MAJOR ARTICLE

Maternal Human Immunodeficiency Virus-Associated Hypergammaglobulinemia Reduces Transplacental Transfer of Immunoglobulin G to *Plasmodium falciparum* Antigens in Cameroonian Neonates

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Background. Human immunodeficiency virus (HIV) infection reduces placental transfer of antibodies from mother to the fetus for many antigens; however, conflicting data exist for transfer of immunoglobulin G (IgG) to malarial antigens. The mechanism(s) underlying reduced placental transfer is unknown.

Methods. Levels of maternal and cord total IgG, IgG subclasses, and cord-to-mother ratios (CMRs) were measured in 107 mother-cord pairs to 3 malarial antigens: circumsporozoite protein (CSP), apical membrane antigen 1 (AMA-1), merozoite surface protein 1 (MSP-1), and tetanus toxoid C-fragment (TTc).

Results. Immunoglobulin G levels to CSP and TTc were lower in HIV+ mothers, and cord IgG to CSP, MSP-1, and TTc were significantly lower in neonates born to HIV+ mothers (all P values <.05). The prevalence of mothers with hypergammaglobulinemia was significantly higher among HIV+ women (68%) compared with HIV− mothers (8%) ($P < .0001$). Maternal hypergammaglobulinemia was associated with reduction in transplacental transfer of antibodies to CSP ($P = .03$), MSP-1 ($P = .004$), and TTc ($P = .012$), and CMRs <1 were found for MSP-1 (odds ratio [OR] = 6.5), TTc (OR = 4.95), and IgG1 to CSP (OR = 3.75, $P = .025$) in statistical models adjusted for maternal IgG.

Conclusions. Data confirmed that HIV infections are associated with lower cord antibody levels to malarial antigens and that hypergammaglobulinemia may contribute to reduced antibody transfer.

Keywords. antibodies; human immunodeficiency virus; hypergammaglobulinemia; malaria; placental transfer.

Placental transfer of antibodies from mother to the fetus is an adaptive mechanism by which deficiencies in neonate immunity are counterbalanced, providing short-term passive protection [1]. The amount of antibodies transferred to the neonate determines the degree and length of protection the infant has from some infections [2]. Transplacental transfer of immunoglobulin G (IgG) is especially important for infections for which vaccines are not available, and clinical protection is mediated by naturally acquired antibodies [3, 4] that are not fully acquired until adulthood [5].

Placental transfer of IgG across placental syncytiotrophoblast cells is mediated by Fc neonatal receptors (FcRn) [6, 7] that are located inside endosomes [8]. Placental transport of IgG depends on maternal IgG levels, IgG subclass (preferential transport of IgG1, followed by IgG4, IgG3, and IgG2), antibody avidity, gestational age, and number and functionality of FcRn [1, 9]. Placental transfer of IgG may be influenced by prematurity, intrauterine growth restriction, maternal human immunodeficiency virus (HIV), placental malaria (PM), hypergammaglobulinemia, and maternal immune deficiencies [1, 10–18].

In sub-Saharan Africa, women disproportionately bear the burden of the HIV epidemic [19, 20]. In Cameroon, the national HIV prevalence is 5.6% in women and 2.9% in men, with a prevalence of 7.8% among pregnant women [21, 22]. Studies have demonstrated that HIV reduces transplacental transfer of maternal IgG to *Streptococcus pneumonia*, *Haemophilus influenza* type b, herpes simplex virus, varicella-zoster virus, streptolysin O, tetanus, measles, and pneumococcal capsular IgG [12, 13, 23–26]. The impact of HIV on transfer of IgG to *Plasmodium falciparum* antigens is less clear, because data from 2 major studies are conflicting [27, 28] and the mechanism responsible for deficient transport of antimalarial IgG in the context of HIV has not been investigated.

This study examined the influence of HIV and hypergammaglobulinemia on placental transfer of IgG to pre-erythrocytic

and erythrocytic-stage malarial antigens in Cameroonian pregnant women. In addition, IgG1 and IgG3 antibodies to malarial antigens were investigated because IgG1 levels to malarial antigens predominate [29, 30] and are preferentially transferred transplacentally [1].

METHODS

Ethical Review

The study was approved by the National Ethics Committee, Cameroon (Number 2013/11/366/L/CNERSH/SP) and the Institutional Review Board, University of Hawaii (CHS 21 370). Written informed consent was obtained from each woman at enrollment.

Study Site and Population

The case-controlled study was carried out in the maternity ward of Central Hospital, Yaoundé, Cameroon (2014–2015). Inclusion criteria included women ≥18 years of age, who did not have pre-existing health conditions that might influence the study (diabetes, preeclampsia, and hemolysis elevated liver enzymes low platelet count syndrome). Women who had spontaneous abortions were also excluded. This study enrolled HIV-positive (HIV+) cases that met inclusion criteria and HIV-negative (HIV−) controls at a 1:2 ratio. A questionnaire was used to record maternal demographic information, clinical history, use of the intermittent preventive treatment and insecticide treated bednets (ITNs), HIV status, and use of antiretroviral therapy (ART). According to the Cameroonian government's guidelines, pregnant women were tested for HIV during pregnancy and received tetanus vaccination. The standard of care is for HIV+ women to receive ART with zidovudine from 14 weeks of pregnancy at government HIV treatment centers for prevention of mother-to-child transmission of HIV. For the few women newly diagnosed with HIV at delivery, a single dose of nevirapine and the first dose of zidovudine were given in the maternity ward, and neonates were placed on nevirapine immediately after birth and linked to the government HIV care facility for follow up. Information recorded for neonates included infant birth weight and Apgar score. Length of gestation was estimated based on date of last menstrual period or ultrasound data when available. Neonates born before 37 weeks were classified as premature. Singletons weighing less than 2500 grams were considered low birth weight (LBW).

Specimen Collection and Processing

Before active labor or after delivery, maternal venous blood samples were collected. After delivery, cord blood and placental intervillous space blood samples were obtained [31]. In addition, a biopsy of placental tissue was retained for parasitological studies.

Human Immunodeficiency Virus Ribonucleic Acid Levels

Information on the women's HIV status was obtained from the Yaoundé Central Hospital medical records. Human immunodeficiency virus copy number was determined at the Chantal-Biya International HIV Reference Center, Yaoundé, when sufficient plasma was available for testing (n = 15 women) using Abbott RealTime polymerase chain reaction HIV-1 kit (Abbott Park, IL). The lower and upper detection limits were <150 copies/mL and 10 000 000 copies/mL, respectively.

Diagnosis of Malaria, Placental Malaria, and Anemia

Peripheral, placental intervillous space, and cord blood samples were evaluated for *P. falciparum* parasites by microscopy [32]. Placental biopsies were fixed in 10% buffered formalin, embedded, stained with hematoxylin-eosin, and examined for parasites. A woman was considered to have PM if infected erythrocytes were detected in blood smears of intervillous space blood, impression smears of villous tissue, or histological sections of the placenta [33]. Maternal hemoglobin (Hb) levels were determined using HemoCue Hb 201 (HemoCue, Sweden). Women with <11 g/dL Hb levels were considered to be anemic (according to the World Health Organization 2012 guidelines).

Laboratory Assays for Total Immunoglobulin G

Total IgG in maternal peripheral and cord plasma was measured at 1:400 000 dilution using a total IgG enzyme-linked immunosorbent assay (ELISA) kit (MabTech, Cincinnati, OH) and standards from the National Institute for Biological Standards and Controls. Hypergammaglobulinemia was defined as having total IgG >1600 mg/dL [13, 34, 35]. Optical density values were converted to total IgG concentration (mg/dL) using a standard curve and corrected for dilution factor of 400 000. To validate IgG ELISA, total IgG levels were compared for 8 North American pregnant women (Hawaii Biospecimen Repository) and 6 Cameroonian women [32] in the ELISA assay and by nephelometry (Clinical Labs of Hawaii).

Recombinant Plasmodium falciparum Antigens

The following antigens were used: recombinant apical membrane antigen 1 ([AMA-1] FVO, expressed in yeast, molecular weight [MW] 83 kDa) and merozoite surface protein 1 ([MSP-1]$_{42}$ FVO, expressed in *Escherichia coli*, MW 42 kDa) (provided by C. Long, Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD); synthetic peptide containing B-cell epitopes from circumsporozoite protein (CSP) (synthesized by AnaSpec, Inc., San Jose, CA) [36]; and tetanus toxin C-fragment ([TTc] from J. K.) plasmid [37].

Measuring Immunoglobulin G Using a Multianalyte Platform Assay

Antigens were coupled to MagPlex-C microspheres (Luminex, Austin, TX) as described previously, with 1 million microspheres coupled with 1 μg of MSP-1, AMA-1, and TTc and 15 μg of CSP [36]. Immunoglobulin G was measured in a multianalyte platform assay as previously described [30, 36]. In brief, 50 μL (2000 microspheres/test) antigen-coupled microspheres were incubated with 50 μL maternal and cord plasma at 1:100 dilution in phosphate-buffered saline (PBS) and 1% bovine serum albumin

(BSA) for 1 hour at 25°C on a shaker. After 3 washes, microspheres were incubated with 100 μL secondary antibody diluted to 2 μg/mL in PBS-1% BSA per well (R-phycoerythrin-conjugated, Affini Pure F(ab′)2 fragment, goat anti-human IgG Fc fragment specific; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hour. Microspheres were washed 3 times, resuspended in 100 μL PBS-1% BSA, and analyzed using a MAGPIX reader (EMD Millipore, Billerica, MA). The results were expressed as median fluorescence intensity (MFI). Positive and negative controls were included on each plate consisting of (1) pool of plasma from 6 Cameroonian adults (positive assay control) and (2) pool of plasma from 13 North Americans (negative assay control). In addition, plasma from 20 North American adults was used to determine the cutoff for antibody positivity.

Immunoglobulin G Subclass Determination to Malaria Antigens

Immunoglobulin G IgG1 and IgG3 subclasses to the antigens were measured in maternal peripheral and cord plasma samples as previously described [29]. In brief, after incubating beads with 50 μL of a 1:100 dilution of plasma, beads were washed and incubated with 100 μL mouse anti-human IgG1 (Molecular Probes) at 1:250 dilution or mouse anti-human IgG3 (clone HP6050 I7260; Sigma) at 1:2000 dilution in PBS-1% BSA for 1 hour at 25°C. After washing, beads were resuspended in 100 μL phycoerythrin-labeled donkey anti-mouse IgG H + L (catalog number 715-116-150, Jackson ImmunoResearch Laboratories) at a 1:250 dilution in PBS-1% BSA for 1 hour, washed, and resuspended in 100 μL PBS-1% BSA. Median fluorescence intensity were determined as described above.

Statistical Analysis

Demographic, clinical, and assay variables were summarized using descriptive statistics; continuous variables were compared using Student t test; and proportions were compared using Fisher's exact test. Maternal and cord antibody levels stratified by HIV status were compared using the Mann-Whitney U test. Proportion of individuals who were seropositive for a given antigen was determined based on mean MFI + 2 standard deviation cutoff for 20 North American individuals. To assess transfer across the placenta, cord-to-mother ratio (CMR) of MFI were calculated and compared stratified by maternal HIV or hypergammaglobulinemia status using Mann-Whitney U test using data only for mothers that were seropositive for a given antigen. Multiple linear regression models were used to assess the effect of maternal HIV or hypergammaglobulinemia status on cord IgG levels (natural logarithm) and CMR. Regression models were built using the forward stepwise method, and the influence of both maternal and neonatal factors was assessed. In addition, logistic regression models were used to assess the odds of having reduced placental antibody transfer defined as CMR <1 for a given antigen, (because in healthy pregnancies, CMR ranges from 1.0 to 1.2 [1]). In addition, the influence of maternal and neonatal factors on log regression models was assessed. All statistical analyses were performed using GraphPad Prism and STATA14; P values of ≤.05 were regarded as statistically significant.

RESULTS

Characteristics of the Study Population

A comparison of pregnancy-related factors between HIV− (n = 76) and HIV+ (n = 31) women showed that HIV+ mothers were slightly older (30 vs 27.5 years, P = .04), but otherwise the women had similar characteristics (Table 1 and Supplementary Table 1). Among the 31 HIV+ women, 93% had received the ART. Analysis of a subset of samples (n = 15) revealed that 60% of HIV+ women had detectable viral loads (>150 copies/mL), with median viral loads of 15 423 copies/mL (minimum 152 copies/mL, maximum 790 190 copies/mL, interquartile range 223 139 copies/mL). CD4 counts were available for only 9 women, with 44.4% having CD4 counts below 350 cells/mm³. Only 3 of 30 HIV+ women were coinfected with PM (Table 1). Thus, there were no major differences in pregnancy-related characteristics between HIV− and HIV+ women, even though some of the HIV+ women had low CD4 counts and high viral loads.

Levels of Maternal and Cord Antimalarial Immunoglobulin G Subclasses at Delivery

The majority of mothers had IgG to the 3 malaria antigens (Supplementary Table 2). Compared with maternal levels, cord IgG levels for AMA-1, MSP-1, and TTc were significantly lower (all P < .05; Supplementary Figure 1); as expected, maternal and cord antibody levels had a positive linear relationship (Supplementary Figure 2). Mothers who were HIV+ had significantly lower IgG levels to CSP and TTc than HIV− mothers (all P values <.05) (Figure 1A). Likewise, HIV-exposed neonates had lower IgG levels to CSP, MSP-1, and TTc than HIV− unexposed neonates (all P values <.05) (Figure 1B). As expected, IgG1 was the predominant subclass for all malaria antigens and TTc. Mothers who were HIV+ had significantly lower IgG1 levels to CSP, MSP-1, and TTc compared with HIV− mothers (all P values <.05) (Figure 1A), and HIV-exposed neonates had lower cord blood IgG1 levels to all antigens (P values <.05) (Figure 1B). Only a few women and neonates had IgG3 to malaria antigens, making it impossible to analyze. Pregnant Cameroonian women in this cohort do not have IgG2 and IgG4 subclasses for CSP and MSP-1 (data not shown); therefore, IgG2 and IgG4 were not included in the study.

Maternal Total Immunoglobulin G Levels at Delivery

Before conducting the study, the commercial ELISA-based total IgG assay was validated by testing plasma with known concentrations of total IgG established by nephelometry (Supplementary Figure 3). Congruent results between the 2 assays were found (Pearson correlation R^2 = 0.75); however, milligram per deciliter values determined by the ELISA tended to be lower than by nephelometry.

Total IgG levels were significantly higher in HIV+ women (P < .0001) and PM+ women (P = .0037) compared with

Table 1. Demographic, Clinical, and Hematological Parameters of 107 Participants Enrolled at Delivery

Characteristic	HIV−	HIV+	P Value
Enrolled participants (n)	76	31	
Maternal Factors			
Age (years)[a]	27.5 ± 6	30 ± 5	.04
Axillary temperature[b] (°C)	37.4 (0.7)	37.3 (0.5)	.2
Fever[c] (>37.5°C)	20/64 (31)	6/26 (23)	.6
BMI[a]	29.0 ± 4	28.5 ± 3.7	.6
Hemoglobin levels[a] (g/dL)	12.1 ± 1.6	11.7 ± 1.7	.4
Anemia[c] (<11 g/dL)	13/65 (20)	6/25 (24)	.8
ART use[c]	n/a	26/28 (93)	n/a
IPT and bednet use[c]	63/70 (90)	30/30 (100)	.1
Number of SP doses[b]	2 (1)	2 (0.5)	.4
Bednet[c]	55/72 (76)	26/31 (84)	.4
Peripheral malaria[c,d]	13/61 (21)	4/27 (15)	.6
Parasite density (parasites/µL)[b,e]	1880 (15 540)	1080 (17 735)	.6
Placental malaria[c,d,f]	14/64 (22)	3/30 (10)	.3
Parasitemia by impression smears (%)[b,e]	0.65 (6.8)	0.23 (0.58)	.4
Gravidity[b]	3 (2)	3 (3)	.4
Primigravidae[c]	17 (23)	6 (19)	.8
Multigravidae[c]	56 (77)	25 (81)	.8
Neonatal Factors			
Length of gestation[b] (weeks)	39.6 (2.3)	39.4 (2.7)	.6
Preterm deliveries[c] (<37 wks)	9/70 (13)	6/31 (19)	.5
Singleton deliveries[c]	3/70 (4)	2/31 (6.5)	.6
Neonate gender[c] (male)	40/73 (55)	20/31 (65)	.4
Placental weight[b] (g)	600 (171)	590 (150)	.5
Neonate weight[a] (g)	3161 ± 591	3127 ± 497	.8
Low birth weight[c] (<2500 g)	7/73 (9.6)	2/31 (6.5)	.7
Apgar[b]; at 1 min, at 5 min	8 (1); 9 (2)	8 .5 (1); 9 (1.5)	.2; 1
Cord malaria infection[d]	0	0	n/a

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IPT, intermittent preventive treatment (with sulfadoxine pyrimethamine and bednets); n/a, not applicable; SP, sulfadoxine pyrimethamine.

[a] Mean ± standard deviation, compared using t test.

[b] Medians (interquartile range) compared using Mann-Whitney U test.

[c] Frequencies are reported as number (percentage in parentheses) and compared using Fisher's exact t test. Numbers may not add up to 107 due to missing responses.

[d] Blood smears were tested by microscopy for presence of *Plasmodium falciparum*, *Plasmodium ovale*, and *Plasmodium malariae*. Only *P falciparum* was detected.

[e] Calculated for smear-positive women only.

[f] Woman was considered placental malaria-positive if malaria-infected erythrocytes were found in the intervillous blood smears, impression smears, or histological slides by microscopy.

HIV-PM− women (Figure 2A). In this study, 86 women were tested for total IgG and 32.6% (28 of 86) were found to have IgG levels >1600 mg/dL, ie, had hypergammaglobulinemia. Among these women, 15 (53.6%) were HIV+, 5 (17.9%) were PM+, 3 (10.7%) were both HIV+ and PM+, and 5 (17.9%) had elevated IgG levels due to other causes (Figure 2C). It is interesting to note that total IgG levels were similar in HIV+ and PM+ mothers (P = .13). Accordingly, the prevalence of hypergammaglobulinemia was higher in HIV+ women (68%) compared with HIV− women (11%) (P < .0001), and 6 of 9 (67%) HIV+ women with detectable viral loads had hypergammaglobulinemia.

Human immunodeficiency virus-exposed neonates had higher total IgG levels compared with HIV-unexposed neonates (P = .02), and some of these neonates had hypergammaglobulinemia (Figure 2B). No significant differences were observed in total IgG levels between preterm and full-term neonates (P = .57); similarly,

no significant difference was observed in IgG levels between neonates with LBW and normal weight (P = .72) (data not shown). When considering CMR of total IgG levels, HIV+ women had lower, but statistically not significant CMR of total IgG (P = .18) compared with HIV− women (Figure 2D). Significantly lower CMR of total IgG (P = .03) was observed in HIV-malaria coinfected mothers compared with controls (Figure 2D).

Influence of Human Immunodeficiency Virus and Associated Hypergammaglobulinemia on Transfer of Immunoglobulin G to Malaria Antigens

Human immunodeficiency virus had a nonsignificant effect on CMR of IgG to malaria antigens, but a significant negative impact on CMR of IgG1 to CSP (P = .02), AMA-1 (P = .03), MSP-1 (P = .02), and TTc (P < .0001) compared with CMR from HIV− mothers (Figure 3A). In linear regression models adjusted for maternal and neonatal factors, maternal HIV
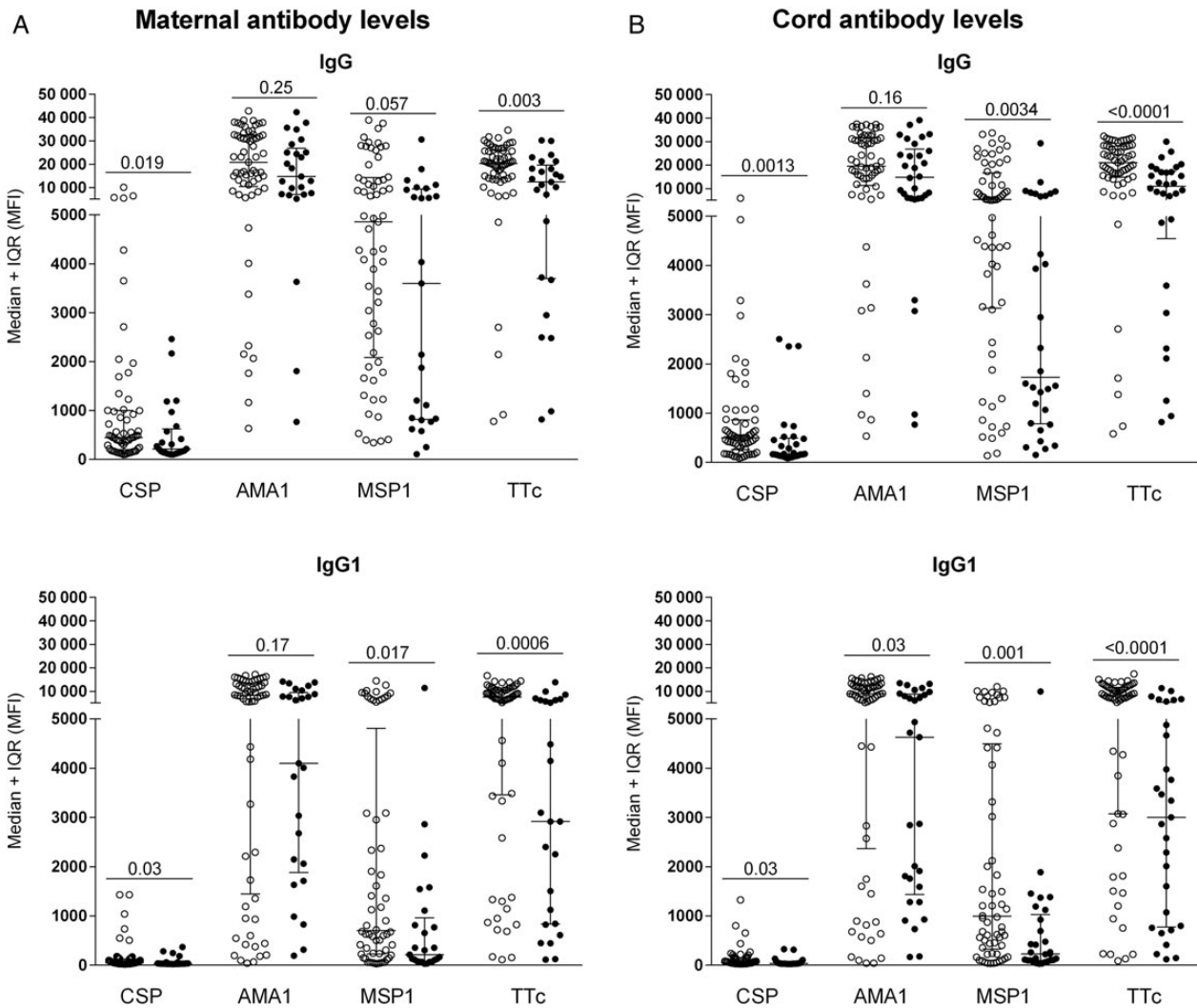
Figure 1. Antibody levels to malaria antigens in maternal peripheral and cord blood. (A) Maternal antibody levels stratified by human immunodeficiency virus (HIV) status; (B) cord antibody levels stratified by maternal HIV status. Medians and interquartile ranges were plotted, and antibody levels were compared using Mann-Whitney *U* test. Open circles represent HIV-negative mothers (A) or neonates born to HIV-negative mothers (B): black circles represent HIV-positive mothers (A) or neonates born to HIV-positive mothers (B). Abbreviations: AMA-1, apical membrane antigen 1; CSP, circumsporozoite antigen; IgG, immunoglobulin G; IQR, interquartile range; MFI, mean fluorescence intensity; MSP-1, merozoite surface protein 1; TTc, tetanus toxoid.

infection was associated with reduced CMR to TTc ($P = .002$), IgG1 to CSP ($P = .01$), and TTc ($P < .0001$) (Table 2). In addition, HIV was associated with likelihood of having CMR <1 of IgG to TTc (odds ratio [OR] = 10, $P < .0001$), IgG1 to AMA-1 (OR = 3, $P = .045$), MSP-1 (OR = 3.5, $P = .017$), and TTc (OR = 6.7, $P < .0001$) in logistic regression models.

Maternal hypergammaglobulinemia had a greater impact than HIV on placental transfer of IgG and IgG1 to all malaria antigens and TTc (all $P < .05$) (Figure 3B). A higher proportion of hypergammaglobulinemic mothers had CMR <1, with a significantly higher proportion of women with CMR < 1 for IgG to MSP-1 ($P = .006$) and TTc ($P = .004$) compared with the hypergammaglobulinemia-negative women (Supplementary Table 3). The influence of hypergammaglobulinemia remained significant after

data were adjusted for maternal and neonatal factors in linear regression models (Table 2); hypergammaglobulinemia was associated with significantly reduced CMR of IgG to CSP ($P = .03$), MSP-1 ($P = .004$), TTc ($P = .012$), IgG1 to CSP ($P = .02$), and TTc ($P = .007$). Finally, hypergammaglobulinemia was associated with increased likelihood of CMR <1 of IgG to MSP-1 (OR = 6.5, $P = .002$) and TTc (OR = 4.95, $P = .002$), IgG1 to CSP (OR = 3.75, $P = .025$), AMA-1 (OR = 3.5, $P = .02$), MSP-1 (OR = 3.6, $P = .01$), and TTc (OR = 3.8, $P = .009$) in logistic regression models.

DISCUSSION

This study (1) examined the influence of maternal HIV infection on transplacental transfer of antibodies to malaria merozoite and sporozoite antigens as well as (2) delineated a potential
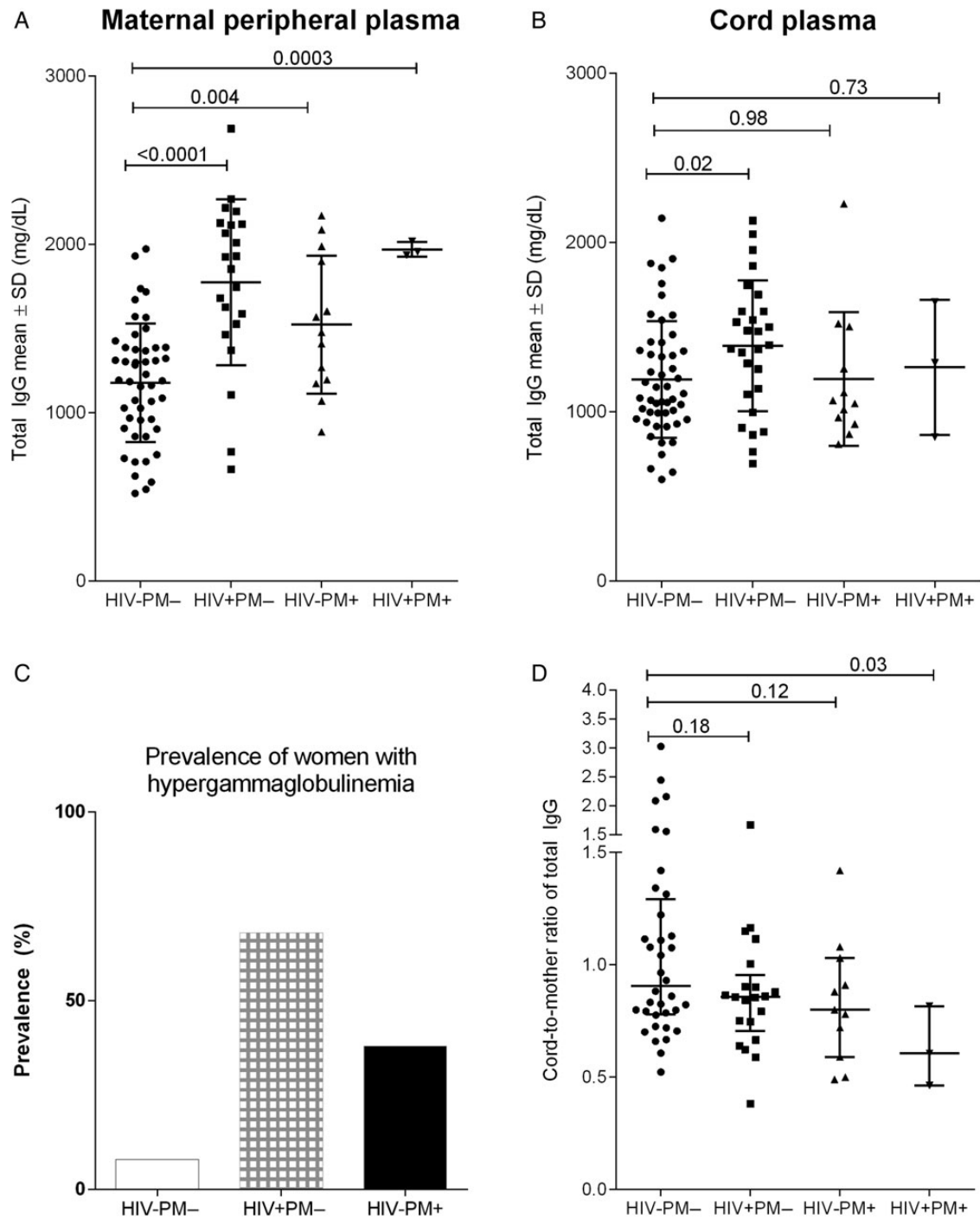
Figure 2. Total immunoglobulin G (IgG) levels in mothers with and without human immunodeficiency virus (HIV). Total immunoglobulin G (IgG) levels were measured in maternal peripheral plasma (A) and cord plasma (B) (mean ± standard deviation plotted). Total IgG levels were compared between 2 groups using *t* test. (C) Prevalence of women with hypergammaglobulinemia (>1600 mg/dL). (D) Cord-to-mother ratio of total IgG levels were compared using Mann-Whitney *U* test (median and interquartile range plotted). Abbreviations: PM, placental malaria; SD, standard deviation.

mechanism underlying reduced placental transfer. Results show that maternal HIV-associated hypergammaglobulinemia significantly reduced transplacental transfer of antibodies to malarial antigens among Cameroonian pregnant women. In this cohort, 68% of HIV+ pregnant women had hypergammaglobulinemia

at delivery. Hypergammaglobulinemia was significantly associated with reduced cord IgG levels to CSP and MSP-1; CMR of IgG to CSP, MSP-1, and TTc; and increased risk of having CMR <1 of antibodies to CSP, AMA-1, MSP-1, and TTc. After adjustments were made for maternal factors including
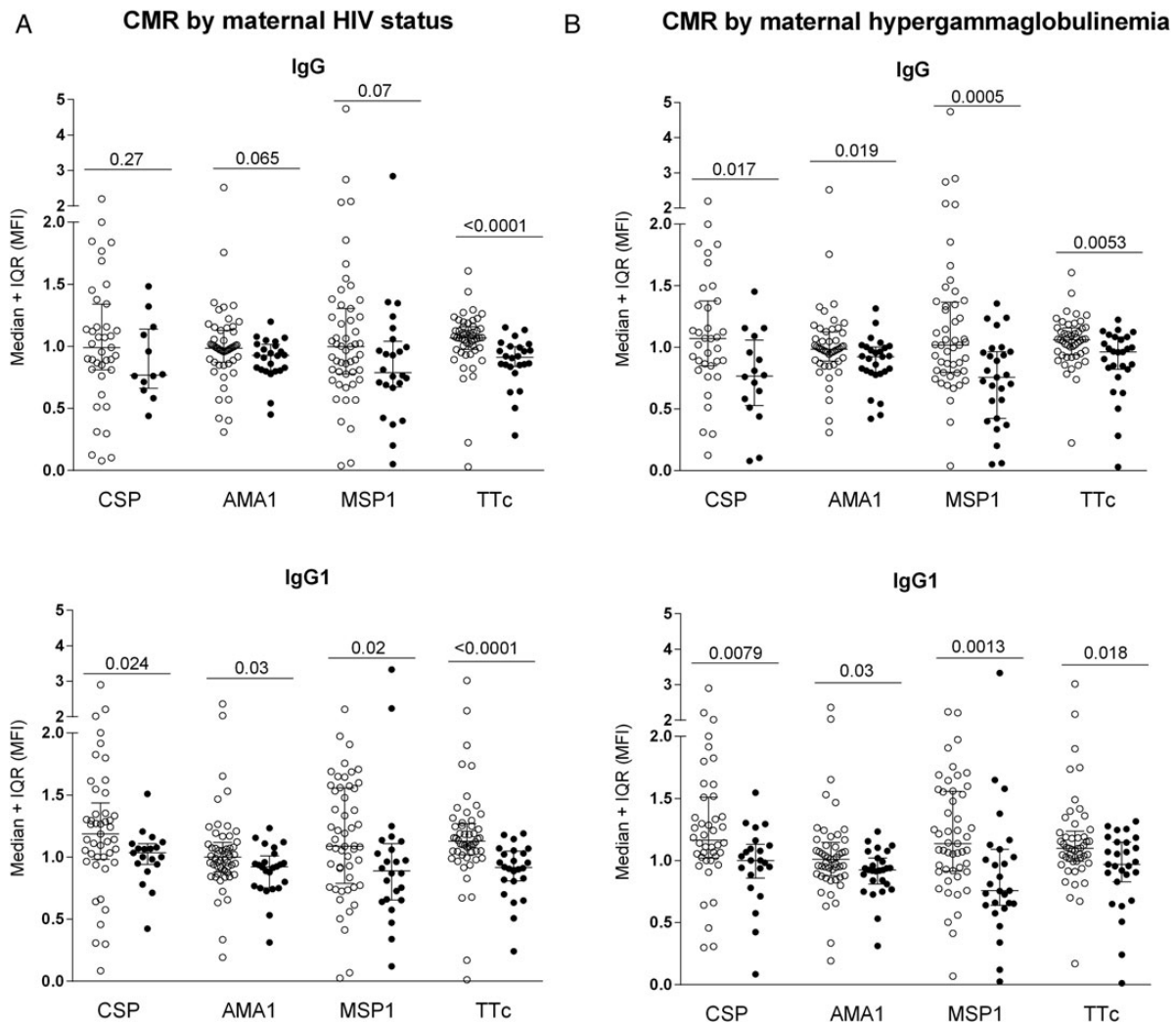
Figure 3. Cord-to-mother ratio (CMR) of antibodies to malaria. Cord-to-mother ratios were calculated for those women who were seropositive for a given antigen. Medians and interquartile range (IQR) of CMR were plotted: (A) stratified by maternal human immunodeficiency virus (HIV) status; (B) stratified by maternal hypergammaglobulinemia status. Antibody levels were compared using Mann-Whitney U test. Open circles represent neonates born to HIV-negative mothers (A) and mothers without hypergammaglobulinemia (B); black circles represent neonates born to HIV-infected mothers (A) and those born to mothers with hypergammaglobulinemia (B). Abbreviations: AMA-1, apical membrane antigen 1; CSP, circumsporozoite antigen; IgG, immunoglobulin G; MFI, mean fluorescence intensity; MSP-1, merozoite surface protein 1; TTc, tetanus toxoid.

antibody levels and PM, the effect of hypergammaglobulinemia was retained.

This is the third study examining the influence of HIV on placental transfer of antibodies to malarial antigens, and the results help resolve discrepancies in the literature. Ayisi et al [27] reported lower cord IgG and CMR only for CSP and not for the erythrocytic stage antigens EBA175, MSP-1–19, MSP2, and MSP3 in neonates born to HIV+ mothers. This contradicts findings by Moro et al [28], who reported lower cord IgG and CMR to MSP-1, EBA175, and AMA-1 in neonates born to HIV+ mothers. These differences could be due to variation in HIV pathology between study populations (CD4 counts, viral loads, ART use), immunological assays, recombinant antigens, study design, and outcome variables. The current study confirms previous results that maternal HIV infection is associated

with reduced transfer of IgG to TTc [24] and both malaria merozoite [28] and sporozoite [27] antigens. The role of hypergammaglobulinemia in reduced placental transfer to vaccine antigens [11–13, 15, 16] has been documented. Previous studies on placental transfer of IgG to malaria did not examine possible mechanisms responsible for placental transfer reduction. To our knowledge, this is the first study demonstrating the role of HIV-associated hypergammaglobulinemia as an important factor of reduced transfer of antimalarial IgG.

The reason why maternal hypergammaglobulinemia reduces placental IgG transfer is not clear. Brambell et al [38] hypothesized that high IgG concentrations produced as a result of polyclonal B-cell activation compete with antigen-specific IgG for binding to the FcRn on syncytiotrophoblasts. Hypergammaglobulinemia independently affected placental IgG transfer in Malawian

Table 2. Antibody Placental Transfer Reduction Due to HIV and Hypergammaglobulinemia

	HIV				Hypergammaglobulinemia			
	Regression Coefficient (95% CI)	Coefficient P	R^2	Model P	Regression Coefficient (95% CI)	Coefficient P	R^2	Model P
Cord Antibody Levels[a]								
IgG CSP	−0.12 (−.39, .16)	0.4	0.62	0.0000	−0.42 (−.73, −.12)	**0.0007**	0.65	0.0000
IgG AMA-1	0.016 (−.17, .2)	0.85	0.85	0.0000	−0.09 (−.32, .13)	0.41	0.85	0.0000
IgG MSP-1	−0.54 (−.95, −.12)	**0.01**	0.68	0.0000	−0.36 (−.77, .06)	0.09	0.67	0.0000
IgG TTc	−0.22 (−.51, .07)	0.13	0.63	0.0000	−0.23 (−.61, .16)	0.24	0.63	0.0000
IgG1 CSP	−0.06 (−.27, .15)	0.55	0.74	0.0000	−0.09 (−.33, .16)	0.47	0.73	0.0000
IgG1 AMA-1	−0.21 (−.45, .02)	0.07	0.90	0.0000	−0.1 (−.33, .12)	0.37	0.89	0.0000
IgG1 MSP-1	−0.2 (−.55, .14)	0.24	0.79	0.0000	−0.4 (−.78, −.007)	**0.046**	0.80	0.0000
IgG1 TTc	−0.29 (−.64, .05)	0.09	0.69	0.0000	−0.42 (−.89, .04)	0.07	0.70	0.0000
IgG3 CSP	0.08 (−.05, .23)	0.21	0.41	0.15	0.16 (−.06, .4)	0.15	0.44	0.19
IgG3 AMA-1	0.05 (−.26, .36)	0.76	0.90	0.0000	−0.1 (−.45, .24)	0.53	0.90	0.0000
IgG3 MSP-1	0.03 (−.46, .39)	0.88	0.70	0.0000	−0.51 (−.99, −.15)	**0.044**	0.72	0.0000
IgG3 TTc	0.01 (−.2, .23)	0.89	0.61	0.0000	−0.11 (−.31, .09)	0.29	0.66	0.0000
CMR[b]								
IgG CSP	−0.14 (−.37, .10)	0.25	0.23	0.012	−0.27 (−.51, −.02)	**0.03**	0.28	0.005
IgG AMA-1	−0.1 (−.22, .02)	0.09	0.12	0.47	−0.09 (−.21, .02)	**0.1**	0.11	0.06
IgG MSP-1	−0.24 (−.57, .09)	0.15	0.13	0.0009	−0.39 (−.66, −.13)	**0.004**	0.17	0.001
IgG TTc	−0.15 (−.25, .56)	**0.002**	0.16	0.011	−0.15 (−.26, −.03)	**0.012**	0.51	0.03
IgG1 CSP	−0.23 (−.41, −.06)	**0.011**	0.19	0.02	−0.25 (−.48, −.04)	**0.02**	0.2	0.02
IgG1 AMA-1	−0.13 (−.26, .004)	0.06	0.1	0.19	−0.11 (−.23, .007)	0.067	0.09	0.19
IgG1 MSP-1	−0.16 (−.46, .13)	0.27	0.11	0.016	−0.23 (−.53, .07)	0.13	0.14	0.01
IgG1 TTc	−0.28 (−.43, −.13)	**0.000**	0.16	0.05	−0.23 (−.39, −.06)	**0.007**	0.12	0.05

P values that were <.05 are depicted in bold.

Abbreviations: AMA-1, apical merozoite antigen 1; CI, confidence intervals; CMR, cord-to-mother ratio; CSP, circumsporozoite protein; HIV, human immunodeficiency virus; IgG, immunoglobulin G; MSP-1, merozoite surface protein 1; R^2, model goodness of fit (coefficient of determination); TTc, tetanus toxoid C fragment.

[a] Linear regression models were adjusted for maternal (ln) antibody levels and placental malaria variables. Maternal factors (ie, age, gravidity, number of SP doses, bednet usage, anemia, and placental weight) and neonatal factors (ie, gestation weeks, preterm birth status, birth weight, and low birth weight status) did not improve models.

[b] Linear regression models were adjusted for maternal age, low birth weight, and placental malaria variables. Maternal factors (ie, gravidity, number of SP doses, bednet usage, anemia, and placental weight) and neonatal factors (ie, gestation weeks, preterm birth status, and birth weight) did not improve models.

mothers; however, because the majority of hypergammaglobu-linemic mothers also had PM, it confounded data interpretation [13]. During PM, changes in placental architecture have been hypothesized to contribute to a reduction in the surface area of syncytiotrophoblasts, resulting in impaired maternofetal transfer of nutrients and antibodies [39–43]. Although, syncy-tiotrophoblasts can become infected with HIV, placental in-flammation, syncytial knotting, and malperfusion are not characteristics of HIV-infected placentas. Human immunodefi-ciency virus infection could have a more overt influence on pla-cental transfer by affecting FcRn expression levels; however, FcRn expression levels in placentas from HIV+ women have not been investigated.

This study had several limitations, including small HIV+ cohort, data on viral load was not available for all participants, and the study design did not allow determination of malaria in-cidence in infants during the first 6 months of life. The use of the gold standard method for measuring total IgG levels, neph-elometry, was not possible, because of the cost and amount of sample required. One concern in total IgG determinations by ELISA is that correcting data for dilution factor of 400 000 would overestimate total IgG levels. However, based on assay validation, ELISA values were congruent or lower compared with nephelometry, thus minimizing the probability that a woman was misclassified as hypergammaglobulinemic.

Despite the fact that 93% of HIV+ mothers received ART, ma-ternal HIV infection was associated with significantly reduced maternal IgG and IgG1 levels to malaria antigens. It is clinically proven that after 6 weeks of ART, one's viral loads drop to unde-tectable levels. Thus, it is likely that many HIV+ women in this study did not adhere to the ART. Prenatal HIV obligatory screen-ing in Cameroon is one of the strategies to detect and treat HIV infection under the Centers for Disease Control and Prevention and President's Emergency Plan for AIDS Relief programs, yet many women are lost to follow up for a number of social and eco-nomic reasons. Furthermore, significantly lower antimalarial IgG1 and CMR were observed in neonates born to HIV+ and hypergammaglobulinemic mothers. Acquisition of IgG1 is important for neonate health, because IgG1 is involved in com-plement activation and efficient opsonization of infectious agents in general [44]. During the first 6 months of life, neonates synthe-size low amounts of IgG1 and IgG2, and adult levels are attained

only by late childhood. Thus, maternally acquired IgG1 are key for protection from infections during infancy, especially during the first 3 to 6 months of life [44], and could leave HIV-exposed infants at an increased risk of not only malaria but also other infections [45]. Of interest, only a few mothers and neonates had IgG3 to malaria antigens in this cohort, as previously described elsewhere [29, 30].

Clinical implications of the results from the current study are unclear, because data on malaria incidence during the first 6 months of life of HIV-exposed uninfected infants are controversial [45–47]. It is not clear whether reduced antibody levels in infants will have a biologically significant impact on incidence of malaria. Naniche et al [48] demonstrated that HIV+ mothers have reduced antimalarial antibody levels; however, in the adjusted models, HIV infection was not associated with cord blood malaria infection. Moro et al [28] report that a 2-fold increase in the CMR of antimalarial antibodies was significantly associated with an increased risk of malaria at 2 months of life [49]. However, it is not specified whether infants born to HIV+ mothers received cotrimoxazole prophylaxis or were exposed to it through breastfeeding. Cotrimoxazole preventive therapy in HIV-exposed infants reduces the risk of malaria infection between 6 and 36 weeks of age by 70% [50] and is currently a standard of care beginning 6th week of life. Thus, studies that evaluate the impact of reduction in antimalarial antibody levels in HIV-exposed infants on the risk of malaria infection will be unethical to conduct.

CONCLUSIONS

Although current therapies to prevent malaria in HIV-exposed neonates (including cotrimoxazole and ITN) are efficacious, placentally transferred antibodies would continue to be important when cotrimoxazole resistance emerges and insecticide resistance spreads. In addition, lower antibody levels at birth could reduce protection from malaria infection in utero, congenital malaria and malaria during the first 6 weeks of life. Finally, lower levels of maternal antimalarial antibodies at birth will have shorter antibody half-lives during the first 6 months of life.

Supplementary Data

Supplementary material is available online at *Open Forum Infectious Diseases* online (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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References

1. Palmeira P, Quinello C, Silveira-Lessa AL, et al. IgG placental transfer in healthy and pathological pregnancies. Clin Dev Immunol **2012**; 2012:985646.
2. de Moraes-Pinto I, Hart CA. Transplacental antibody transfer and neonatal immunity. Br J Hosp Med **1997**; 58:317–9.
3. Cohen S, McGregor IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature **1961**; 192:733–7.
4. McGregor IA. The passive transfer of human malarial immunity. Am J Trop Med Hyg **1964**; 13:237–9.
5. Marsh K, Snow RW. Host-parasite interaction and morbidity in malaria endemic areas. Philos Trans R Soc Lond B Biol Sci **1997**; 352:1385–94.
6. Simister NE, Mostov KE. An Fc receptor structurally related to MHC class I antigens. Nature **1989**; 337:184–7.
7. Simister NE, Rees AR. Isolation and characterization of an Fc receptor from neonatal rat small intestine. Eur J Immunol **1985**; 15:733–8.
8. Vaughn DE, Bjorkman PJ. Structural basis of pH-dependent antibody binding by the neonatal Fc receptor. Structure **1998**; 6:63–73.
9. Avanzini MA, Pignatti P, Chirico G, et al. Placental transfer favours high avidity IgG antibodies. Acta Paediatr **1998**; 87:180–5.
10. Caceres VM, Strebel PM, Sutter RW. Factors determining prevalence of maternal antibody to measles virus throughout infancy: a review. Clin Infect Dis **2000**; 31:110–9.
11. Okoko BJ, Wesumperuma LH, Hart AC. Materno-foetal transfer of H. influenzae and pneumococcal antibodies is influenced by prematurity and low birth weight: implications for conjugate vaccine trials. Vaccine **2001**; 20:647–50.
12. de Moraes-Pinto MI, Almeida AC, Kenj G, et al. Placental transfer and maternally acquired neonatal IgG immunity in human immunodeficiency virus infection. J Infect Dis **1996**; 173:1077–84.
13. de Moraes-Pinto MI, Verhoeff F, Chimsuku L, et al. Placental antibody transfer: influence of maternal HIV infection and placental malaria. Arch Dis Child Fetal Neonatal Ed **1998**; 79:F202–5.
14. Wesumperuma HL, Perera AJ, Pharoah PO, Hart CA. The influence of prematurity and low birthweight on transplacental antibody transfer in Sri Lanka. Ann Trop Med Parasitol **1999**; 93:169–77.
15. Gendrel D, Richard-Lenoble D, Blot P, Fribourg-Blanc A. [Transfer of measles immunoglobulins and antibodies from mother to child in Africa and Europe]. Presse Med **1988**; 17:1633–6.
16. Gendrel D, Richard-Lenoble D, Massamba MB, et al. [Placental transfer of tetanus antibodies and protection of newborn infants]. Arch Fr Pediatr **1990**; 47:725–9.
17. Catty D, Drew R, Seger R. Transmission of IgG subclasses to the human foetus. In: Hemmings WA, ed. Protein Transmission Through Living Membranes. London: Elsevier/North Holland Biomedical Press; **1979**; pp. 64–70.
18. de Moraes-Pinto MI, Farhat CK, Carbonare SB, et al. Maternally acquired immunity in newborns from women infected by the human immunodeficiency virus. Acta Paediatr **1993**; 82:1034–8.
19. UNAIDS. GAP Report 2014. Children and Pregnant Women Living with HIV. Geneva. Available at: http://www.unaids.org/en/resources/campaigns/2014/2014gapreport/gapreport. Accessed 1 February 2015.

20. UNIADS. UNAIDS Global Plan Progress Report 2013. Geneva. Available at: http://www.unaids.org/sites/default/files/media_asset/20130625_progress_global_plan_en_0.pdf. Accessed 1 February 2015.

21. Demographic and health survey MICS. In: Services DoH, ed. Yaounde, Cameroon: Ministry of Health, 2011.

22. Annual Report of the National AIDS Control Committee. Cameroon, 2012.

23. Gaensbauer JT, Rakhola JT, Onyango-Makumbi C, et al. Impaired haemophilus influenzae type b transplacental antibody transmission and declining antibody avidity through the first year of life represent potential vulnerabilities for HIV-exposed but -uninfected infants. Clin Vaccine Immunol 2014; 21:1661–7.

24. Cumberland P, Shulman CE, Maple PA, et al. Maternal HIV infection and placental malaria reduce transplacental antibody transfer and tetanus antibody levels in newborns in Kenya. J Infect Dis 2007; 196:550–7.

25. Scott S, Cumberland P, Shulman CE, et al. Neonatal measles immunity in rural Kenya: the influence of HIV and placental malaria infections on placental transfer of antibodies and levels of antibody in maternal and cord serum samples. J Infect Dis 2005; 191:1854–60.

26. Gupta A, Mathad JS, Yang WT, et al. Maternal pneumococcal capsular IgG antibodies and transplacental transfer are low in South Asian HIV-infected mother-infant pairs. Vaccine 2014; 32:1466–72.

27. Ayisi JG, Branch OH, Rafi-Janajreh A, et al. Does infection with human immunodeficiency virus affect the antibody responses to Plasmodium falciparum antigenic determinants in asymptomatic pregnant women? J Infect 2003; 46:164–72.

28. Moro L, Bardaji A, Nhampossa T, et al. Malaria and HIV infection in Mozambican pregnant women are associated with reduced transfer of antimalarial antibodies to their newborns. J Infect Dis 2015; 211:1004–14.

29. Babakhanyan A, Fang R, Wey A, et al. Comparison of the specificity of antibodies to VAR2CSA in Cameroonian multigravidae with and without placental malaria: a retrospective case-control study. Malar J 2015; 14:480.

30. Babakhanyan A, Leke RG, Salanti A, et al. The antibody response of pregnant Cameroonian women to VAR2CSA ID1-ID2a, a small recombinant protein containing the CSA-binding site. PLoS One 2014; 9:e88173.

31. Suguitan AL Jr, Cadigan TJ, Nguyen TA, et al. Malaria-associated cytokine changes in the placenta of women with pre-term deliveries in Yaounde, Cameroon. Am J Trop Med Hyg 2003; 69:574–81.

32. Tako EA, Zhou A, Lohoue J, et al. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am J Trop Med Hyg 2005; 72:236–42.

33. Tutterrow YL, Avril M, Singh K, et al. High levels of antibodies to multiple domains and strains of VAR2CSA correlate with the absence of placental malaria in Cameroonian women living in an area of high Plasmodium falciparum transmission. Infect Immun 2012; 80:1479–90.

34. Okoko BJ, Wesuperuma LH, Ota MO, et al. Influence of placental malaria infection and maternal hypergammaglobulinaemia on materno-foetal transfer of measles and tetanus antibodies in a rural west African population. J Health Popul Nutr 2001; 19:59–65.

35. Brair ME, Brabin BJ, Milligan P, et al. Reduced transfer of tetanus antibodies with placental malaria. Lancet 1994; 343:208–9.

36. Fouda GG, Leke RF, Long C, et al. Multiplex assay for simultaneous measurement of antibodies to multiple Plasmodium falciparum antigens. Clin Vaccine Immunol 2006; 13:1307–13.

37. Franz B, May KF Jr, Dranoff G, Wucherpfennig K. Ex vivo characterization and isolation of rare memory B cells with antigen tetramers. Blood 2011; 118:348–57.

38. Brambell FWR. The transmission of passive immunity from mother to young. In Frontiers of Biology, North Holland: Amsterdam; American Elsevier New York, 1970; 18.

39. Bulmer JN, Rasheed FN, Morrison L, et al. Placental malaria. II. A semi-quantitative investigation of the pathological features. Histopathology 1993; 22:219–25.

40. Leopardi O, Naughten W, Salvia L, et al. Malaric placentas. A quantitative study and clinico-pathological correlations. Pathol Res Pract 1996; 192:892–8.

41. Bulmer JN, Rasheed FN, Francis N, et al. Placental malaria. I. Pathological classification. Histopathology 1993; 22:211–8.

42. Rogerson SJ, Beeson JG. The placenta in malaria: mechanisms of infection, disease and foetal morbidity. Ann Trop Med Parasitol 1999; 93:S35–42.

43. Galbraith RM, Faulk WP, Galbraith GM, et al. The human materno-foetal relationship in malaria: I. Identification of pigment and parasites in the placenta. Trans R Soc Trop Med Hyg 1980; 74:52–60.

44. Jefferis R, Kumararatne DS. Selective IgG subclass deficiency: quantification and clinical relevance. Clin Exp Immunol 1990; 81:357–67.

45. Kourtis AP, Wiener J, Kayira D, et al. Health outcomes of HIV-exposed uninfected African infants. AIDS 2013; 27:749–59.

46. Osterbauer B, Kapisi J, Bigira V, et al. Factors associated with malaria parasitaemia, malnutrition, and anaemia among HIV-exposed and unexposed Ugandan infants: a cross-sectional survey. Malar J 2012; 11:432.

47. Ezeamama AE, Spiegelman D, Hertzmark E, et al. HIV infection and the incidence of malaria among HIV-exposed children from Tanzania. J Infect Dis 2012; 205:1486–94.

48. Naniche D, Serra-Casas E, Bardaji A, et al. Reduction of antimalarial antibodies by HIV infection is associated with increased risk of Plasmodium falciparum cord blood infection. J Infect Dis 2012; 205:568–77.

49. Menendez C, Bardaji A, Sigauque B, et al. A randomized placebo-controlled trial of intermittent preventive treatment in pregnant women in the context of insecticide treated nets delivered through the antenatal clinic. PLoS One 2008; 3:e1934.

50. Davis NL, Barnett EJ, Miller WC, et al. Impact of daily cotrimoxazole on clinical malaria and asymptomatic parasitemias in HIV-exposed, uninfected infants. Clin Infect Dis 2015; 61:368–74.