) were detected positive for malaria by microscopy during their pregnancy (Table 1). The median age was similar between primiparous and nulliparous (19 years old for infected women and 18.5 years old for non-infected women). At delivery, malaria peripheral infection was diagnosed by microscopy in 3 women (9.1%). The mean parasite density was estimated at 540.9 [95% CI: 0 - 5584] parasites/µL. In addition, 5 women (more than 20%) had placental malaria (PM), although most of them had malaria pigment, without parasites. Anaemia (Hb < 11 g/dL) was reported in 6 primiparous women with *Plasmodium falciparum* infection during their pregnancy (35.3%) compared 5 women among uninfected ones (31.3%). Anaemia was reported in 2nulliparous women (13.3%). The analysis of the IPTp-SP compliance and malaria attacks data showed that there were more malaria cases among the women who received > 2 doses of IPTp-SP (n = 10) compared to the women who received ≤ 2 doses of IPTp-SP (n = 7).
The majority of nulliparous (93.3%) were recruited during the dry season. A total of 20 women delivered during the transmission season (July-December), indicating that the majority had a chance of being infected late in pregnancy (Table1).

**Effect of malaria in pregnancy on specific antibody responses to DBL5 and ID1-ID2a at delivery**

Women who experienced malaria infection during their pregnancy had significant higher levels of antibody against

**Table 1.** Characteristicsofthe study population.

| Characteristics | | Nulliparous | Primiparous | |
|---|---|---|---|---|
| | | | Malaria infection during pregnancy | No malaria infection during pregnancy |
| **Numberofwomenpergroup–** n (%) | | 15 | 17 (51.5) | 16 (48.5) |
| **Medianage**(IQR) | | 19 (18 - 20.5) | 19 (18 - 19) | 18.5 (18 - 20.2) |
| **Meanhaemoglobin level**(SD) | | 11.9 (1.5) | 11.6 (35.3) | 11.4 (1.5) |
| **RDT results at delivery –** n (%) | Negative | - | 14 (82.4) | 15 (93.8) |
| | Positive | - | 3 (17.7) | 1 (6.3) |
| **Parasite density of peripheral infection at delivery –** Parasite/µL, Mean (min - max) | | - | 540.9 (0 - 5584) | 0 (0.0) |
| **Placental malaria at delivery (N = 21) –** n (%) | Active | - | 1 (9.1) | 0 (0.0) |
| | Past only | - | 2 (18.2) | 2 (20.0) |
| | No infection | - | 8 (72.7) | 8 (80.0) |
| **Anaemia–** n (%) | | 2 (13.3) | 6 (35.3) | 5 (31.3) |
| **IPTp-SP–** n (%) | ≤ 2 doses | - | 7 (41.2) | 10 (62.5) |
| | >2 doses | - | 10 (58.8) | 6 (37.5) |
| **Recruitment season–** n (%) | High trans. | 1 (6.7) | 12 (70.6) | 8 (50.0) |
| | Low trans. | 14 (93.3) | 5 (29.4) | 8 (50,0) |

IQR: interquartile range; SD: standard deviation; Hb: haemoglobin; IPTp-SP: intermittent preventive treatment with sulfadoxine-pyrimethamine given during pregnancy. High trans.: High transmission season (July and December); Low trans.: Low Transmission season (January to June).

both DBL5 and ID1-ID2a antigens at delivery. The mean values of total IgG (Fig. 1a and Fig. 1b), IgG1 (Fig. 1c and Fig. 1d) and IgG3 (Fig. 1e and Fig. 1f) specific to DBL5 and ID1-ID2a were higher in infected women during pregnancy than non-infected women. These findings indicate that during the course of pregnancy, malaria infections could have induced the production of specific antibodies to VAR2CSA recombinant fragments through eventual placenta infections.

**Relationship between specific VAR2CSA recombinant fragments antibodies and characteristic variables at delivery.**

To further check the relationship between the season of delivery, anaemia status at delivery, the number of IPTp-SP doses received during pregnancy, malaria infection status during pregnancy and levels of VAR2CSA recombinant fragments antibodies and subclasses, Spearman's rank-order correlation coefficients were determined (Table 2).

Data showed positive correlations between specific VAR2CSA recombinant fragments antibodies and malaria during pregnancy (all the p-values were <0.001; Table 3). A similar correlation was also seen between DBL5 IgG1 and anaemia ($\sigma$ = 0.37**,** p = 0.033). On the other hand, data showed negative correlation between DBL5 IgG3 ($\sigma$ = -0.47**,** p = 0.006), ID1-ID2a IgG3 ($\sigma$ = -0.55**,** p = 0.001) and the season of delivery. However, the number of IPTp-SP doses received during pregnancy did not significantly correlate with any specific VAR2CSA recombinant fragments antibody.

**The distribution of sera DBL5 and ID1-ID2a-specific antibodies at delivery, and at 1- and 3-months post delivery**

Data related to the distribution of sera DBL5 and ID1-ID2a-specific antibodies are reported in Table 3.

At delivery, the proportions of serum samples with positive levels of DBL5 IgG, DBL5 IgG3, ID1-ID2a IgG and ID1-ID2a IgG3 were similar between infected and non-infected women. However, proportions were higher for DBL5 and ID1-ID2a specific IgG1 in malaria infected women than non-infected women (p-values were 0.001 and < 0.001 respectively).

At 1 month post-delivery, the number of malaria cases were low (n = 3), consequently, higher proportions of seropositive individuals were found in the non-infected group (n = 28).

A similar trend was found at 3 months post-delivery. At this time-point, seven malaria cases were registered and

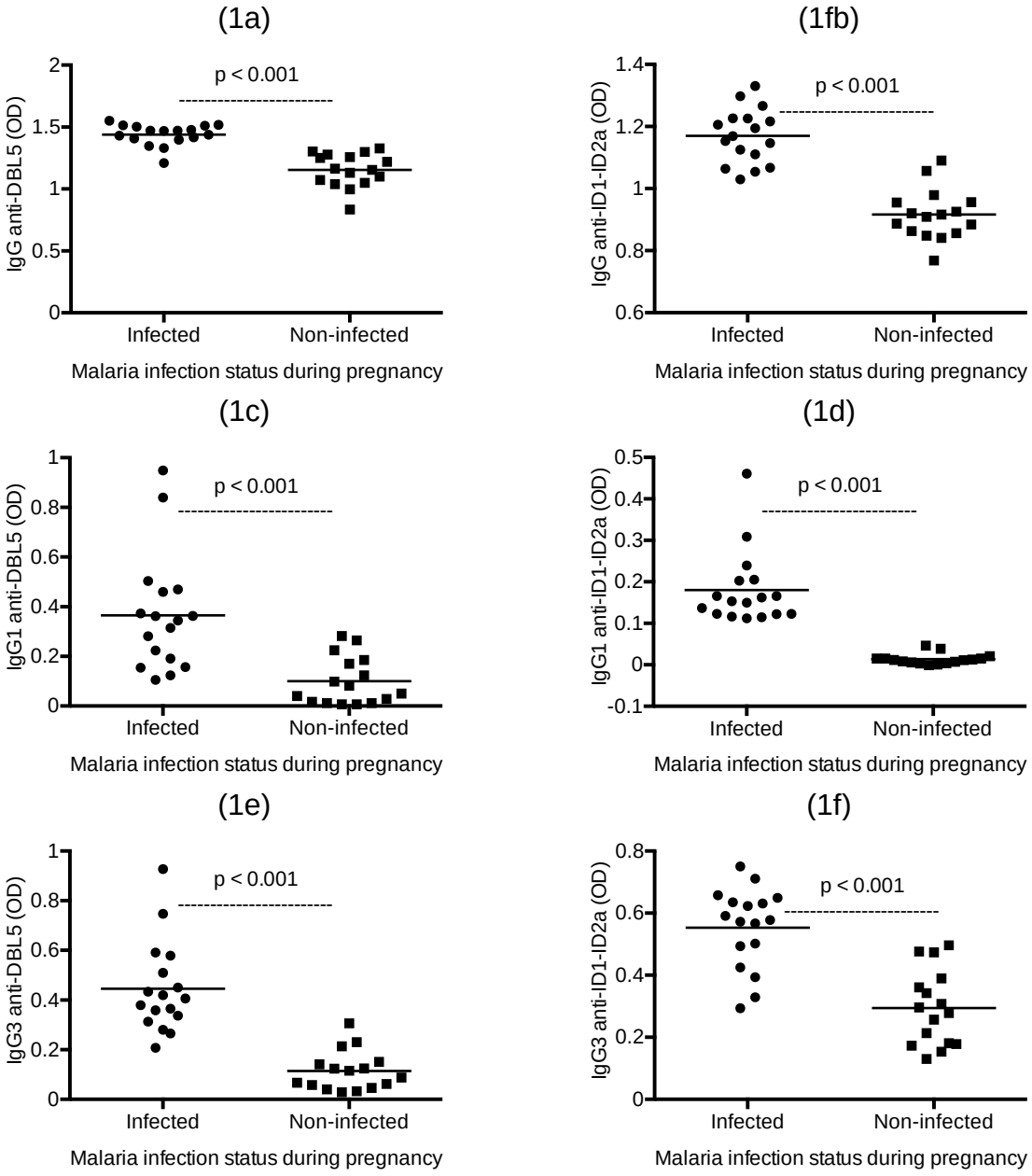


**Figure 1.** Antibody levels to VAR2CSA domains stratified by malaria infection status during pregnancy. (1a) levels of DBL5 IgG, (1b) levels of ID1-ID2a IgG, (1c) levels of DBL5 IgG1, (1d) levels of ID1-ID2a IgG1, (1c) levels of DBL5 IgG3 and (1f) levels of ID1-ID2a IgG3. Statistical differences between infected and non-infected primigravidae are indicated with the p-values obtained using the Mann Whitney test.

higher proportion were found in non-infection group (n = 25).

**Dynamics of sera DBL5 and ID1-ID2a specific antibodies during the follow up time period**

The trend of the distribution of sera DBL5 and ID1-ID2a-specific antibodies at delivery, 1 and 3 months post-delivery, regardless of women's malaria status is illustrated in Figure 2.

Compared to those reported at delivery, inconstant proportions of seropositive DBL5 and ID1-ID2a-specific antibody subclasses were observed. Proportions of seropositive to DBL5-specific IgG3 individuals decreased significantly between delivery and 1-month post-delivery, ($p < 0.001$). A similar decrease was observed between delivery and 3 months post-delivery ($p<0.001$). For ID1-ID2a IgG3, the difference was statistically significant between proportions at delivery and 3-months post-delivery ($p = 0.001$).

However, we observed a slight but not significant increase in proportions of seropositive to ID1-ID2a-specific IgG1 (delivery versus 1-month delivery: $p = 0.832$; delivery versus 3 months delivery: $p = 0.167$).

**Table 2.** Relationships between season of delivery, anaemia at delivery, number of IPTp-SP doses, infection during pregnancy and levels of VAR2CSA recombinant fragments antibodies (Spearman's rank-order correlation coefficients).

| | Season of delivery | | Anaemia at delivery | | Number of IPTp-SP doses | | Infection during pregnancy | |
|---|---|---|---|---|---|---|---|---|
| | σ | p | σ | p | σ | p | σ | p |
| **DBL5 IgG** | -0.14 | 0.426 | 0.11 | 0.540 | 0.07 | 0.699 | 0.82 | **<0.001** |
| **DBL5 IgG1** | -0.24 | 0.177 | 0.37 | **0.033** | 0.07 | 0.699 | 0.68 | **<0.001** |
| **DBL5 IgG3** | -0.47 | **0.006** | 0.07 | 0.719 | 0.25 | 0.163 | 0.83 | **<0.001** |
| **ID1-ID2a IgG** | -0.29 | 0.098 | 0.22 | 0.216 | 0.20 | 0.271 | 0.83 | **<0.001** |
| **ID1-ID2a IgG1** | -0.20 | 0.260 | 0.22 | 0.216 | 0.19 | 0.287 | 0.87 | **<0.001** |
| **ID1-ID2a IgG3** | -0.55 | **0.001** | 0.07 | 0.719 | 0.02 | 0.916 | 0.73 | **<0.001** |

σ: Spearman rho

## DISCUSSION

Our study showed that more than half of study participants experienced at least one malaria episode during their pregnancy. This finding confirms the report from a previous study evaluating the importance of malaria during pregnancy recently conducted in the same settings (Natama *et al.* 2017). The immediate consequence of malaria during pregnancy is parasite sequestration in the placenta, i.e. PM (Mens *et al.* 2010). Primigravidae are more susceptibility to PM due to the lack of specific antibodies to placental malaria antigens (Dechavanne *et al.* 2015; Salanti *et al.* 2004).

The study population consisted of primigravidae only. The higher levels of antibody at delivery in women infected during pregnancy confirm that malaria infection during pregnancy plays an important role in the establishment of immune responses against VAR2CSA (Elliott *et al.* 2005). Actually, the majority of placental infection detected at delivery were past infections, i.e. characterized by only the presence of hemozoin. The mechanism underlying this effect could be linked firstly to the sequestration of *P. falciparum* parasites in the placenta, followed by the recognition and binding of the *Pf*EMP1 variant VAR2CSA at the surface of the infected erythrocytes to the placenta's chondroit in sulphate A (CSA) receptor (Achur *et al.* 2000; Beeson *et al.* 2000; Fried & Duffy 1996; Ismail *et al.* 2000). Therefore, the final step of this mechanism is the production by the immune system of infected pregnant women, of specific antibodies to VAR2CSA in order to defend their body against PM (Salanti *et al.* 2004).

Furthermore, our data showed positive correlations between specific VAR2CSA recombinant fragments antibodies and malaria during pregnancy. This finding indicates that during the course of pregnancy, malaria infections could have induced the production of specific antibodies to VAR2CSA recombinant fragments through eventual placenta infections. This could suggest that each malaria infection during pregnancy can induce antigenic exposure and therefore immune responses to targeted antigens (Salanti *et al.* 2004). This is probably patent, since by reducing the risk of infection during pregnancy by regular uptake of IPTp-SP (at least 2 doses), no significant correlation was found between specific VAR2CSA recombinant fragments antibodies and this factor (Staalsoe *et al.* 2004). In addition, both anaemia and antibodies production are the consequences of *Plasmodium falciparum* infection. Therefore, the interactions between the two outcomes are confirmatory of the assumption that malaria during pregnancy is intimately linked to PM (Omer *et al.* 2017).

Compared to other IgG subclasses, IgG3 has features which are characteristics to enhance opsonization of malaria-infected erythrocytes and promotion of effect or functions (Mathiesen *et al.* 2013; Stapleton *et al.* 2011). The finding related to negative correlations between DBL5 IgG3, ID1-ID2a IgG3 and the season of delivery, has several implications. This finding could be an indication that during the high transmission season lower levels of IgG3 are produced. On the other hand, IgG3 is known to be highly polymorphic, with distinct variants that give rise to allotypes (Dechavanne *et al.* 2017). During the high transmission season, women are highly exposed to repeated malaria attacks, and also to the multiplicity of antigens. In these conditions, it is plausible that only a subset of IgG3 allotypes specific to VAR2CSA recombinant fragments have been quantified at the time

**Table 3.** Proportions of sera sample with positive levels of specific VAR2CSA recombinant fragments antibodies.

| Delivery | Cut-off * | All primiparous(N = 33) (%) | Infected during pregnancy (n= 17) (%) | Non-Infected during pregnancy (n = 16) (%) | p |
|---|---|---|---|---|---|
| DBL5 IgG | 0.01 | 100.0 | 51.5 | 48.5 | - |
| DBL5 IgG1 | 0.12 | 66.7 | 72.7 | 27.3 | 0.001 |
| DBL5 IgG3 | 0.02 | 100.0 | 51.5 | 48.5 | - |
| ID1-ID2a IgG | 0.03 | 100.0 | 51.5 | 48.5 | - |
| ID1-ID2a IgG1 | 0.05 | 54.6 | 94.4 | 5.6 | <0.001 |
| ID1-ID2a IgG3 | 0.03 | 100.0 | 51.5 | 48.5 | - |
| **1 month after delivery** | **Cut-off** | **All primiparous(N = 31) (%)** | **Infected (n = 3) (%)** | **Non-Infected (n = 28) (%)** | |
| DBL5 IgG | 0.01 | 100.0 | 9.7 | 90.3 | |
| DBL5 IgG1 | 0.12 | 58.1 | 11.1 | 88.9 | 0.751 |
| DBL5 IgG3 | 0.02 | 41.9 | 15.4 | 84.6 | 0.361 |
| ID1-ID2a IgG | 0.03 | 100.0 | 9.7 | 90.3 | |
| ID1-ID2a IgG1 | 0.05 | 58.1 | 11.1 | 88.9 | 0.751 |
| ID1-ID2a IgG3 | 0.03 | 96.8 | 10.0 | 90.0 | 0.814 |
| **3 months after delivery** | **Cut-off** | **All primiparous(N = 31) (%)** | **Infected (n = 7) (%)** | **Non-Infected (n = 25) (%)** | |
| DBL5 IgG | 0.01 | 100.0 | 21.9 | 78.1 | - |
| DBL5 IgG1 | 0.12 | 62.5 | 25.0 | 75.0 | 0.581 |
| DBL5 IgG3 | 0.02 | 56.3 | 16.7 | 83.3 | 0.419 |
| ID1-ID2a IgG | 0.03 | 100.0 | 21.9 | 78.1 | - |
| ID1-ID2a IgG1 | 0.05 | 75.0 | 20.8 | 79.2 | 0.805 |
| ID1-ID2a IgG3 | 0.03 | 71.9 | 21.7 | 78.3 | 0.976 |

*****Levels were considered positive if they exceeded the cut-off values calculated from results obtained with the 15 control sera samples collected in the nulliparous.

of sampling. Malaria-specific IgG3 is known to be strongly associated with specific malaria immunity (Dobbs & Dent 2016). It's also known to have a shorter half-life (Vidarsson *et al.* 2014).

In this study, the proportion of seropositive subjects to both DBL5 and ID1-ID2a at delivery was higher in women who experienced at least one episode of malaria during pregnancy compared to non-infected ones. This was verified for the IgG1 subclass in particular. A previous study (Megnekou *et al.* 2005) has also reported increasing levels of VAR2CSA-specific IgG1 associated with gestational age. The role of this cytophilic antibody in the protection against clinical malaria is well described in the general population (Lusingu *et al.* 2005; Ndungu *et al.* 2002; Oeuvray *et al.* 2000).

Moreover, previous investigations in non-pregnant women living in an area of stable malaria transmission, have reported that VAR2CSA-specific IgG levels and B cell numbers progressively increase during pregnancy and decrease during postpartum in both primigravidae and multigravidae (Ampomah *et al.* 2014; Staalsoe *et al.* 2001). We investigated the dynamics of anti-VAR2CSA antibodies from delivery to 3 months post-delivery, and the results further support the hypothesis that VAR2CSA is exclusively expressed by placental parasites. Indeed, higher antibody levels were found at delivery in women who experienced at least one *P. falciparum* malaria infection during their pregnancy. In addition, these anti-VAR2CSA antibody responses decreased during the post-partum, suggesting that in the absence of placenta, parasites expressing epitopes similar to those expressed on VAR2CSA domains are absent. There was no boost of the immune responses to these antigens as reported elsewhere (Ramharter *et al.* 2005a). Since no boosting was observed in seroprevalences from delivery to 3 months post-delivery, these findings support that antibodies

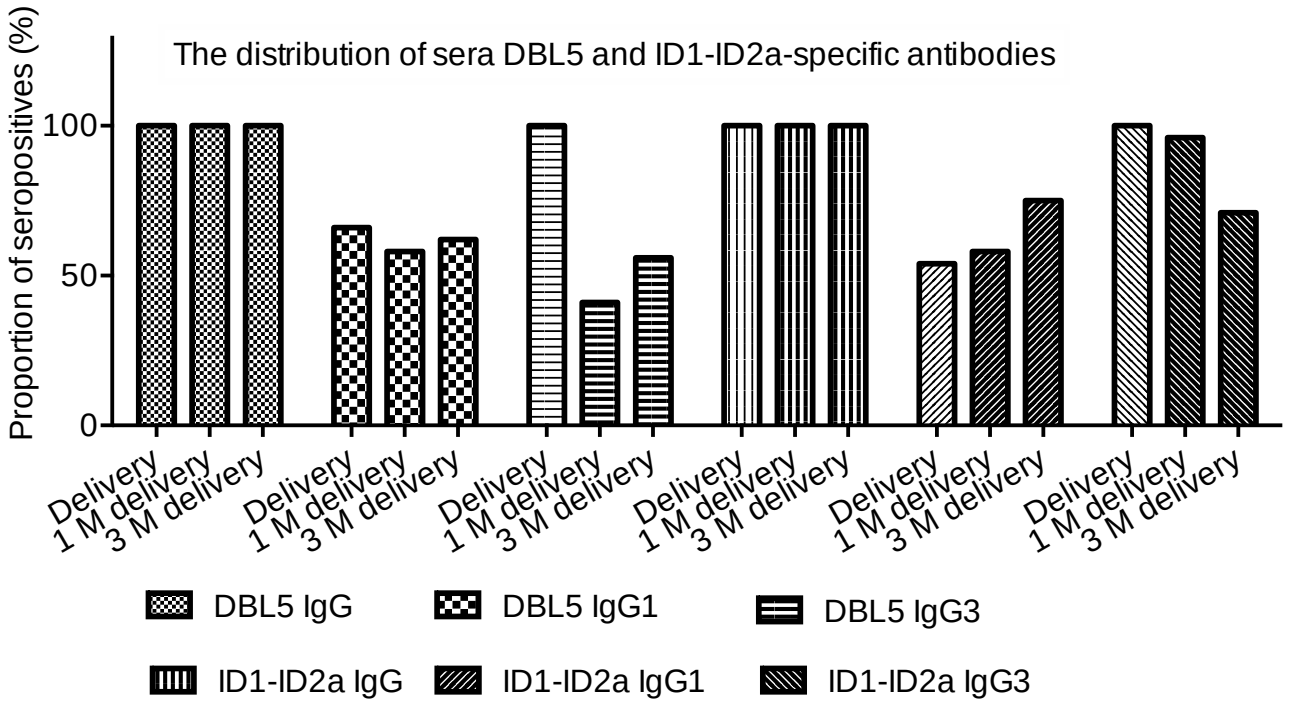


**Figure 2.** Distribution of plasma levels of VAR2CSA recombinant fragments specific antibodies in primigravidas. 1 M delivery: 1 months after delivery; 3 M delivery: 3 months after delivery. The proportions of sera DBL5 and ID1-ID2a-specific antibodies at delivery, 1 and 3 months after delivery, independently to women's malaria status. Each triplet constitutes a separated bar graph illustrating the evolution of proportions of seropositive individuals to a particular antigen (DBL5 and ID1-ID2a) from delivery to 3 months after delivery.

to DBL5 and ID1-ID2a are specific to malaria during pregnancy.

The limited number of participants can be considered as a limitation of this study. This was due to financial constraints. In addition, the small number of placental infections detected have limited the power of our analyses which could induce statistical biases. A similar study in a larger sample size will probably provide better estimations. Nevertheless, the analyses performed in this study could contribute to improve the knowledge on this topic.

## CONCLUSION

The results of our study confirmed that malaria infection during pregnancy contributes to establish specific humoral immunity to placental malaria antigens in women living in endemic areas. While acknowledging the limitation of this study, we provide some indications that in the absence of placenta, malaria infection during the postpartum cannot boost the specific immune response to PM antigens. Understanding the mechanism that underlies the decay of humoral immunity against PM antigens in primigravidas will probably be useful for the reliable scheme of a VAR2CSA vaccine, appropriate for use in different malaria-endemic areas.

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