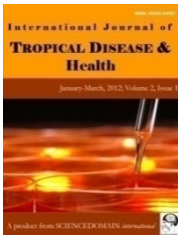

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Plasmodium falciparum Specific IgE, IgG and Anti-GPI IgG Antibodies in Cameroonian Children with Severe and Uncomplicated Malaria

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Research Article

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ABSTRACT

Aims: We investigated the role of antibodies in the pathogenesis of severe malaria in children by measuring and comparing plasma levels of antibodies to glycosyl phosphatidy linositol (GPI) and crude *Plasmodium falciparum* extract.
Study Design: Cross-sectional case-control study.
Place and Duration of Study: Five health institutions in two towns and seven primary schools in the South West region of Cameroon between April 2003 and December 2005.
Methodology: A total of 649 children including 25, 156 and 233 cases of cerebral malaria (CM), severe malaria anaemia (SMA) and uncomplicated malaria (UM) respectively were recruited from health institutions and 233 apparently healthy controls (HC) from schools using predefined inclusion criteria. Malaria parasitaemia was determined by light microscopy using Giemsa-stained thick blood smears, haemoglobin level using a haemoglobinometer and blood cell count using a haemocytometer. The levels of total IgE,

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P. falciparum IgG, IgE and anti-GPI IgG antibodies were measured from plasma by the ELISA technique.
Results: The mean white blood cell count (WBC) was higher in the severe malaria group compared with the HC group. Geometric mean parasite densities were significantly different (P<0.001) amongst the study groups but similar in the two severe malaria groups (Severe Malaria Anaemia and Cerebral Malaria). Seropositivity for IgG antibodies to *P. falciparum* was different within the study groups (P<0.001) and higher in the clinical cases compared to the HC group. Mean levels of anti-GPI IgG and *P. falciparum* specific IgE and IgG antibodies were significantly different among the study participant categories. Mean plasma levels of these antibodies were higher in the UM and HC groups when compared with the severe malaria groups. There was a significant positive correlation between the age of the participant and levels of anti-GPI IgG (P<0.001), *P. falciparum* IgE (P = 0.027) and total IgE (P = 0.020) antibodies.
Conclusion: Our observation of lower levels of anti-GPI and *P. falciparum* specific IgE antibodies in the severe group compared with the control group suggest a protective role of these antibodies in the pathogenesis of severe malaria. The correlation observed between *P. falciparum* IgE, IgG and GPI IgG antibody levels with age confirm previous reports that immunity to malaria develops with age and is partially dependent on antibody production.

Keywords: Antibodies; glycosylphosphatidylinositol; severe malaria; P. falciparum.

1. INTRODUCTION

Infection with the malaria parasite may result in asymptomatic, mild or severe disease. Mortality is mainly due to severe malaria, and death may occur within 48 hours of hospital admission, especially in children under 5 years of age. The frequency and severity of *falciparum* malaria generally decreases over successive malaria exposure, suggesting that immunity is exposure related (Chaiyaroj et al., 2004). Antibody-dependent mechanisms are presumed to play an important role in protection and a wide range of antigen-specific antibodies as well as polyclonal antibody production have been implicated (Bereczky et al*.,* 2004; Lucchi et al., 2008).

The mechanisms and agents leading to the pathogenesis of severe malaria are not clearly understood although previous studies seem to indicate the importance of parasite and immunological factors. Different pathways are thought to be involved including glycosylphosphatidylinositol (GPI) of *Plasmodium falciparum* and Immunoglobulin E (IgE) (Clark and Schofield, 2000).

Malaria GPI-anchored proteins expressed by *Plasmodium* species have a potentially important role in the induction of innate immune responses to malaria, such as macrophage activation, parasite phagocytosis and killing and may thus contribute to rapid control of blood stage infections (de Souza et al., 2002). In a previous study, monoclonal antibodies to GPI completely blocked induction of TNF-α from macrophages by crude extracts of *Plasmodium* and the passive transfer of monoclonal antibodies raised against these antigens prevented cerebral malaria in a murine malaria model (WHO, 2001). Parasite specific GPI act as a toxin and is able to induce tumour necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine and interleukin 1 (IL-1) (Schofield et al., 1993) amongst others. In synergy with

interferon-gamma (IFN-γ), GPI increases expression of intercellular adhesion molecule 1 (ICAM-1), nitric oxide synthase (iNOS) and nitric oxide (NO), which are implicated in the aetiology of cerebral malaria (Schofield et al., 1996). Antibodies against GPI may therefore reduce the pathology mediated through this pathway. The role and mechanism(s) by which parasite specific GPI initiate or mediate the pathogenesis of severe malaria therefore warrants further investigation.

The induction of TNF-α cannot be attributed to parasite derived toxins of GPI origin alone, since it has been shown that IgE efficiently activates monocytes to kill parasites by inducing surface expression and cross-linking of CD23, the low affinity receptor for IgE (Vouldoukis et al., 1995). This cross-linking leads to activation of iNOS and the genes coding for TNF-α (Jacobs et al., 1996; Anstey et al., 1999). Previous studies point to a role of IgE as well as anti-malarial IgE antibodies in both the pathogenesis/protection from severe disease and/or the regulation of malaria-specific immune responses (Desowitz et al., 1993; Perlmann et al., 1999, 2000; Bereczky et al., 2004; Farouk et al., 2005; Duarte et al., 2007). In *P. falciparum* malaria, it has been shown that IgE elevation is most pronounced in cerebral malaria and that IgE elevation parallels elevated levels of TNF-α in serum (Perlmann et al., 1999). Furthermore, the deposition of IgE in microvessels has been correlated with parasite sequestration in post-mortem brain tissues (Maeno et al., 2000). IgE antibodies in the form of immune complexes are thought to cross-link the IgE receptors (CD23) *in vivo*, thereby triggering local overproduction of TNF-α and NO (Perlmann et al., 1997, Perlmann et al., 1999; Maeno et al., 2000; Perlmann and Troye-Blomberg, 2000).

In this study, we investigated the role of antibodies in the pathogenesis of severe malaria by measuring and comparing plasma levels of anti-GPI IgG and *P. falciparum* specific IgE and IgG in cerebral malaria, severe malaria anaemia, uncomplicated malaria and healthy control children below 14 years of age.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out between April 2003 and December 2005 in Fako division of South West Cameroon, where malaria transmission is perennial. The study sites included hospitals (Regional Hospital Limbe and Bota District Hospital Limbe), health centres (Mount Mary Health Centre Buea, Bokova Health Centre Buea and PMI Down Beach Limbe) and primary schools (Catholic School (CS) Muea, CS Buea Station and CS Great Soppo, Government School (GS) Bolifamba, GS Bonduma, Government Practising School (GPS) Molyko I and II, GPS Muea I and II). This region experiences two seasons: the dry (November - March) and the rainy (March-October, when malaria transmission is more intense) seasons. *P. falciparum* causes more than 90% of malaria infections in this area and *Anopheles gambiae* is the dominant vector species (Wanji et al., 2003). Malaria prevalence attains 70-85% during the peak transmission season, which drops to 30% during the low transmission season, the dry season (Nkuo-Akenji et al., 2004).

2.2 Study Population

The study participants consisted of sick and healthy children 1-14 years old reporting for medical attention at health care facilities or attending the primary schools respectively. Ethical clearance was obtained from the Institutional Review Board of the University of Buea,

Cameroon and authorization to conduct the school surveys was obtained from the Regional Delegate of Basic Education, South West region. In addition, at each school, the approval of the teachers were sought and informed consent forms explaining in details the project objectives, protocols and benefits were sent through the children to their parents/guardians. Only children whose parents consented in writing were enrolled into the study. During the school surveys, information relating to gender, age and area of residence were recorded. The axillary temperature of each pupil was obtained using a digital thermometer. Only apparently healthy children with a body temperature below 37.5ºC with or without malaria parasitaemia were enrolled as controls.

For the sick cases informed consent was obtained from the parent and/or guardian at consultation in the respective health institutions. Using a well structured case report form, volunteers were enrolled into the following groups after due consideration of the criteria indicated below:

2.2.1 Case definition

Inclusion criteria for malaria patients were: fever (Temperature ≥37.5ºC) measured within 24 hours of admission, malaria parasitaemia and at least one other sign of malaria (vomiting, diarrhoea, malaise etc). Study participants homozygous for the sickle cell trait were excluded.

2.2.1.1 Specific Inclusion and exclusion criteria

Cerebral malaria (CM) cases: Unconscious, with a Blantyre coma score of < 3 and duration of coma > 60 minutes, any haemoglobin value, no record of recent severe head trauma and other cause of coma or neurological diseases.

Severe Malaria Anaemia (SMA) cases: Haemoglobin < 5g/dL and no incidences of severe bleeding reported.

Uncomplicated Malaria (UM) cases: Haemoglobin value > 8g/dL and fully conscious

2.3 Sample Collection and Processing

Venous blood (2-5ml) was collected from the study participants into EDTA vacutainer tubes for parasitological, haematological and serological studies. The blood samples were collected from the sick children on hospital admission and before treatment. The cases were mostly given quinine sulphate (79.9%) or artesunate (15.8%) and then rescued with artesunate (73.9%) or amodiaquine (20.9%) in accordance with national guidelines. The blood samples were centrifuged (Beckman TJ-6, USA) at 2500 x g for 5 minutes and plasma supernatants stored in eppendorf tubes (Brinkman Instrument Inc., Westbury, USA) at -86ºC until analysed. For some of the participants, the sample volume was too small to run all the analyses.

2.4 Parasitological Examination

Thick blood films were prepared for all participants, air-dried, stained with Giemsa (Sigma, St. Louis, USA) and examined for malaria parasites by light microscopy. Parasitaemia density was estimated by counting parasites against a minimum of 200 white blood cells and

assuming a mean leucocyte count of 8000/μL (Rooth et al., 1991). Fifty high power fields (HPF) were scanned to confirm malaria negative slides. Urine samples were also collected from each sick case and examined for schistosome eggs by microscopy. None of the patients were positive for schistosoma.

2.5 Haematological Measurements

2.5.1 Packed Cell Volume (PCV) and Hb Measurements

Haemoglobin (Hb) levels of the study children were determined using a haemoglobinometer (HemoCue, Anglholm, Sweden). PCV values were also determined using a microhaematocrit centrifuge (HHC-24, Hanshin Medical Co. Ltd, Korea) and read off using a Hawksley microhaematocrit reader. White Blood Cell (WBC) and Red Blood Cell (RBC) counts were determined using a haemocytometer as described by Cheesbrough (2006).

2.5.2 Sickle Cell Test

The rapid screening test described by Cheesbrough (2006) was used to determine the sickle cell status of study participants eligible for recruitment into the study. A drop of blood was placed on a slide and mixed with an equal volume of freshly prepared 2% w/v sodium metabisulphite. The wet preparation was placed in a petri dish with a damp piece of tissue. After 10-20 min the slides were examined microscopically for sickled cells. Positive cases were confirmed by haemoglobin electrophoresis using cellulose acetate membrane.

2.6 ELISA Seroreactivities to Crude Blood Stage Antigens of *P. falciparum* and GPI

2.6.1 Anti-GPI Immunoglobulin G (IgG) ELISA

Anti-GPI IgG analysis was conducted as previously described by de Souza et al. (2002), with some modifications as prescribed by the donor of the GPI antigen. Lyophilised GPI (a kind donation from Dr. Channe Gowda, Pennsylvania State University, USA) stock solution was prepared in water saturated 1-butanol (0.1 μg/ml). The stock solution was diluted to a working concentration of 2 ng/50μl with methanol. Briefly, microtitre plates were coated with 50 μL/well of the dilute GPI (2 ng/well), dried at 37ºC and blocked with 150 μL/well of 1% BSA in PBS for 1h. Plasma samples were diluted 1:100 in PBS-0.05% Tween added to the blocked plates (50 μL/well) and incubated for 2h at room temperature. Plates were incubated at room temperature for 1h with 50 μL/well of HRP-conjugated goat anti-human IgG (Caltag Laboratories, Burlingame, U.K.) diluted 1:2000 in 1% BSA-PBS. The plates were incubated with 50 μL/well of K-Blue substrate (R&D Systems, USA) for an hour in the dark. Optical densities were read from an ELISA microplate reader (Thermo Labosystems, China) set at 450 nm. Plates were washed four times between incubation steps with PBS-Tween 20 (0.05% Tween).

2.6.2 *Plasmodium falciparum* Specific IgG and IgE ELISA

Total IgE, *P. falciparum*-specific IgE and IgG antibodies were measured by ELISA using total blood stage parasite (F32) extract (schizont enriched) as capture antigen. ELISA for the determination of the isotypic malaria specific antibodies was done using the method described by Perlmann et al. (1989). The IgG antibodies were measured using goat anti-

human IgG (Fc-specific) (Sigma, St. Louis, Missouri) while total and malaria specific IgE was measured using affinity purified rabbit IgG antibodies specific for human IgE (5 μg/ml; Miab, Uppsala, Sweden). From checkerboard titration, it was determined that the optimal concentration for coating the crude blood stage antigens was 10 μg/ml for IgG and 5 μg/ml for Pf IgE. The test plasma was screened at 1/200 dilution for IgG and 1/100 for IgE.

Microtitre plates (Corstar, Cambridge, MA) coated with crude antigens in carbonate buffer (50 μl/well) were incubated overnight at 4ºC and then blocked for 3 hours at 37ºC with phosphate-buffered saline (PBS) containing 2% Bovine Serum Albumin (BSA). Duplicate wells were subsequently incubated with 50 μl/well of test serum in PBS-0.05% Tween 20 and isotype specific anti-sera conjugated to alkaline phosphatase (ALP). All incubations were done at 37ºC for 1 h. Since detection of IgE antibodies in the presence of excess IgG antibodies requires prolonged interaction times (Elghazali et al., 1997) incubation was at room temperature overnight. Bound IgE was assayed with biotinylated, IgE-specific rabbit antibodies followed by ALP-conjugated streptavidin (Mabtech, Stockholm, Sweden) as described in detail by Perlmann et al. (1994). Para-nitrophenol phosphate (Sigma, St Louis, USA) was added and the absorbance at wavelength 405nm (OD_{405}) read with a multiscan ELISA plate reader.

Test samples with OD_{405} values in excess of the mean + 2SD of the values of 25 non-immune European donors were defined as seropositive. Sera from adult African and European donors (British and Swedish not exposed to malaria) served as positive and negative controls respectively.

2.7 Statistical Analysis

Analyses were performed using the software package SPSS Statistics 17 (SPSS Inc., Chicago, IL). Malaria parasite density and antibody optical density values were log transformed before analyses. The Pearson Chi-Square test was used to evaluate group differences in parasite and antibody seropositivity while differences in group means were assessed using the Students' t–test and analysis of variance (ANOVA). Multivariate analysis was undertaken using logistic and linear regression using as covariate, age. Correlation between variables was determined using Pearson correlation. Statistical significance was set at $P \leq 0.05$.

3. RESULTS

3.1 Characteristics of the Study Participants

A total of 649 (348 males and 298 females) children below 14 years were enrolled into the study consisting of 181 with severe malaria (25 CM cases and 156 SMA cases), 233 UM cases and 233 apparently healthy controls (HC). The mean age of the children was 5.30 ± 3.22 (range: 1–13 years). CM children (3.92 ± 1.94 years) were significantly older (P=0.008) than SMA children (2.61 ± 2.25 years). There was no significant association between gender and study participant category (P=0.227).

3.2 Haematological Indices

The mean (± SD) age, Hb level, packed cell volume (PCV), white blood cell count (WBC) and body temperature (ºC) of the different study participant categories are shown in Table 1.

Children in the HC and UM groups were significantly older than severe malaria groups. Body temperature (degree of fever) was not an indicator of the severity of malaria and as expected the lowest Hb level occurred in the SMA group. There was a significant difference in the mean WBC count between the different study categories. The mean WBC count was lowest in the HC group and highest in the severe malaria anaemia group with the UM group being intermediate. Furthermore, the mean WBC count (8903 ± 5529 /μl of blood) of the combined severe malaria groups (CM + SMA) was higher when compared with that of the UM (P = 0.011) and HC (P < 0.001) groups.

Table 1. Mean (± SD) Hb (g/dl) level, packed cell volume (PCV; %), white blood cell count (WBC, /μl of blood) and body temperature (ºC) of the different study participant categories

Participant Category	n	Mean ± SD				
		Age (years)	Hb (g/dL)	*PCV (%)*	WBC (/μl)	Body Temp. (ºC)
CM	25	3.9±1.9	7.50±2.2	22.78±6.78	8596±3309	38.89±1.05
SMA	155	2.6±2.3	4.20±1.2	12.6 ±3.73	8950±5806[†]	38.69±0.85
UM	230	4.6±3.4[*]	9.73±1.3	29.03±4.36	7695±4044	38.86±1.27
HC	233	7.6±1.8[♣]	12.3±1.3	36.84±3.82	6762±2689	37.11±0.35[‡]
Significance of Difference		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

[♣]Age significantly higher than the corresponding values for CM (P<0.001), SMA (P<0.001) and UM (P<0.001).

**Age significantly higher than the corresponding values for SMA (P<0.001).*

[†]WBC Significantly higher than the corresponding values for UM (P=0.022) and HC (P<0.001).

[‡]Body temperature significantly lower than the corresponding values for CM (P<0.001), SMA (P<0.001) and UM (P<0.001).

NB: Hb and PCV values are significantly different between the participant categories (P<0.001 each); For some of the participants, the sample volume was insufficient to run all the analyses.

3.3 Malaria Parasitaemia

The parasite density of the different study participant categories is shown in Figure 1. Although there was a significant difference in malaria parasitaemia among the different participant categories (P<0.001), values were significantly higher (P<0.001 each) in patient categories (CM, SMA and UM) compared to healthy controls but similar among patient categories. When the severe malaria groups were combined (CM + SMA) and their mean parasite density compared with the UM group the difference was not significant (P=0.056). One hundred and twenty-nine (55.4%) of the children in the healthy control group had malaria parasites. In all, 22 (4.4%) of the study children had hyperparasitaemia, with most of these (54.5%) being patients with uncomplicated malaria.

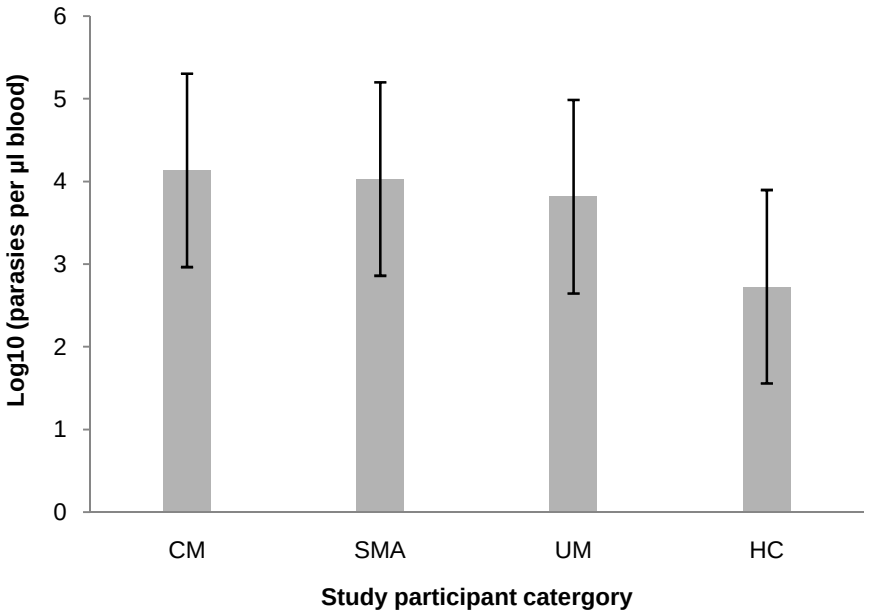

Fig. 1. Malaria parasite density in the different study participant categories
Cerebral Malaria – CM, Severe Malaria Anaemia – SMA, Uncomplicated Malaria – UM and Healthy Controls – HC.

3.4 *Plasmodium falciparum* Specific IgG, IgE and GPI Antibodies

The seroreactivity of antibodies to the GPI molecule was very low in the total population while only a small fraction of the total IgE antibodies measured were associated with *P. falciparum* infection. As expected, most of the children had developed IgG antibodies against *Plasmodium.* Seropositivity for antibodies to the GPI, total IgE and *P. falciparum* specific IgE was not significantly different in the different study categories (Figure 2). In contrast, seropositivity for IgG antibodies to the crude antigen of *P. falciparum* was significantly different between the categories ($P<0.001$) being higher in the clinical cases as compared to the healthy controls. A similar observation was obtained when the seropositivity for the different antibodies were compared between the severe malaria combined categories (CM + SMA), the UM and HC groups (Data not shown).

With the exception of total IgE, the mean ELISA OD value for IgG antibodies to the GPI and *P. falciparum* specific IgE and total IgG antibodies was significantly different among the study participant categories (Table 2).

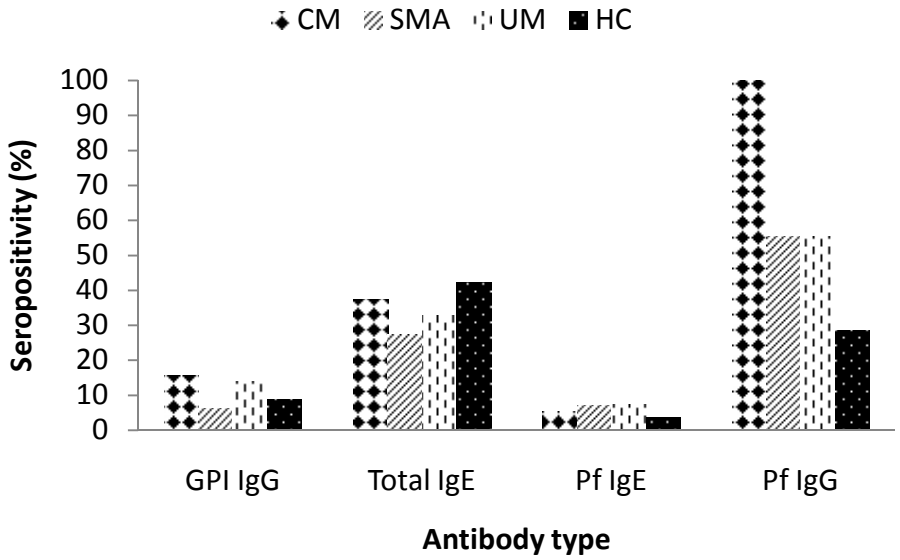

Fig. 2. Percentage Seropositivity for the GPI-IgG, total IgE, *P. falciparum* specific IgE and IgG antibodies in cerebral malaria (CM), severe malaria anaemia (SMA), uncomplicated malaria (UM) and healthy control children from South Western Cameroon.

Mean OD values for GPI-IgG, *P. falciparum* specific IgE and IgG antibodies were significantly higher in the UM and HC groups when compared with the severe malaria group (CM + SMA). When the mean ELISA OD values for the different antibodies were compared between the combined severe malaria group (SMA + CM) and the UM group, differences were obtained only in the mean GPI-IgG and *P. falciparum* IgE/IgG levels (Table 3). Mean GPI-IgG and *Pf* IgE levels were lower in the severe malaria group compared with the UM group. Anti-GPI IgG remained significantly higher (P=0.029) while *Pf* IgE levels were similar (P=0.075) in UM compared to the severe malaria group after controlling for age. *Pf*IgG levels were higher in the severe group compared with the HC group.

We investigated the possibility of existence of a correlation between plasma levels of the different antibodies measured and age and the results are shown in Table 4. A significant positive correlation was obtained between logarithmically transformed OD values for GPI-IgG and total IgE and age of the child. Similarly *P. falciparum*-specific IgE and IgG levels were found to increase with age of the child. Furthermore, a significant (P=0.02) negative correlation was obtained between GPI-IgG antibody level and malaria parasite density in the study participants.

Table 2. Mean (± SD) ELISA OD_{405} GPI-IgG, total IgE, *P. falciparum* specific IgE and IgG antibodies in CM, SMA, UM and HC study participants

Participant Category	n	GPI-IgG Mean ± SD	n	Total IgE Mean ± SD	n	*Pf* Specific IgE Mean ± SD	n	*Pf* Specific IgG Mean ± SD
CM	19	0.052 ± 0.085	16	0.208 ± 0.212	18	0.022 ± 0.024	03	0.140 ± 0.020
SMA	127	0.049 ± 0.060	91	0.174 ± 0.193	125	0.031 ± 0.027♯	83	0.126 ± 0.103
UM	187	0.095 ± 1.268‡	91	0.195 ± 0.197	187	0.035 ± 0.022	135	0.137 ± 0.104
HC	205	0.095 ± 0.104†	71	0.259 ± 0.293	213	0.032 ± 0.026	192	0.083 ± 0.054♣
Significance of Difference		P<0. 001		P=0.322		P=0.009		P<0.001

‡ GPI-IgG level significantly higher than the corresponding values for SMA (P=0.018) and HC (P=0.002)
† GPI-IgG level significantly higher than the corresponding value for CM (P=0.018), SMA (P < 0.001)
♯*Pf* Specific IgE level significantly lower thanthe corresponding value for UM (P=0.004)
♣*Pf* Specific IgG levelsignificantly lower than the corresponding values for SMA (P=0.012) and UM (P<0.001)
NB: For some of the participants, the sample volume was insufficient to run all the analyses.

Table 3. Mean (± SD) ELISA OD_{405} GPI-IgG, total IgE, *P. falciparum* specific IgE and IgG antibodies in severe malaria (CM + SMA) compared with uncomplicated malaria (UM) and/or healthy control (HC) children

Participant Category	GPI-IgG Mean ± SD (n)	P value	Total IgE Mean ± SD (n)	P value	Pf Specific IgE Mean ± SD (n)	P value	Pf Specific IgG Mean ± SD (n)	P value
CM + SMA	0.049 ± 0.064(146)	†REF	0.180 ± 0.196(93)	†REF	0.030 ± 0.267(143)	†REF	0.126 ± 0.101(86)	REF
UM	0.095 ± 0.127(187)	0.006	0.195 ± 0.197(91)	0.320	0.035 ± 0.022(187)	0.001	0.137 ± 0.104(135)	0.721
HC	0.095 ± 0.104(205)	<0.001	0.259 ± 0.293(71)	0.077	0.032 ± 0.028(213)	0.124	0.083 ± 0.054(192)	0.002

†Reference group; NB: For some of the participants, the sample volume was insufficient to run all the analyses.

Table 4. Correlation between the log_{10} of OD_{405} value for anti-GPI IgG, total IgE, Pf specific IgE, IgG antibodies and age of the study children

Variable	Correlation coefficient and P value (n)		
	Log_{10}Total IgE	**Log_{10}Pf IgE**	**Age (Years)**
Log_{10}GPI IgG	0.133*; P=0.036 (251)	0.016; P=0.718 (509)	0.237**; P<0.001 (468)
Log_{10}Total IgE		-0.014; P=0.835 (238)	0.149; P=0.020 (246)
Log_{10}Pf IgE			0.103*; P=0.027 (462)

**and ** indicate significant correlations at 0.05 and 0.01 significant level.*
NB: For some of the participants, the sample volume was insufficient to run all the analyses.

4. DISCUSSION

In this study, we sought to investigate the role of antibodies that have been suggested in previous studies to explain the pathogenesis of severe malaria in children. We measured and compared plasma levels of anti-GPI IgG antibodies and *P. falciparum* specific IgE and IgG antibodies in cerebral malaria, severe malaria anaemia, uncomplicated malaria and healthy control children below 14 years of age.

To gain insight into whether the severity of disease in some cases was as a result of differences in parasite density (PD) and body temperature, mean PD and axillary temperature were compared with age and patient groups. The observation of similar peripheral blood PD and axillary temperature among patient categories suggests that other factor(s) may contribute to disease severity. These results are consistent with those obtained in the acute care study in Uganda (Nussenblatt et al., 2001) and corroborate the observation that differences in disease severity are not due mainly to differences in parasite load. Additionally, sequestered parasites have been implicated in the pathology of severe malaria manifestations so that peripheral malaria parasite densities are not expected to show significant associations with clinical manifestations.

The mean WBC count was lowest in the HC group and highest in the severe malaria group with the UM group being intermediate. This finding is expected since infections, including malaria may result in increased white blood cell production or deployment in an attempt to combat the attack.

The role of anti- *P. falciparum* IgG in protection against malaria has been documented for nearly forty years by passive transfer experiments and in epidemiologic studies (Calissano et al., 2003). We observed that antibody levels increased with age and that seropositivity for IgG antibodies to *P. falciparum* crude antigens was significantly different between the categories, being higher in the clinical cases than in the healthy controls. Our mean ELISA OD_{405} values for *P. falciparum* specific IgG antibodies were also significantly higher in the severe malaria/SMA group when compared with the HC group. In a study by Titanji et al. (2002) total IgG levels were significantly higher in parasite-bearing individuals compared to the non-infected. Shigidi et al. (2008) reported that anti-MSP 1 antibodies were significantly higher in patients with CM and UM compared with clinically healthy asymptomatic volunteers. This is contrary to the finding of lower mean IgG levels in infected volunteers compared to control subjects by Nmorsi et al. (2008).

Glycosylphosphatidylinositol (GPI), the anchor molecules of some membrane proteins of *Plasmodium* species, has been implicated in the induction of immunopathology during malaria infections. Hence, neutralization of GPIs by antibodies may reduce the severity of clinical attacks of malaria (Tachado et al*.,* 1996). We observed that anti-GPI IgG antibodies was highest in UM children compared with severe malaria group. Furthermore, a negative though insignificant correlation was obtained between GPI antibody level and malaria parasite density. All these observations lend support to previous reports of a protective role for GPI especially to developing malaria (Boutlis et al., 2002; de Souza et al., 2010). There are conflicting reports, however, with regards to the protective role of GPI antibodies. In one study, anti-GPI antibodies were found not to prevent placental malaria infections and malaria associated poor birth outcomes (Suguitan et al., 2004). Similarly these antibodieshave been shown not to confer resistance to mild or severe malaria (de Souza et al., 2002)and to be highest among children with cerebral malaria and children who died compared with healthy controls (Cissoko et al, 2006). In other studies, it was observed that high levels of persistent anti-GPI antibodies was associated with clinical resistance to malaria (Naik et al., 2006) or protection against cerebral malaria (Perraut et al., 2005). It is possible that anti-GPI antibodies may be important in the development of anti-disease immunity in the category of children investigated. These findings may have implications in vaccine development. Further prospective case-control studies are required to elucidate the role of anti-GPI antibodies in protection against symptomatic malaria.

Malaria infections are associated with elevations of malaria-specific and total IgE; up to 5% of total IgE is malaria specific (Perlmann et al., 1999). Antibody production and especially IgE induction reflects a switch from Th1 to Th2, due to repeated exposure of the immune system to the parasites and involves a shift from IgM/G- to IgE that increase with age till puberty (Perlmann et al., 1999). The possible role of IgE in protective immunity or pathogenesis in *P. falciparum* malaria remains to be elucidated, as recent results are still contradictory. Perlmann and colleagues showed that both total and anti-malarial IgE levels were significantly higher in patients with severe malaria than in those with UM (Perlmann et al., 1997), suggesting that IgE has a role in disease severity. Verra et al., 2004 found no difference in *P. falciparum*-specific IgE levels between severe and uncomplicated malaria patients. Our results, on the contrary, show that the mean *P. falciparum*-specific IgE levels in UM were higher than the severe malaria group. This is in agreement with other studies in Tanzania (Bereczky et al., 2004) and Mali (Farouk et al., 2005) which showed that elevated anti-malarial IgE in asymptomatic individuals is associated with a reduced risk for subsequent clinical malaria. *P. falciparum*- specific IgE could thus play a role in protection from severe disease. Furthermore, *P. falciparum*-specific IgE might be involved in the mechanisms accounting for low susceptibility to malaria disease in some ethnic groups like the Fulani, in which IgE levels were higher compared to the more susceptible sympatric tribes. As such, IgE production is also under genetic control and there is a possibility that there is a relationship between severe malaria, IgE production and a variation in the IL-4 promoter region (Verra et al., 2004; Farouk et al., 2005). Total IgE does not seem to play a role in protection from severe disease. Though the control group had the highest mean level, there was no significant difference in mean levels among the patient categories.

If among the roles of IgE is the induction of TNF-α and NO (Perlmann et al., 1999), two molecules involved in the pathogenesis of malaria, then over-producers of TNF-α and NO may have an increased risk of susceptibility to severe disease while individuals with low TNF-producing IgE antibodies might help protect against disease. To solve this discrepancy, it is suggested that the mechanism of action of IgE is probably different in the maintenance of protective immunity in asymptomatic individuals, compared with the immune activation in

acute clinical or severe disease (Bereczky et al., 2004). This leaves the action of TNF-α and NO induction to the non-malaria specific IgE. Further studies including measurements of IL-4 and TNF-α levels are thus needed to clarify the roles of total and *P. falciparum*-specific IgE in immune reactions against malaria.

Antibodies to molecules on the merozoite surface (IgE inclusive) may break the blood cycle by blocking merozoites invasion of new RBCs, and thus prevent their destruction, and will consequently prevent anaemia. Also antibodies to the GPI portion of GPI-anchored proteins, one of the putative immune stimulatory molecules, could suppress the activation of macrophages, resulting in failure to produce pro-inflammatory cytokines (Hisaeda et al*.,* 2005) such as TNF-α which is believed to cause anaemia, fever, tissue lesions and other symptoms of malaria.

5. CONCLUSION

It is evident from our study that seroposivity for GPI-IgG antibodies, total IgE and Pf specific IgE were similar in the different study participants. However, *Pf* specific IgG antibody seropositivity was higher in the clinical categories compared with the control groip. Furthermore, Pf IgG antibody levels were higher in the clinical groups compared with the controls reflecting the boosting of antibody production, following infection, in children who have already been primed to malaria antigens.

Our observation of lower levels of GPI and Pf IgE antibodies in the severe group compared with the control group suggest a protective role for these antibodies in the pathogenesis of severe malaria. The correlation obtained between Pf IgE, IgG and GPI antibody levels with age confirm previous reports that immunity to malaria develops with age.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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