ELSEVIER

# Variation in the relationship between anti-MSP-1$_{19}$ antibody response and age in children infected with *Plasmodium falciparum* during the dry and rainy seasons

Y.O. Omosun [a], C.I. Anumudu [a], S. Adoro [a,1], A.B. Odaibo [a], O. Sodeinde [b], A.A. Holder [c], M. Nwagwu [a], R.I. Nwuba [a,*]

[a] *Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Ibadan, Nigeria*
[b] *Department of Paediatrics, University College Hospital, Ibadan, Nigeria*
[c] *National Institute for Medical research, Mill Hill, London, UK*

## Abstract

Malaria remains a major parasitic disease in Africa, with 300–500 million new infections each year. There is therefore an urgent need for the development of new effective measures, including vaccines. *Plasmodium falciparum* merozoite surface protein-1$_{19}$ (MSP-1$_{19}$) is a prime candidate for a blood-stage malaria vaccine. Blood samples were collected from children aged 10 days to 15 years in the months of January–March ($N = 351$) and October–November ($N = 369$) corresponding to the dry and rainy seasons, respectively. *P. falciparum* infection was determined by microscopy and enzyme linked immunosorbent assay (ELISA) was used to determine the total IgG and IgG subclasses. There was a significant increase in the mean anti-MSP-1$_{19}$ antibody titre in the dry season ($p < 0.05$), compared to the rainy season. A significantly positive correlation between the anti-MSP-1$_{19}$ antibody titre and parasite density ($p < 0.01$, $r = 0.138$) was observed. In the rainy season, unlike in the dry season, *P. falciparum* positive children had higher anti-MSP-1$_{19}$ antibody titres than *P. falciparum* negative children and this difference was significant ($p < 0.05$). When all individuals were grouped together, the anti-MSP-1$_{19}$ antibody titre increased with age in both seasons ($r = 0.186$ and $0.002$), this increase was more apparent in the dry season. However, when the study population was divided into *P. falciparum* positive and negative groups, it was observed that in the rainy season, there was a negative correlation between anti-MSP-1$_{19}$ titre and age in *P. falciparum* positive individuals, while those who were *P. falciparum* negative had a positive correlation between anti-MSP-1$_{19}$ titre and age. Analysis of anti-MSP-1$_{19}$ IgG subclass showed that IgG1 and IgG3 mean titres were highest in both the dry and rainy seasons with an increase in the mean antibody titres for IgG1, IgG2 and IgG3 in the rainy season. In the dry season there was a positive correlation between IgG1, IgG2, and IgG3 titres with age, while IgG4 was negative, whereas in the rainy season there was a positive correlation between IgG2 and IgG4 (non-cytophilic antibodies) with age and

---

* Corresponding author. Tel.: +234 2 8107547; fax: +234 2 8103118; mobile: +234 8033715621.
  *E-mail address:* rnwuba@yahoo.com (R.I. Nwuba).
[1] Present address: Immunology Graduate Group, University of Pennsylvania.

a negative correlation for IgG1 and IgG3 (cytophilic antibodies) with age. Seasonal differences in the level of MSP-$1_{19}$ IgG subclass titres were observed for *P. falciparum* negative and positive individuals. Only samples, which were positive for IgG2 and IgG4, showed positive correlation between parasitemia and total IgG. The incidence of *P. falciparum* infection, which increases during the rainy season, might be an important determinant of anti-MSP-$1_{19}$ antibody levels in children living in Igbo-Ora and the results point to the fact that non-cytophilic antibodies to MSP-$1_{19}$ in children might be associated with an increase in total IgG and parasitemia.

## 1. Introduction

MSP-$1_{19}$ has been shown to be a target of protective immunity and is a leading vaccine candidate currently under development. Some MSP-$1_{19}$ specific monoclonal antibodies that inhibit merozoite invasion also inhibit the secondary processing of MSP-1 (Blackman et al., 1994) and this is proposed to be the basis of their protective mechanism. Processing inhibitory antibodies have also been reported to occur in individuals naturally exposed to malaria (Nwuba et al., 2002). In humans it has been shown that antibodies against MSP-$1_{19}$ comprise a large component of the total invasion inhibitory response in *Plasmodium falciparum* infected individuals (Wipasa et al., 2002).

The prevalence and concentration of antibodies to both the C- and N-terminal regions of MSP-1 have been shown to increase with age (Al-Yaman et al., 1996; Egan et al., 1995, 1996). The levels of IgG antibodies against the MSP-1 C-terminal region can serve as a good surrogate measure of protective immunity; there is a negative correlation between antibody levels and clinical malaria (Al-Yaman et al., 1996). A study showed that 96.2% of mothers and 80.2% of their infants had MSP-$1_{19}$ specific antibodies, and there was a lower risk of developing clinical malaria during the first year of life in infants with high levels of anti-MSP-$1_{19}$ antibodies at birth, with the level of specific anti-MSP-$1_{19}$ antibodies being correlated with protection (Hogh et al., 1995). This was corroborated by Branch et al. (1998) who associated the protection against parasitemia and febrile illness with anti-MSP-$1_{19}$ antibodies. However some authors could not show a relation between the prevalence of antibodies against MSP-1 with the reduction in clinical malaria (Shi et al., 1996; Dodoo et al., 1999). In areas that are endemic for malaria, antibodies of cytophilic IgG1 or IgG3 isotype are associated with lower parasitemia or lower risk of malaria attack (Taylor et al., 1995). In contrast antibodies of IgG4 isotype display a blocking function in this system (Perlman and Bjorkman, 2000). It has been suggested that the IgG1 and IgG3 to IgG2 and IgG4 ratio might be important to asexual stages of *P. falciparum*. Tebo et al. (2001) described how IgG3 might play a role in controlling parasitemia via an antibody dependent cellular immunity (ADCI) mechanism involving monocyte-derived mediators.

Ferreira et al. (1998) showed that the overall properties of IgG subclass to MSP-1 were cytophilic, and the concentration of each IgG-subclass to *P. falciparum* correlated positively with age. Protection against the asexual stage of malaria seems to rely largely on specific IgG1 and IgG3 antibodies (Diallo et al., 2001). The study by Cavanaugh et al. (2001) presented results that showed strikingly distinct subclass preferences of antibody responses to two different regions of MSP-1. Seven of the individuals studied consistently produced IgG3 to block 2 and equally consistently, IgG1 to MSP-$1_{19}$ in response to their clinical malaria infections, this result is consistent with that of Jouin et al. (2001) who showed that anti-block 2 antibodies were mostly of the IgG3 isotype, and Egan et al. (1995) who showed that MSP-$1_{19}$ appears to induce predominantly IgG1 antibodies. Unlike other asexual blood stage antigens MSP-$1_{19}$ seems to elicit a restricted set of antibody responses in immune populations with elevated IgG1/IgG3 and little if any IgG2 and IgG4 (Diallo et al., 2001). Here we determined the factors (total IgG, IgG subtype, parasitemia and seasonal variations) important in the immune response against MSP-$1_{19}$ of *P. falciparum* a leading malaria vaccine candidate in children living in Igbo-Ora, southwestern Nigeria, a malaria endemic region.

## 2. Materials and methods

### 2.1. MSP-1$_{19}$ antigen

Wild type MSP-1$_{19}$ recombinant proteins were obtained from Dr. Tony Holder, National institute for Medical Research (NIMR), Mill Hill, London, UK.

### 2.2. Study area

Igbo-Ora and Idere towns in Ibarapa local government area of Oyo state in southwestern Nigeria were chosen as the study site. Igbo-Ora lies in savannah country, which is heavily cultivated with numerous small streams. Three rivers, River Opeki, River Ofiki and River Ayin pass through this area. *Anopheles gambiae* and *A. funestus* are the mosquito species found in this area (Lawrence, 1965). Igbo-Ora is about 100 km from Ibadan, the state capital; the majority of the population is Yoruba. The climate consists of a warm dry season (November–March) and a cooler rainy season (April–October). The main occupation of the men is farming and hunting while the women are peasant farmers and retail traders (Achidi et al., 1996).

### 2.3. Study design

The cross-sectional survey was carried out during the dry season (January–March) and at the end of the rainy season (October–November). The study protocol was reviewed and approved by the Joint Ethical Committee of the College of Medicine and the University College Hospital, Ibadan. The subjects of the study included infants and children from 10 days to 15 years. Three hundred and fifty-one and 369 individuals were enrolled in the dry and rainy seasons, respectively. Criteria for inclusion into the survey included the age, sex, length of time spent in the study site and informed consent.

### 2.4. Blood collection

Blood (1–2 ml) was collected by venipuncture from the arm by qualified medical doctors. For children less than 1-year-old blood was withdrawn from the femoral vein and finger pricking where necessary. The blood was then stored in sample tubes with 0.12 M trisodium citrate in them and properly labelled, the tubes containing the blood was then stored in ice, and then transported to the Cellular Parasitology Laboratory in Ibadan within 3 h, where it was the centrifuged at 8000 rpm for 2 min in a Biofuge table top centrifuge (Hereaus instruments, Kendro Lab product GmbH, Lagenselbold, Germany). The plasma obtained from it was stored at $-80\,^{\circ}$C (Forma Scientific, Marietta, OH, USA).

### 2.5. Parasitology

Blood was spotted on the slide and thick and thin films were prepared. The slides were labelled, allowed to dry, stored in a slide rack and transported to Ibadan. The thick film was then stained in 10% Giemsa solution (pH 7.2 buffered distilled water) in a Wheaton staining jar for 20 min and then allowed to dry. The thin film was first fixed in methanol before being immersed in 10% Giemsa solution for 20 min. The parasites were counted with a microscope (Leitz Laborux-11, Germany) using the thick film on the basis of number of parasites per 200 white blood cells; this was converted to the number of parasites per $\mu$l of blood (WHO, 1985).

### 2.6. Determination of anti-MSP-1$_{19}$ total IgG by ELISA

The serum samples from 351 subjects (dry season) and 369 (rainy season) were analyzed by ELISA for antibodies to MSP-1$_{19}$. Ninety-six-well plates (Immulon 4; Dynatech Labs, Chantilly, VA) were coated with $100\,\mu$l MSP-1$_{19}$ ($0.5\,\mu$g of MSP-1$_{19}$/ml of sodium carbonate buffer) and incubated overnight at $4\,^{\circ}$C. The plates were washed three times with 0.05% Tween-20 in phosphate buffered saline (PBS-Tween-20) and blocked with 5% bovine serum albumin (BSA) for 1 h at $37\,^{\circ}$C. Washing was repeated (three times with PBS-Tween-20) and serum samples (1:50) were added to the first row of wells and then diluted serially to 1:6400 for the last row of wells in PBS containing 1% BSA at a working volume of $100\,\mu$l. The plates were incubated for 1 h at $37\,^{\circ}$C in an incubator (Forma Scientific, Marietta, OH, USA) followed by washing (three times with PBS-Tween-20). Horseradish peroxidase-conjugated goat anti-human IgG 1:1000 (Kierkegaard and Perry) in 1% BSA was added and the plates incubated at $37\,^{\circ}$C, for 2 h. The plates were washed (three times with PBS-Tween-

20) and 2,2′-azino-di(3-ethyl-benzthiazoline sulfonate (ABTS) substrate/$H_2O_2$ (Kierkegaard and Perry) was diluted 1:1 and 100 µl of the substrate was added to the wells. The reaction was allowed to develop in the dark for 30 min at 37 °C and the absorbance read at 650 nm with a microplate reader (Molecular Devices, Menlo Park, CA, USA). The assays included a reference positive sample (FA 28) from an earlier study (Nwagwu et al., 1998) known to have a high antibody titre against *P. falciparum* circumsporozoite protein and also a high anti-MSP-1$_{19}$ antibody titre in this study. Negative serum samples obtained from naïve Caucasian residents of UK were initially used as control. When the study progressed negative samples were later obtained from the cohort (ID 77 and ID 53). The end point titre was defined as the highest dilution that gave an absorbance value above the highest absorbance of the negative control. The reciprocal end titres were log transformed and expressed as log reciprocal titres.

## 2.7. Determination of anti-MSP-1$_{19}$ IgG subtypes by ELISA

MSP-1$_{19}$ specific IgG subtypes (1–4) were determined by ELISA to demonstrate which of the subtypes recognised the recombinant antigen. One hundred and twenty-six samples chosen randomly within the age groups (77 from the dry season January–March and 49 from the rainy season October–November 1999) were analyzed. Flat bottom 96-well polyvinyl chloride plates (Corning Incorporated-Life Sciences, MA, USA) were coated with 50 µl of MSP-1$_{19}$ (1 µg of MSP-1$_{19}$/ml of sodium carbonate buffer) per well and incubated overnight at 4 °C. The antigen was poured off and the plates were then blocked with 150 µl of 5% bovine serum albumin (BSA) for 1 h at 37 °C in an incubator (Forma Scientific, Marietta, OH, USA). The plates were washed three times with PBS-Tween-20. Sera diluted 1:50 in 1% BSA was added to the plates and the whole incubated for 1 h at 37 °C. The plates were washed three times with PBS-Tween-20 and 50 µl of mouse anti-human monoclonal antibody diluted in 1% BSA (anti-IgG1, anti-IgG2 and anti-IgG4, 1:400 while anti-IgG3 1:800, Caltag) was added to the plates The plates were washed three times with PBS-Tween-20 and 50 µl of 1:1000 dilution of goat anti-mouse peroxidase conjugate (H + L) (Caltag), was added to the plates

which were then incubated for 1 h at 37 °C. The plates were finally washed three times with PBS-Tween-20. A 50 µl of 2,2′-azino-di(3-ethyl-benzthiazoline sulfonate (ABTS) substrate/$H_2O_2$ (Kierkegaard and Perry) was added to the plates and the colour allowed to develop at 37 °C in the incubator (Forma Scientific, Marietta, OH, USA) for 30 min. The absorbance was read at 650 nm with a microplate reader (Molecular Devices, Menlo Park, CA, USA) without stopping the reaction. Negative serum samples were obtained from the study sites. The cut-off was determined using mean (average) + 2 S.D. of the optical density (OD) of [ID 77 and ID 53].

The total IgG subtype ELISA was included as a control. Flat bottom polyvinyl chloride plates (Corning Incorporated-Life Sciences, MA, USA) were coated with 50 µl/well of IgG subtype monoclonal diluted in carbonate buffer (1:400 for anti-IgG1, anti-IgG2 and anti-IgG4 and 1:800 for anti-IgG3) and incubated for 18 h at 4 °C. The monoclonal antibodies were poured off and the plates blocked with 150 µl of 5% bovine serum albumin (BSA) for 1 h at 37 °C. The plates were washed three times with PBS-Tween-20. Sera diluted 1:50 in 1% BSA was added to the plates that were then incubated for 1 h at 37 °C in an incubator (Forma Scientific, Marietta, OH, USA). The plates were washed three times with PBS-Tween-20 and 50 µl of 1:2000 dilution of goat anti-human peroxidase conjugate (Fab)$_2$; (Dako), was added to the plates which were then incubated for 1 h at 37 °C. The plates were then finally washed three times with PBS-Tween-20. A 50 µl 2,2′-azino-di(3-ethyl-benzthiazoline sulfonate) (ABTS) substrate/$H_2O_2$ (Kierkegaard and Perry) was added to the plates and the colour allowed to develop at 37 °C for 30 min in the incubator (Forma Scientific, Marietta, OH, USA). The absorbance was read at 650 nm with a microplate reader (Molecular Devices, Menlo Park, CA, USA) without stopping the reaction.

## 2.8. Statistical analysis

The antibody titres were log transformed and the results analyzed using correlation, logistic regression, ANOVA and Student's *t*-test. The levels of significance were estimated at $p < 0.05$ for logistic regression, ANOVA and Student's *t*-test, while $p < 0.01$ for correlation. The log reciprocal antibody titres were expressed at log base 10. The software packages used were Microsoft EXCEL and SPSS.

Table 1
The prevalence of *P. falciparum* in the dry and rainy seasons

|  | *P. falciparum* negative $N$ (%) | *P. falciparum* positive $N$ (%) |
|---|---|---|
| Dry season ($N = 343$[a]) | 198 (58) | 145 (42) |
| Rainy season ($N = 362$[a]) | 189 (52) | 173 (48) |

The number and percentages of individuals that were *P. falciparum* positive and negative during the dry and rainy seasons were shown.

[a] The number of individuals stated here vary from that in the text, due to correction based on missing values.

## 3. Results

The results showed that in the dry season, 42% of the individuals studied were *P. falciparum* positive while at the end of the rainy season 48% of the children were *P. falciparum* positive (Table 1). There was significant increase in the anti-MSP-$1_{19}$ total IgG in the dry season ($p < 0.05$). The mean anti-MSP-$1_{19}$ antibody titre was 2.57 in the dry season and 2.44 in the rainy season. There was no significant difference in anti-MSP-$1_{19}$ total IgG titres for *P. falciparum* positive or negative individuals in the dry season. However in the rainy season there was a significant difference in the anti-MSP-$1_{19}$ total IgG between individuals who

were *P. falciparum* positive or negative, the mean antibody titre for *P. falciparum* positive individuals was 2.50 while the mean antibody titre for *P. falciparum* negative individuals was 2.39 ($p < 0.05$). There was a positive correlation between anti-MSP-$1_{19}$ total IgG and age with the total IgG increasing with age in the dry season ($r = 0.186$) and at the end of the rainy season and ($r = 0.002$) (Figs. 1 and 2). This relationship was significant only in the dry season. Table 2 shows the age grouping used in this study. In the dry season the 61–120 months old group had the highest mean anti-MSP-$1_{19}$ total IgG titre, while the children less than 12 months old had the lowest mean anti-MSP-$1_{19}$ total IgG titre (Fig. 3). At the end of the rainy season, the 61–120 months old group had the highest mean anti-MSP-$1_{19}$ total IgG titre while 120–180 months old children had the lowest mean anti-MSP-$1_{19}$ total IgG titre (Fig. 4). Total IgG increased with parasitemia, and the relationship was significant ($r = 0.138$).

There was a positive correlation between anti-MSP-$1_{19}$ total IgG and age for *P. falciparum* positive children in the dry season ($r = 0.199$) (Fig. 5). There was however a negative correlation in the rainy season ($r = -0.083$) (Fig. 7). There was positive correlation between anti-MSP-$1_{19}$ total IgG and age in *P. falci-*
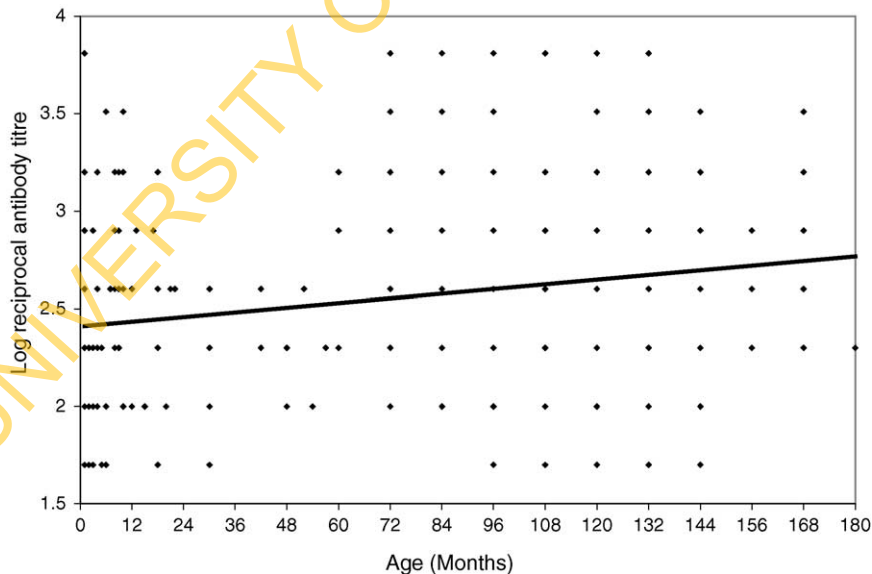


Fig. 1. Correlation between anti-MSP-$1_{19}$ total IgG and age in the dry season. Scatter diagram showing the relationship between age and anti-MSP-$1_{19}$ IgG antibody titre for children in the dry season. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.
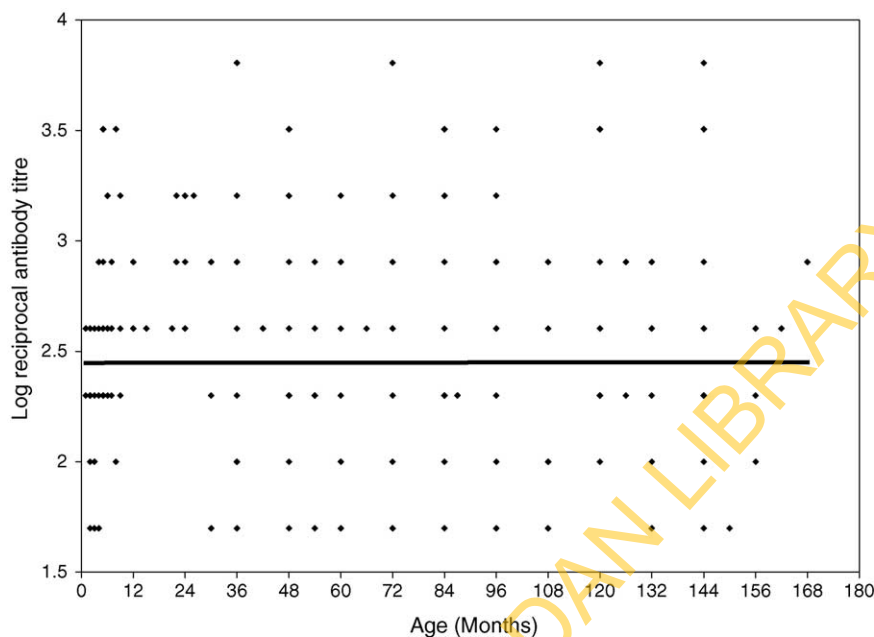
Fig. 2. Correlation between anti-MSP-1$_{19}$ total IgG and age at the end of the rainy season. Scatter diagram showing the relationship between age and anti-MSP-1$_{19}$ IgG antibody titre for children at the end of the rainy season. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

*parum* negative individuals in the dry season ($r = 0.200$) (Fig. 6) and rainy season ($r = 0.165$) (Fig. 8). The influence of anti-MSP-1$_{19}$ IgG, age and sex on malaria was analyzed using regression analysis. The regression showed that age was important in predicting *P. falciparum* positive individuals in the dry season; whereas, in the rainy season it was not important in predicting *P.*

*falciparum* positive individuals. Results from the IgG subtype assay showed that 84%, 66%, 84% and 56% of the subjects were positive for anti-MSP-1$_{19}$ IgG1, IgG2, IgG3 and IgG4 in the dry season, while in the rainy season 87%, 74%, 98% and 46% of the subjects

Table 2
Age grouping of children

| Age group | Number of samples | |
|---|---|---|
| | Dry season | Rainy season |
| Total IgG | | |
| <12 | 58 | 47 |
| 12–60 | 36 | 113 |
| 61–120 | 165 | 130 |
| 120–180 | 86 | 74 |
| IgG subtypes | | |
| <12 | 14 | 11 |
| 12–60 | 20 | 19 |
| 61–120 | 20 | 13 |
| 120–180 | 23 | 4 |

The age group distribution of individuals who were analyzed for anti-MSP-119 antibodies were shown.
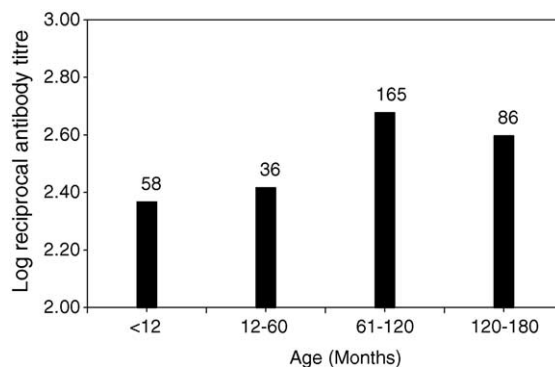


Fig. 3. Mean anti-MSP-1$_{19}$ total IgG and age in the dry season. Histogram showing the relationship between age and anti-MSP-1$_{19}$ IgG titre for children in the dry season. The mean anti-MSP-1$_{19}$ IgG titre was determined for each age group. Antibody titre is expressed as log reciprocal antibody titre, while age is in months. The numbers on the bars represent number of individuals for each age group.
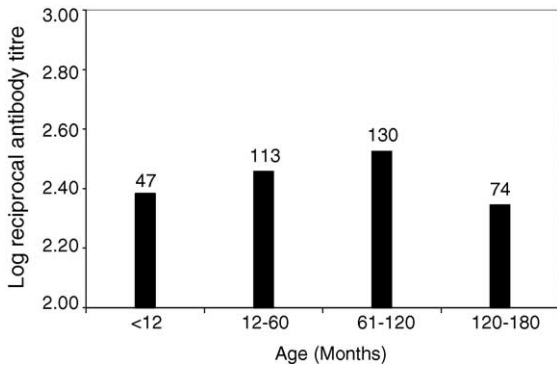
Fig. 4. Mean anti-MSP-$1_{19}$ total IgG and age at the end of the rainy season. Histogram showing the relationship between age and anti-MSP-$1_{19}$ IgG titre for children in the dry season. The mean anti-MSP-$1_{19}$ IgG titre was determined for each age group. Antibody titre is expressed as log reciprocal antibody titre, while age is in months. The numbers on the bars represent number of individuals for each age group.

were positive for anti-MSP-$1_{19}$ IgG1, IgG2, IgG3 and IgG4 (Fig. 9). IgG1 and IgG3 had the highest mean antibody titres in the dry season and at the end of the rainy season. There was an increase in mean antibody titres for IgG1, IgG2 and IgG3 at the end of the rainy season. This increase was only significant for anti-MSP-$1_{19}$ IgG3 ($p < 0.05$). IgG4 had the lowest mean titres in both seasons (Fig. 9).

In the dry season, there was a positive correlation between IgG1, IgG2 and IgG3 and age ($r = 0.137$, $0.243$ and $0.420$, respectively) while there was a negative correlation between IgG4 and age ($r = -0.009$) (Fig. 10). At the end of the rainy season there was positive correlation between IgG2 ($r = 0.202$) and IgG4 ($r = 0.332$) with age, while there was a negative correlation between IgG1 ($r = -0.165$) and IgG3 ($r = -0.06$) with age (Fig. 11).

The mean IgG1 and IgG3 titres for *P. falciparum* negative individuals were higher than those of *P. falciparum* positive individuals in the dry season. Also the mean IgG2 and IgG4 titres for *P. falciparum* positive individuals were higher than those of *P. falciparum*
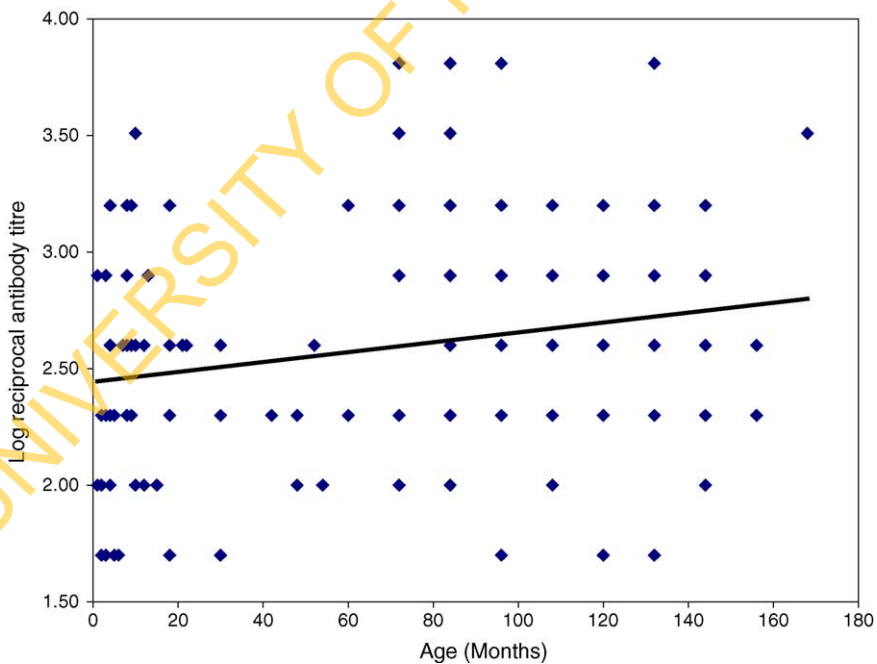


Fig. 5. Correlation between anti-MSP-$1_{19}$ IgG and age for samples positive for *P. falciparum* parasites in the dry season. Scatter diagram showing the relationship between age and anti-MSP-$1_{19}$ IgG antibody titre for children whose giemsa stained blood smears were *P. falciparum* positive. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.
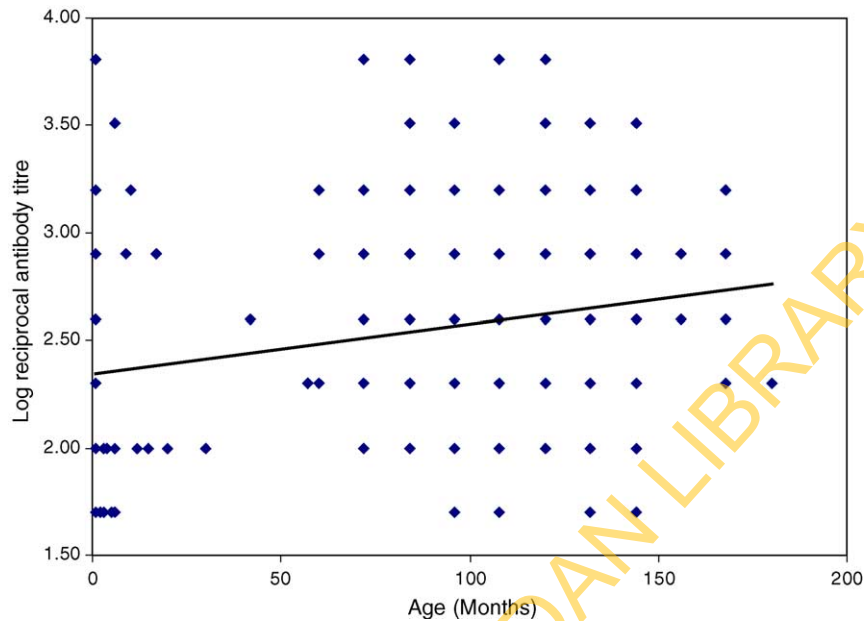
Fig. 6. Correlation between anti-MSP-1$_{19}$ IgG and age for samples negative for *P. falciparum* in the dry season. Scatter diagram showing the relationship between age and anti-MSP-1$_{19}$ IgG antibody titre for children whose giemsa stained blood smears were *P. falciparum* negative. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

negative individuals (Fig. 12). There was a significant difference in the anti-MSP-1$_{19}$ IgG4 subtypes between individuals positive or negative for *P. falciparum* parasites in the dry season ($p < 0.05$) (Fig. 12).

The mean IgG1 and IgG4 titres for *P. falciparum* negative individuals were higher than those of *P. falciparum* positive individuals at the end of the rainy season. Also the mean IgG2 and IgG3 titres for *P. falciparum* positive individuals were higher than those of *P. falciparum* negative individuals (Fig. 13). There was a significant difference in the anti-MSP-1$_{19}$ IgG4 subtypes between individuals positive or negative for *P. falciparum* parasites at the end of the rainy ($p < 0.05$) (Fig. 13). Subjects positive for anti-MSP-1$_{19}$ IgG2 and anti-MSP-1$_{19}$ IgG4 had a positive relationship between total IgG with parasitemia ($r = 0.079$ and $0.197$) while those positive for anti-MSP-1$_{19}$ IgG1 and anti-MSP-1$_{19}$ IgG3 had a negative relationship ($-0.007$ and $-0.039$ Pearson's correlation). Anti-MSP-1$_{19}$ IgG1 and IgG4 had a negative relationship with parasitemia ($r = -0.213$ and $-0.349$), while IgG2 and IgG3 had a positive relationship with parasitemia ($r = 0.285$ and $0.318$). The influence of anti-MSP-1$_{19}$ subtypes in malaria outcome was shown using regression analysis.

The regression showed that none of the subtypes had an effect in predicting *P. falciparum* positive individuals in the dry and rainy seasons.

## 4. Discussion

A major determinant of malaria epidemiology is the pattern of acquired immunity to *P. falciparum* infection, with individuals that can tolerate parasitemias living in malaria endemic areas (Bradley, 1991). Epidemiological studies have demonstrated that the burden of morbidity and mortality is concentrated among the youngest age groups under conditions of intense, perennial, stable transmission. The precise relationship between frequencies of parasite exposure, functional immunity and disease risk remains ill defined (Snow et al., 1999). Antibody responses directed against surface proteins of the merozoite may function either by blocking red blood cell invasion or by making the merozoite susceptible to phagocytosis. Parasite antigen-specific antibody plays an important role in controlling parasitemia via antibody dependent cellular inhibition (ADCI), whereby binding of antibodies

to phagocytes via Fc receptors leads to inhibition of parasite growth (Tebo et al., 2001). MSP-1$_{19}$ specific antibodies not only inhibit secondary processing of the MSP-1 precursor but also bind to MSP-1$_{19}$ thus preventing merozoites from binding to the red blood cell surface (Wipasa et al., 2002). Children living in Igbo-Ora have been reported to have antibodies that inhibit the processing of MSP-1 (Nwuba et al., 2002). Studies have linked anti-MSP-1$_{19}$ antibodies in infants with protection from clinical malaria (Hogh et al., 1995; Branch et al., 1998). This would imply that there is a transfer of maternal antibodies to these children. Unpublished results by Nwuba et al., show the transfer of specific anti-MSP-1$_{19}$ antibodies from mother to child in Igbo-Ora. This may be the reason for the high anti-MSP-1$_{19}$ in the children studied.

Igbo-Ora is located in the savannah region where malaria though seasonal is also persistent throughout the year, most individuals living in this area are constantly exposed to infection, with the levels of infection increasing in the rainy season. Results from this study mirror this argument that the prevalence of *P. falciparum* infection is higher in the rainy season (Table 1). The results however showed that age is more important in estimating the immune response of individuals in the dry season than at the end of the rainy season when other factors such as increased exposure to the parasite are considered. There was an increase in anti-MSP-1$_{19}$ IgG antibody titre with age in both the dry season (Fig. 1) and the rainy season (Fig. 2). The decrease in the mean IgG antibody titre in children between 61 and 120 months was more pronounced in the dry season (Figs. 3 and 4). There was a significant decrease in the anti-MSP-1$_{19}$ IgG antibodies produced at the end of the rainy season compared with the dry season. Braga et al. (2002) showed that in individuals with long-term exposure to malaria, a higher percentage that was asymptomatic for malaria recognised MSP-1$_{19}$. This is similar to the result obtained from this study, since the incidence of malaria infection is higher in the rainy season. It is a widely accepted fact that individuals living in an endemic area develop immunity against malaria infection, as they grow older and they produce specific antibodies, due to their constant exposure to parasite antigens. Al-Yaman et al. (1996) reported that the prevalence and concentration of antibodies to parasite derived MSP-1 as well as to both the C- and N-terminal regions increased with age. The MSP-1$_{19}$
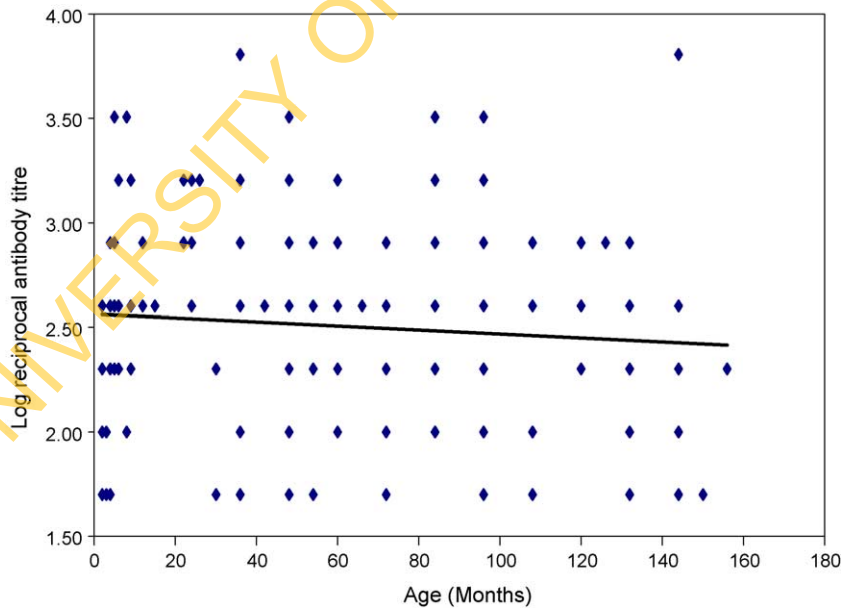


Fig. 7. Correlation between anti-MSP-1$_{19}$ IgG and age for samples positive for *P. falciparum* at the end of the rainy season. Scatter diagram showing the relationship between age and anti-MSP-1$_{19}$ IgG antibody titre for children whose giemsa stained blood smears were *P. falciparum* positive. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

recombinant antigen used in this study had the two EGF motif of MSP-1 which had been shown by Egan et al. (1995, 1996) to have increased prevalence of antibodies against it with age.

Analysis on individuals who were either *P. falciparum* positive or negative in the dry season revealed that the total anti-MSP-$1_{19}$ antibodies correlated with age (Figs. 5 and 6) and there was no significant difference in the anti-MSP-$1_{19}$ IgG titres between the two groups. In contrast, at the end of the rainy season the antibody titres of *P. falciparum* negative individuals positively correlated with age, while the MSP-$1_{19}$ antibody titres of those who were *P. falciparum* positive correlated negatively with age, at the end of the rainy season (Figs. 7 and 8). There was a significant increase in the anti-MSP-$1_{19}$ IgG titres in *P. falciparum* positive individuals in the rainy season, compared with those who were *P. falciparum* negative. The results imply that parasitic infection rather than age might be a strong determining factor for *P. falciparum* positive individuals, in assessing MSP$1_{19}$ antibody levels at the end of the rainy season as observed in the dry season. Branch

et al. (1998) stated that untreated infants who went on to clear their parasitemia without requiring treatment had significantly higher anti-MSP-$1_{19}$ IgG levels one month prior to infection than untreated infants who did not clear their parasitemia and they associated this with protection against parasitemia. There was a significant positive relationship between the anti-MSP-$1_{19}$ IgG and parasite density. This implies that the level of antibodies against MSP-$1_{19}$ increased with increasing parasite densities. It could be agued that the high IgG titre in *P. falciparum* positive individuals in the rainy season could be due to the increased parasite infection and subsequent production of anti-MSP-$1_{19}$ antibodies, which would eventually aid in parasite clearance. The requirement for high titre would reflect the fact that plasma antibodies must act quickly between rupture of one erythrocyte and invasion of another (Good and Doolan, 1999). Also this relatively high antibody response against MSP-1 C-terminal could be given the necessary cognate help by T-cell epitopes outside the MSP-$1_{19}$ region (Quin and Langhorne, 2001). Egan et al. (1996) reported that anti-MSP-$1_{19}$ antibodies tend
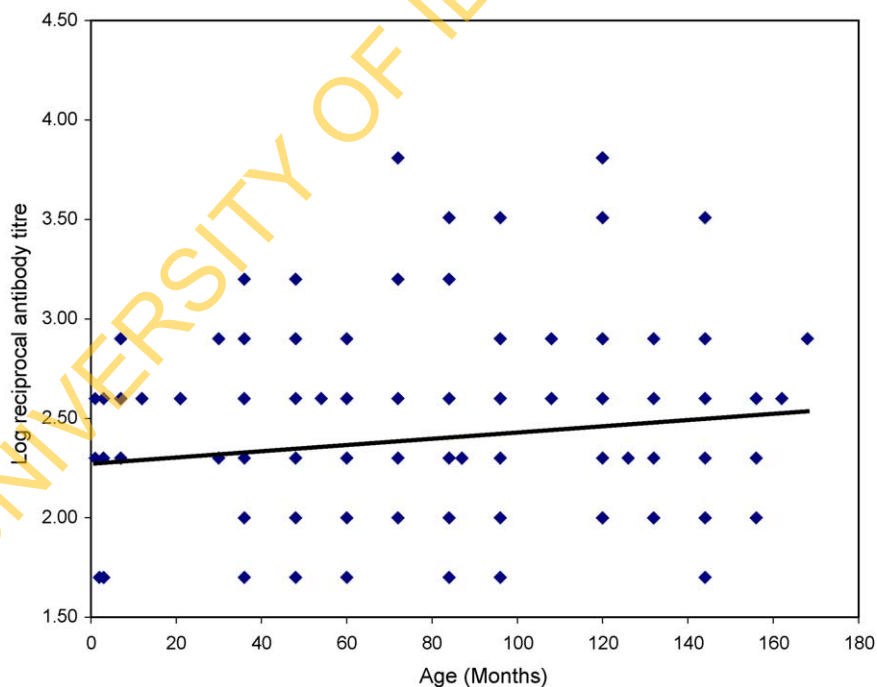


Fig. 8. Correlation between anti-MSP$1_{19}$ IgG and age for samples negative for *P. falciparum* at the end of the rainy season. Scatter diagram showing the relationship between age and log anti-MSP-$1_{19}$ IgG antibody titre for children whose giemsa stained blood smears were *P. falciparum* negative. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

to be higher in children who experienced only asymptomatic infections than in children who experienced clinical infections.

Results from this study showed that in the dry season all four subclasses of IgG were present in agreement with Aucan et al. (2001) who showed that IgG1-IgG4 from most of their study subjects recognised the antigens RESA, MSP-1 and MSP-2. In the dry season, the cytophilic antibodies IgG1 and IgG3 were more predominant (84.4%) in all the children surveyed, with anti-MSP-$1_{19}$ IgG4 as the least prevalent (55%) (Fig. 9). The same trend was observed at the end of the rainy season (Fig. 1) with anti-MSP-$1_{19}$ IgG1 and anti-MSP-$1_{19}$ IgG3 being predominant (87% and 97.8%, respectively). Shi et al. (1996) and Nguer et al. (1997) had also showed IgG1 and IgG3 to be predominant in children and adults living in a malaria endemic area. Comparing the mean IgG subtype antibody titres for both seasons it was observed that while the anti-MSP-$1_{19}$ IgG1, IgG2, and IgG3 increased at the end of the rainy season, anti-MSP-$1_{19}$ IgG4 decreased (Fig. 9). This increase was
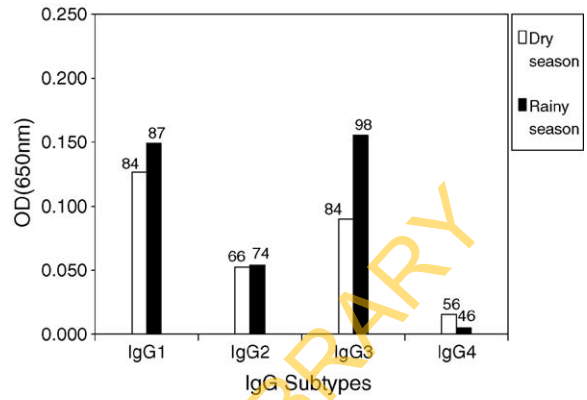


Fig. 9. Mean subtype titre in the dry season and at the end of the rainy season. The mean absorbance values for each of the IgG subtypes was determined for the dry season and at the end of the rainy season and compared. The numbers on the bars represent the percentage of individuals positive for the subtypes in each season.
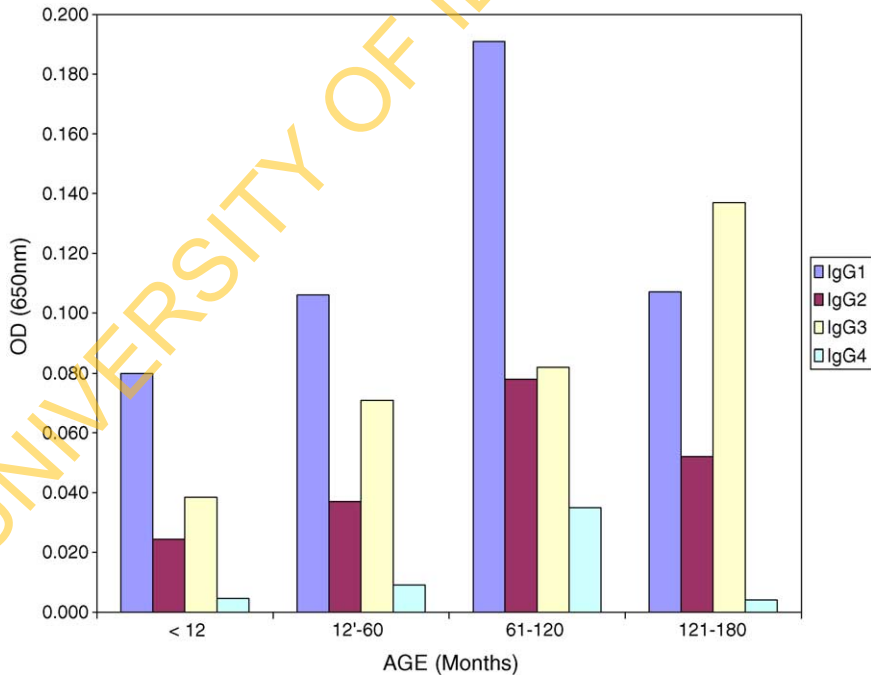


Fig. 10. Relationship between anti-MSP-$1_{19}$ IgG1, IgG2, IgG3 and IgG4 with age in the dry season. The relationship between the absorbance values of the IgG subtypes (IgG1, IgG2, IgG3 and IgG4) and the age of the children was determined. This relationship was described in the dry season and at the end of the rainy season. The absorbance value is expressed as optical density while the age is in months.

significant only for anti-MSP-$1_{19}$ IgG3. Braga et al. (2002) related the presence of anti-MSP-$1_{19}$ IgG1 with long time exposure and anti-MSP-$1_{19}$ IgG3 with short time exposure to malaria. This might also help explain the higher IgG3 levels noticed at the end of the rainy season in this study, though both cytophilic antibodies are functionally equivalent. Transmission level tends to have a greater (but still transient) impact upon the isotype distribution, whereas the duration of exposure to the parasite had a maximum influence upon the isotype level in children (Aribot et al., 1996). In the dry season anti-MSP-$1_{19}$ IgG1, anti-MSP-$1_{19}$ IgG2 and anti-MSP-$1_{19}$ IgG3 increased with age (Fig. 10), with anti-MSP-$1_{19}$ IgG3 having the strongest correlation with age ($p < 0.01$, $r = 420$). Shi et al. (1996) reported that the prevalence and levels of response of IgG1 and IgG3 increased with age. At the end of the rainy season this was reversed; only anti-MSP-$1_{19}$ IgG2 and anti-MSP-$1_{19}$ IgG4 correlated positively with age (Fig. 11). This could explain at least in part, a higher incidence of malaria at the end of the rainy season than in the dry season. It could therefore be inferred that in this study, age and seasonal variation

influenced the anti-MSP-$1_{19}$ IgG subclass production. This is in contrast to the work by Egan et al. (1996) who showed that there was no detectable association between anti-MSP-$1_{19}$ IgG subclass and age.

There is a negative correlation between parasite density and anti-MSP-$1_{19}$ IgG1 IgG4. It should be noted that the levels of anti-MSP-$1_{19}$ IgG4 were, however very low. Wipasa et al. (2002) reported that the level of anti-MSP-$1_{19}$ IgG1 was inversely correlated with parasite density, while Braga et al. (2002) demonstrated a high level of anti-MSP-$1_{19}$ IgG1 in asymptomatic patients suggesting that anti-MSP-$1_{19}$ IgG1 antibody response may play a role in protection in humans. The results from this study showed that anti-MSP-$1_{19}$ IgG1 titres and anti-MSP-$1_{19}$ IgG3 titres were higher in individuals that were *P. falciparum* negative in the dry season, while anti-MSP-$1_{19}$ IgG2 and anti-MSP-$1_{19}$ IgG4 titres were higher in *P. falciparum* positive individuals (Fig. 12). The anti-MSP-$1_{19}$ IgG1 and anti-MSP-$1_{19}$ IgG4 titres were higher in individuals that were *P. falciparum* negative at the end of the rainy season, while anti-MSP-$1_{19}$ IgG2 and anti-MSP-$1_{19}$ IgG3 were higher in *P. falciparum* positive individu-
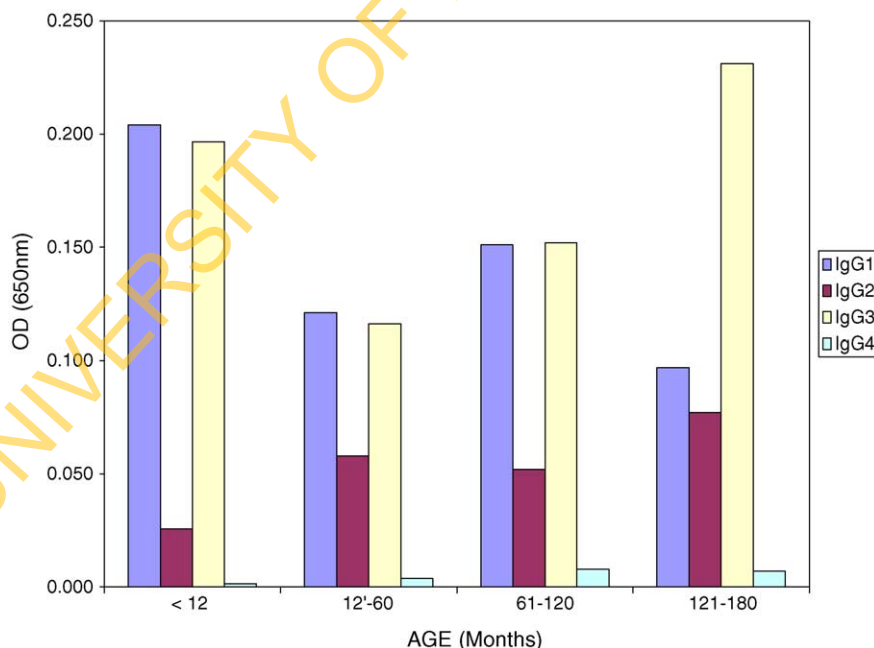


Fig. 11. Relationship between anti-MSP-$1_{19}$ IgG1, IgG2, IgG3 and IgG4 with age at the end of the rainy season. The relationship between the absorbance values of the IgG subtypes (IgG1, IgG2, IgG3 and IgG4) and the age of the children was determined. This relationship was described in the dry season and at the end of the rainy season. The absorbance value is expressed as optical density while the age is in months.
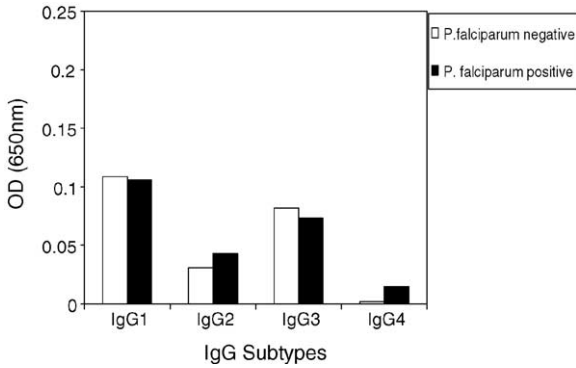
Fig. 12. Mean absorbance values of IgG subtypes for individuals positive or negative for *P. falciparum* in the dry season. The mean absorbance values of IgG subtypes for individuals whose smears were positive and negative for *P. falciparum* were compared for each season.

als (Fig. 13). The results also showed that there was a negative correlation between samples with anti-MSP-$1_{19}$ IgG1 and anti-MSP-$1_{19}$ IgG3 with parasite density, while there was a positive correlation of parasite density with samples that were positive for anti-MSP-$1_{19}$ IgG2 and anti-MSP-$1_{19}$ IgG4. Shi et al. (1996) and Wipasa et al. (2002) reported that IgG1 and IgG3 were protective, while IgG4 and IgG2 block the effect of the cytophilic antibodies. The present study supports
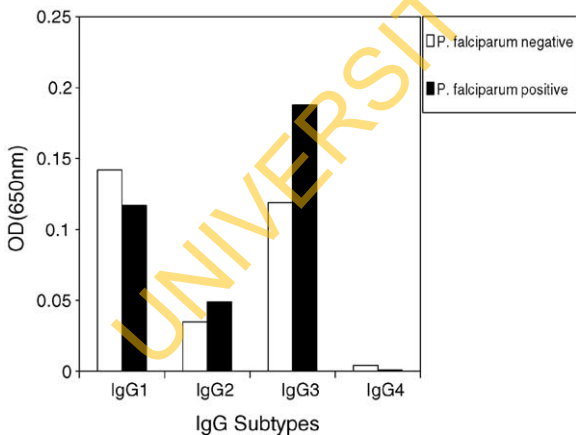


Fig. 13. Mean absorbance values of IgG subtypes for individuals positive or negative for *P. falciparum* at the end of the rainy season. The mean absorbance values of IgG subtypes for individuals whose smears were positive and negative for *P. falciparum* were compared for each season.

the earlier result and showing that anti-MSP-$1_{19}$ IgG1 and IgG3 might be protective against *P. falciparum* in the children studied. This result points to the fact that non-cytophilic antibodies are associated with increased parasitemia in the rainy season.

The results strongly suggest that the children living in Igbo-Ora have some form of immunity against malaria due to the observed high prevalence of antibodies against MSP-$1_{19}$. It has been found that seasonal variations affects the kind of IgG subclass being expressed by children in Igbo-Ora, and its effect on anti-MSP-$1_{19}$ IgG production should be considered as an significant factor when reporting correlation experiments between antibody responses and age due to the increased number of younger children with *P. falciparum* infection in the rainy season. As shown from the results anti-MSP-$1_{19}$ IgG1 and IgG3 antibodies are important in parasite clearance and the dynamics of the anti-MSP-$1_{19}$ IgG1, IgG2, IgG3 and IgG4 antibodies could play an essential part in protecting children living in this area from malaria. Therefore the production of an MSP-$1_{19}$ based vaccine that would produce more IgG1 and IgG3 antibodies might prove beneficial.

## Acknowledgements

## References

Achidi, E.A., Salimonu, L.S., Asuzu, M.C., Berzins, K., Walker, O., 1996. Studies on *Plasmodium falciparum* parasitemia and

development of anemia in Nigerian infants during their first year of life. Am. J. Trop. Med. Hyg. 55 (2), 138–143.

Al-Yaman, F., Genton, B., Kramer, K.J., Chang, S.P., Hui, G., Baisor, M., Alpers, M.P., 1996. Assessment of the role of naturally acquired antibody levels to *Plasmodium falciparum* Merozoite surface protein-1 in protecting Papua New Guinean Children from malaria morbidity. Am. J. Trop. Med. Hyg. 54 (5), 443–448.

Aribot, G., Rogier, C., Sarthou, J., Trape, J., Balde, A.T., Druilhe, P., Roussilhon, C., 1996. Pattern of immunoglobulin isotype response to *Plasmodium falciparum* blood-stage antigens in individuals living in a holoendemic area of Senegal [Dielmo, West Africa]. Am. J. Trop. Med. Hyg. 54 (5), 449–457.

Aucan, C., Traore, Y., Fumoux, F., Rihet, P., 2001. Familial correlation of immunoglobulin G subclass responses to *Plasmodium falciparum* antigens in Burkina Faso. Infect. Immun. 69 (2), 996–1001.

Blackman, M.J., Scott-Finnigan, T.J., Shai, S., Holder, A.A., 1994. Antibodies inhibit the protease-mediated processing of a malaria merozoite surface protein. J. Exp. Med. 180, 389–393.

Bradley, D.J., 1991. Malaria. In: Feachem, R.G., Jamison, D.T. (Eds.), Disease and Mortality in Sub-Saharan Africa. Oxford University Press/International Bank for Reconstruction and Development/The World Bank, pp. 190–202.

Braga, M.E., Barros, R.M., Reis, T.A., Fontes, C.F., Morais, G.C., Martins, M.S., Krettli, A.U., 2002. Association of the IgG response to *Plasmodium falciparum* merozoite protein (C-terminal 19 kDa) with clinical immunity to malaria in the Brazilian Amazon region. Am. J. Trop. Med. Hyg. 66 (5), 461–466.

Branch, O.H., Udhayakumar, V., Hightower, A.W., Oloo, A.J., Hawley, W.A., Nahlen, B.L., Bloland, P.B., Kaslow, D.C., Lal, A.A., 1998. A longitudinal investigation of IgG and IgM antibody responses to 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.

Cavanaugh, D.R., Dobano, C., Elhassan, I.M., March, K., Elhassan, A., Hviid, L., Khalil, E.A.T.G., Theander, T.G., Arnot, D.E., McBride, J.S., 2001. Differential patterns of human immunoglobulin G subclass responses to distinct regions of a single protein, the merozoite surface protein 1 of *Plasmodium falciparum*. Infect. Immun. 69 (2), 1207–1211.

Dodoo, D., Theander, T.G., Kurtzhals, J.A.L., Koram, K., Riley, E., Akanmori, B.D., Nkrumah, F.K., Hviid, L., 1999. Levels of antibody to conserved parts of *Plasmodium falciparum* merozoite surface protein-1 in Ghanaian children are not associated with protection from clinical malaria. Infect. Immun. 67 (5), 2131–2137.

Diallo, T.O., Spiegel, A., Diouf, A., Perraut, R., Kaslow, D.C., Garraud, O., 2001. Short report: IgG1/IgG3 antibody responses to various analogs of recombinant yPfMSP119—a study in immune adults living in areas of *Plasmodium falciparum* transmission. Am. J. Trop. Med. Hyg. 64 (3/4), 204–206.

Egan, A.F., Chappel, J.A., Burghaus, P.A., Morris, J.S., McBride, J.S., Holder, A.A., Kaslow, D.C., Riley, E.M., 1995. Plasma antibodies from malaria-exposed people recognize conserved epitopes formed by the two epidermal growth factor motifs of MSP1₁₉, the carboxyl-terminal fragment of the major merozoite surface protein of *Plasmodium falciparum*. Infect. Immun. 63 (2), 456–466.

Egan, A.F., Morris, J., Barnish, G., Allen, S., Greenwood, B.M., Kaslow, D.C., Holder, A.A., Riley, E.M., 1996. Clinical immunity to *Plasmodium falciparum* malaria is associated to the 19-kDa C-terminal fragment of the merozoite surface antigen, Pf MSP-1. J. Infect. Dis. 173, 765–769.

Ferreira, M.U., Kimura, E.A.S., Katzin, A.M., Santos-Neto, L.L., Ferrari, J.O., Villalobos, J.M., De Carvalho, M.E., 1998. The IgG-subclass distribution of naturally acquired antibodies to *Plasmodium falciparum*, in relation to malaria exposure and severity. Ann. Trop. Med. Parasitol. 92 (3), 245–256.

Good, M.F., Doolan, D.L., 1999. Immune effector mechanisms in malaria. Curr. Opin. Immunol. 11, 412–419.

Hogh, B., Marbianh, N.T., Burghaus, P.A., Andersen, P.K., 1995. Relationship between maternally derived anti-*Plasmodium falciparum* antibodies and risk of infection and disease in infants living in an area of Liberia, West Africa, in which malaria is endemic. Infect. Immun. 63 (10), 4034–4038.

Jouin, H., Rogier, C., Trape, J.F., Mercereau-Puijalon, O., 2001. Fixed, epitope-specific, cytophilic antibody response to the polymorphic block 2 domain of the *Plasmodium falciparum* merozoite surface antigen MSP-1 in humans living in a malaria-endemic area. Eur. J. Immunol. 31, 539–550.

Lawrence, B.R., 1965. Medical entomology at Igbo-Ora, western Nigeria. J. Nig. Med. Ass. 2, 198–205.

Nguer, C.M., Diallo, T.O., Diouf, A., Tall, A., Dieye, A., Perraut, R., Garraaud, O., 1997. *Plasmodium falciparum* – and merozoite surface protein 1 – specific antibody isotype balance in immune Senegalese adults. Infect. Immun. 65 (11), 4873–4876.

Nwagwu, M., Anumudu, C.A., Sodeinde, O., Ologunde, C.A., Obi, T.U., Wirtz, R.A., Gordon, D.M., Lyon, J.A., 1998. Identification of a subpopulation of immune Nigeria adult volunteers by antibodies to the circumsporozoite protein of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. 58 (5), 684–692.

Nwuba, R.I., Sodeinde, O., Anumudu, C.I., Omosun, Y., Odaibo, A.B., Holder, A.A., Nwagwu, M., 2002. The human immune response to *Plasmodium falciparum* includes production of antibodies that inhibit MSP-1 secondary processing. Infect. Immun. 70 (9), 5328–5331.

Perlman, P., Bjorkman, A., 2000. Malaria research: host–parasite interactions and new developments in chemotherapy, immunology and vaccinology. Curr. Opin. Infect. Dis. 13, 431–443.

Quin, S.J., Langhorne, J., 2001. Different regions of the malaria merozoite surface protein 1 of *Plasmodium chabaudi* elicit distinct T-cell and antibody isotype responses. Infect. Immun. 69 (4), 2245–2251.

Shi, Y.P., Sayed, U., Qari, S.H., Roberts, J.M., Udhayakumar, V., Oloo, A.J., Hawley, W.A., Kaslow, D.C., Nahlen, B.L., Lal, A.A., 1996. Natural immune response to the C-terminal 19-kilodalton domain of *Plasmodium falciparum* merozoite surface protein 1. Infect. Immun. 64 (7), 2716–2723.

Snow, R.W., Craig, M., Deichmann, U., Marsh, K., 1999. Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. Bull. WHO 77 (8), 624–639.

Taylor, R.R., Allen, S.J., Greenwood, B.M., Riley, E.M., 1995. IgG3 antibodies to *Plasmodium falciparum* merozoite surface protein 2[MSP2]: increasing prevalence with age and association with clinical immunity to malaria. Am. J. Trop Med. Hyg. 58, 406–413.

Tebo, A.E., Kremsner, P.G., Luty, A.J.F., 2001. *Plasmodium falciparum*: a major role for IgG3 in antibody-dependent monocyte-mediated cellular inhibition of parasite growth in vitro. Exp. Parasitol. 98 (1), 20–28.

WHO, 1985. Bench aids for the diagnosis of malaria. Plate Numbers 1–8.

Wipasa, J., Elliot, S., Xu, H., Good, M.F., 2002. Immunity to asexual blood stage malaria and vaccine approaches. Immunol. Cell Biol. 80, 401–414.