

## Original Article

# Humoral immune IgG response to *Plasmodium falciparum* in adults: the role of subclass-IgG in clinical immunity to cerebral malaria

Mazin M T Shigidi, MBBS\*, MD, Rihab A Hashim, BSc, MSc\*,  
Maowia M Mukhtar, B VSc, MSc, PhD\*\*, Mohamed NA Idris, MD, DCN\*,  
Tag-Eldin O Sokrab, MD, PhD.\*\*\*

Department of Medicine, Faculty of Medicine, University of Khartoum, Sudan\*  
Institute of Endemic Diseases, University of Khartoum, Sudan\*\*  
Department of Medicine, Hamad General Hospital, Doha, Qatar\*\*\*

### Abstract

#### Background

Although antibodies are essential mediators of immunity, high levels of IgG antibodies against a wide range of blood-stage antigens of *P. falciparum* are poor predictors of clinical protection. It is the qualitative and the functional specificity of the antibodies to malaria antigens that predict the development of a clinically potent protective immunity.

The objective of this work is to study the pattern of IgG sub-class in healthy and malaria-infected adults resident in a malaria-endemic area in Sudan

#### Methods

Total plasma IgG and IgG subclasses (IgG1, 2, 3 and 4) against the C-terminal region of the MSA-1<sub>19</sub> antigen of *Plasmodium falciparum* were measured by a quantitative enzyme-linked immunosorbent assay (ELISA) in 30 adult patients presenting to the emergency department with cerebral malaria (CM). The levels of IgG antibody profile in CM patients were compared with those in patients with uncomplicated acute malaria (n=20) and in clinically healthy asymptomatic volunteers (n=20).

**Results** Total plasma IgG level was significantly higher in CM patients. The level of the sub-class IgG1 antibody against MSA-119 was significantly lower in patients infected with *P. falciparum*; the lowest values being observed in CM patients and the highest values in the clinically healthy volunteers.

**Conclusion** Our data suggest that acquisition of IgG1 antibody to MSA-1<sub>19</sub> is associated with a clinically protective immunity and that low production or defective IgG1 response may be associated with severe form of malaria in adults.

**Key words:** cerebral malaria, plasmodium falciparum, IgG antibody, adults, Sudan

#### Introduction

In people living in endemic areas, acquisition of protective immunity against malaria is an age-dependant process achieved by repeated exposure to *Plasmodium* species over the years. Seroprevalence and seropositivity increase and reach their peak in adolescence while the incidence of clinical episodes and parasite density in blood gradually decline<sup>(1)</sup>. However, on the other hand, malaria parasites can readily evade the host immune attached by undergoing antigenic variation.

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#### Correspondent author:

Dr. Mohamed Nagib Abdalla

**Email:** Nagib\_01@yahoo.com

The parasites possess a large family of genes that encode variable antigens, the differential expression of which helps them to evade the host immune defenses<sup>(2,3)</sup>.

Two major *P. falciparum* merozoite surface antigens, MSA-1 and MSA-2, appear to elicit unique patterns of naturally acquired IgG immune responses that inhibit parasite invasion and growth in vitro<sup>(4,5)</sup>. This privilege allowed nominating those antigens as promising vaccine candidates<sup>(6,7)</sup>. MSA-1 is the precursor of the major merozoite surface coat antigen of *P. falciparum*. It is synthesized during schizogony by intraerythrocytic parasites as a high molecular-weight protein and is cleaved into smaller fragments, which are found on the surface of the merozoite<sup>(8,9)</sup>. The majority of the antigen is shed during invasion of the erythrocytes and only a small portion is retained on the newly invading ring stage. This protein, which is known as the Mr 42000 polypeptide, contains the C-terminal 19-kDa fragment MSA-1<sub>19</sub> which is retained on the surface of merozoites throughout the invasion of erythrocytes<sup>(10)</sup>. MSA-1<sub>19</sub> was found to be highly immunogenic and induce protective antibodies that inhibit parasite growth in vitro<sup>(6)</sup>. The antibodies inhibit parasite expansion through co-operation with a monocyte-macrophage series by binding to parasitized erythrocytes and then activate Fc receptors on the immune effector cells<sup>(4,5)</sup>. Although antibodies are essential mediators of immunity, high levels of IgG antibodies against a wide range of blood-stage antigens of *P. falciparum* are poor predictors of clinical protection<sup>(11)</sup>. It is however, the qualitative and the functional specificity of the antibodies to malaria antigens that predict the development of a clinically potent protective immunity<sup>(5)</sup>.

In this study we investigated total and subclass IgG (IgG1, 2, 3 and 4) antibodies to the C-terminal fragment MSA-119 of *P. falciparum* in 3 categories of adult residents of a malaria-endemic area in Sudan. The groups comprised patients with cerebral malaria (CM), patients with uncomplicated malaria (UM) and asymptomatic volunteers (AS).

### Materials and Methods

**Study area.** The study was conducted in Khartoum Teaching Hospital, the largest referral hospital in the region located in Khartoum, the federal capital of Sudan and the capital of Khartoum State. The surface area of the state is over 2,8000 km<sup>2</sup> inhabited by a population of 5 million. The smaller central urban zone (about 1,000 km<sup>2</sup>) is surrounded by large canal-irrigated agricultural lands that are characterized by rural topography. The winter months October -- February are characterized by an outbreak of *P. falciparum* malaria due to peak transmission following the preceding rainy season July -- September. Low-grade transmission and case incidence are maintained throughout the rest of the year. In the study area, malaria constitutes a major health concern leading to over 300,000 cases and 500 deaths each year<sup>(12)</sup>.

**Patients.** Seventy adult men and women, all coming from the same area were recruited in the different study groups, during the period from October 1998 to November 1999. The groups included patients with CM (n = 30), patients with UM (n = 20) and AS group (n = 20). Patients with CM were recruited at presentation to the emergency department and followed up in hospital. The diagnosis of CM was made according to the World Health Organization (WHO) definition<sup>(13,14)</sup>.

The uncomplicated malaria group presented to the emergency department with fever, had positive blood film for asexual *P. falciparum* and had no other explanation for their illness. They exhibited no WHO criteria for severe malaria and were treated as outpatients. The AS group included clinically healthy asymptomatic volunteers attending the emergency department at the same time and coming from the same residing area of the other groups and they did not recall any recent history of symptomatic malaria. Blood film smears from the AS group were negative for malaria by light microscopy. The UM and AS groups were age- and sex-matched with the CM group. Informed consent was obtained from all participants either directly or from attending guardians if they were unconscious. The study was approved by the ethical committee of the Graduate Medical Studies Board of the Faculty of Medicine of the University of Khartoum.

**Enzyme-linked immunosorbent assay (ELISA).** Human sera were tested by the indirect ELISA technique for the presence of IgG antibodies against the MSA-1<sub>19</sub> fragment, as described by<sup>(15)</sup>. Ninety-six-well polystyrene flat bottom plates (Numc), were coated with 100µl/well of *P. falciparum* MSA-1<sub>19</sub> antigen (0.625µg/ml in 0.05M Sodium Carbonate / Citrate buffer, PH 9.6). Since the antigen was conjugated to Glutathione-S- Transferase (GST), 16 wells in each plate were coated with 100µl/well of GST at concentration of 1500µg/ml to determine the background reaction. The plates were incubated overnight at 4°C. The wells were blocked by adding 100µl/well of 1% bovine serum albumin (BSA) in prepared buffer solution (PBS), and incubated for 1 hour at room temperature. The plates were then decanted. 100µl/well of

tested sera (1/200 dilution in PBS, 1% w/v BSA, 0.05% Tween 20) were placed; in each plate sera of two normal controls were also added. The plates were incubated at room temperature for one hour, and then washed three times for 5 minutes each with washing buffer (PBS, 0.05% T20). 100µl/well of 1/2000 diluted goat anti-human IgG peroxidase conjugate (Sigma-Aldrich, Gillingham, Dorset, United Kingdom) were added. The plates were incubated for 2 hours at room temperature. Following three times washes 5 minutes each with washing buffer; the reaction was developed by adding 100µl/well of substrate solution (O-phenylenediamine dihydrochloride -OPD-) (Sigma-Aldrich) dissolved in sodium citrate buffer (pH 5.0, with 0.05% of 30% hydrogen peroxides). Following the incubation of the plates at room temperature for 30 minutes in the dark, the reaction was stopped by adding 50µl/well of 20% sulphuric acid to each well. The color density was measured at 492 nm filter (Universal Microplate Reader BIO-TEK, Model EL X800). The average GST optic density (OD) values were used to determine background reactions.

The cut-off point of serum reactivity (1/200) was determined based on the results of the checkerboard titration. Positive sera were further analyzed for levels of IgG subclasses as described by<sup>(16)</sup>. Basically the same ELISA technique described above was used, with the following modifications. The blocking was done at room temperature for 5 hours. Tested sera were added at 1/200 dilution and the plates were incubated overnight at 4°C. Following three times washes with washing buffer, 100µl/well of mouse monoclonal anti-human IgG1 (Sigma No.19388), IgG2 (Sigma No.19513), IgG3

(Sigma No.17260) and IgG4 (Sigma No.19888) peroxidase conjugate were added at a dilution 1:2000, and incubated for 2 hours at room temperature. The plates were then washed three times with washing buffer, and 100ul/ well of goat anti-mouse IgG peroxidase conjugate (Fc specific) (Sigma No. A0168) were added and the plates were incubated for 1 hour at room temperature. OD values were measured and recorded as above.

#### Statistical Analysis

Statistical analysis of the data was carried using Statistical Package for Social Sciences (SPSS/PC). Frequencies were completed for all variables and the results were expressed as mean  $\pm$  standard deviation. The comparison between quantitative values was assessed using Chi-square and student t-test with a statistical significance of p value  $<$  0.05.

#### Results

All CM and UM patients had microscopically detectable *P. falciparum* parasites in Giemsa-stained blood films. None of the AS individuals had a positive blood film for malaria.

The mean OD values of plasma levels of total and subclass IgG in CM, UM and AS are shown in Table 1 and Figure 1 High level of the total IgG was detected in 25 (83.3%) of CM patients, 12 (60%) of UM patients and in 8 (40%) of AS group. The level of anti- MSA-1 IgG antibodies were significant high in patients with CM and UM compared with AS individuals, Chi-square 10.74 and the p value  $<$  0.05. The highest mean value of total IgG was also observed in CM followed by UM patients.

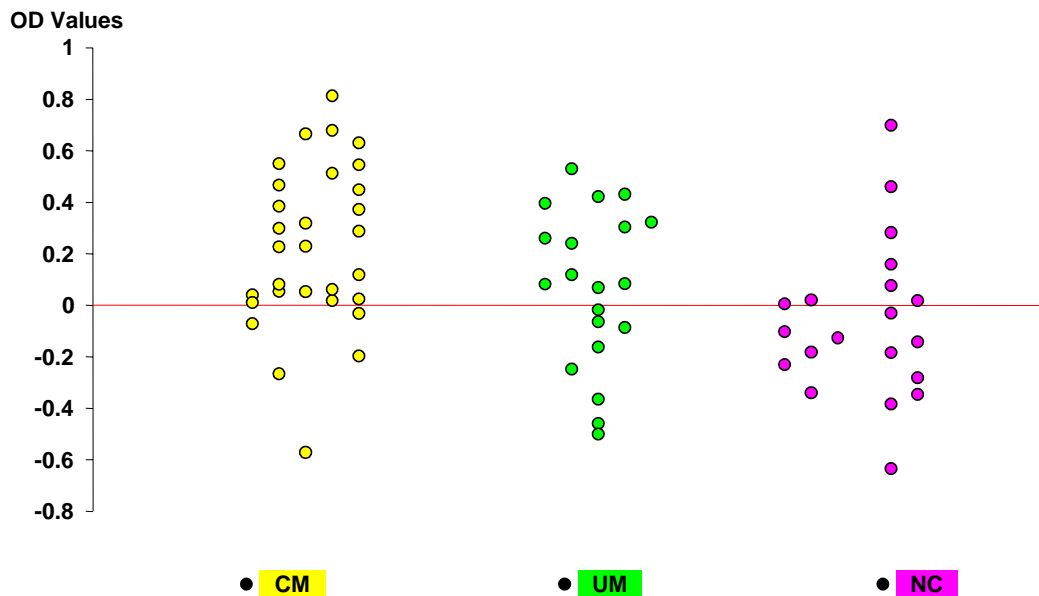
When subclass-IgG profile was assayed in patients and controls with elevated total IgG the highest values for IgG1 were observed in the AS controls whereas the lowest values were seen in patients with CM. That was statistically significant with Chi-square 14.44 and the p value  $<$  0.05. There was a trend for IgG2 to be low in patients with CM and high in AS subjects but the difference was not statistically significant (p value  $>$  0.05). No statistically significant differences were seen when comparing IgG3 and IgG4 levels between the groups.

**Table 1.** Mean plasma levels of total IgG and the IgG subclasses in patients with cerebral malaria (CM), with uncomplicated malaria (UM) and asymptomatic volunteers (AS) measured in optical density (OD).

	CM	UM	AS
<b>Total IgG</b>	0.225 $\pm$ 0.31*	0.068 $\pm$ 0.30	-0.064 $\pm$ 0.30
<b>IgG1</b>	0.017 $\pm$ 0.02*	0.034 $\pm$ 0.04	0.051 $\pm$ 0.06
<b>IgG2</b>	0.121 $\pm$ 0.17	0.245 $\pm$ 0.18	0.372 $\pm$ 0.46
<b>IgG3</b>	0.018 $\pm$ 0.02	0.013 $\pm$ 0.02	0.033 $\pm$ 0.05
<b>IgG4</b>	0.021 $\pm$ 0.01	0.003 $\pm$ 0.003	0.039 $\pm$ 0.05

\* p value  $<$  0.05 Values = OD  $\pm$  SD

**Figure 1:** Plasma IgG values measured in optical density (OD) in patients with cerebral malaria (CM), with uncomplicated malaria (UM) and asymptomatic volunteers (AS).



### Discussion

This study supports our previous proposal that host factors can operate in determining the different manifestation of disease severity in patients with a uniform exposure to malaria parasite in the same geographical location and transmission seasons<sup>(17)</sup>. The plasma IgG antibody against MSA-1<sub>19</sub> fragment was found to be significantly elevated in both groups of clinical malaria and the levels were more higher in CM than in UM patients. It is recognized that acute IgG response occurs in clinically ill and convalescent individuals; however, such response is not persistent and is expected to decline a few months after treatment and

parasite clearance<sup>(6)</sup>. Elevated total IgG was also observed in 40% of our normal controls suggesting recent exposure or presence of asymptomatic sub-patent parasitaemia. Several previous reports have also shown that asymptomatic residents of holoendemic areas may show seropositivity to malarial antigens without any antecedent morbidity<sup>(6,11,17)</sup>. The majority of these “pooled” antibodies play no effective role in immunity against infection and hitherto estimated total IgG level is considered as a poor predictor of clinical protection even though it indicates a past or present exposure<sup>(11,7)</sup>. In fact, it is the functional specificity of the antibody that seems to play

an important role in immune protection<sup>(5)</sup>. It has been proposed that effective immunity against malaria requires antibodies predominance of the subclass IgG1 and IgG3 against MSA-1 because the latter were found to be specific for mature schizonts and predominated in individuals protected from clinical malaria<sup>(5)</sup>.

When assaying IgG subclasses we could also observe specific qualitative differences in the profile composition between the study groups. There was a significantly low IgG1 in the CM group compared to UM and AS. A trend for a lower IgG2 in CM compared to AS was also observed, though the difference did not mount to a statistical significance. The reciprocal pattern of IgG1 distribution in CM and AS denoted a clinical protective role for this specific antibody in our adult community. Previous reports have shown that IgG1 and IgG2 tend to be significantly

elevated in normal individuals after several years of exposure to holoendemic malaria, and a low IgG1 is associated with severe malaria<sup>(4,11)</sup>. This is applied to our AS subjects who are expected to develop intermediate patterns of immune responses secondary to repeated exposures to different strains and antigen variants during subsequent transmission seasons. This longitudinal dynamic premonition will lead to an increase in the prevalence of antibodies to the antigens encountered and can produce a subclass composition that is protective from clinical malaria<sup>(4,5,17)</sup>.

In conclusion, in adult malaria the parasite-host immune interaction plays an important role in determining the clinical presentation of the disease and that acquisition of IgG1 antibodies to MSA-1 may interfere with susceptibility to CM.

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