

Antibody Response to *Plasmodium falciparum* Novel Synthetic Peptides and Protection Against Malaria in a Malaria Endemic Area in Burkina Faso

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Abstract: Antibody immune response play key role in the naturally acquired immunity to malaria. However, due to the complex life cycle and *Plasmodium* antigen diversity, there still is a need to search for protective antigens. We have used multiplex protein microarrays of 92 novel *Plasmodium falciparum* alpha-helical coiled coil protein motifs to screen plasma samples obtained from children and adults belonging to three sympatric ethnic groups from malaria endemic area in Burkina Faso. Samples collected during a cross-sectional survey allowed to draw the IgG antibody response profile related to concurrent parasitaemia and clinical status at baseline. Samples collected from children under 5 years old who received longitudinal follow-up allowed to identify protective malaria antigens. The study results showed that the concurrent parasitemia influenced the antibody response to *Plasmodium falciparum*. The number of recognized antigens and the IgG antibody intensity were higher in uninfected volunteers compared to the infected people. The number of recognized antigens and the IgG antibody level were higher in asymptomatic individuals at baseline compared to symptomatic volunteers. The study results have shown that the IgG antibody response to six antigens (LR150B, LR163, MR232, MR259C, MR261A and MR282) were associated to protection against clinical malaria in children under five years old.

Keywords: malaria, *Plasmodium falciparum*, microarrays, Burkina Faso

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1. Introduction

Malaria remains a major public health problem worldwide. There were an estimated 249 million cases and 608,000 related deaths due to malaria in 2022 [1]. In addition to the current malaria control measures, the recent discovery of malaria vaccines (RTS, S and R21/Matrix-M) will significantly contribute to malaria

control or elimination. Despite its moderate efficacy [2], the World Health Organization (WHO) recommended the use of RTS, S as the first malaria vaccine since October 2021. The R21/Matrix-M is the second malaria vaccine to be recommended by the WHO since October 2023. The R21 vaccine is known to have higher efficacy [3]. Both vaccines only target the liver stage of *Plasmodium* life cycle meaning that there is a need to continue the search of others potentials malaria vaccine candidates which are targeting the erythrocytic either stage of *Plasmodium* or

can block the transmission. This will enable to have multistage vaccines and will definitely increase the efficacy of the current available vaccines. The achievement of the next generation vaccine goal as stated in the revised malaria vaccine technology roadmap [4], will necessitate new strategies to add blood stage or transmission blocking immunity. The complete sequencing of the genome of the most virulent malaria parasite *Plasmodium falciparum* [5] and the description of its proteome [6] offer a huge opportunity to identify genes and their products as potential malaria vaccine candidates. Moreover, the availability of a high throughput technology such as protein microarrays offer the ability to screen a large panel of malaria antigens and to faster the identification of potential target antigens that could undergo clinical development as potential malaria vaccine candidates. Most of the blood stage candidates showed long-standing difficulties of blood stage antigens, such as polymorphism and redundancy. The chemical synthesis of alpha-helical coiled coil segments with none or limited polymorphism and good solubility may be an alternative. Peptides 30-50 aa long can be easily synthesized and are well recognized by antibodies from people naturally exposed to malaria [7,8]. The naturally acquired immunity (NAI) developed in people naturally exposed to malaria parasites in endemic area is a well-known model of immunity showing that a malaria vaccine is feasible [9,10]. NAI can be subdivided into anti-disease immunity and anti-parasite immunity. Anti-disease immunity (defined as absence of symptoms) develops quickly, sometimes requiring only one or two infections in high transmission areas [11,12]. However, individuals living in high transmission areas develop low non-sterile anti-parasite immunity resulting in low-level parasitaemia and asymptomatic infections. This immunity is acquired much more slowly than anti-disease immunity, may require repeated infections depending on the transmission rate, and is rarely sterilizing infections [13]. Identifying antibody targets that are associated with infection, disease or immunity will support the development of vaccines, diagnostics, and tools for sero-surveillance [14]. To identify novel blood-stage malaria antigens associated with protection, we used a high throughput protein microarrays of *Plasmodium falciparum* novel synthetic peptides to screen plasma samples obtained from people living in a malaria endemic area and presenting different infection status and clinical pattern.

2. Materials and Methods

Ethical statement

The samples used in our study are part of a sero-epidemiological study, which received approval from the Ethical committee of Health ministry of Burkina Faso (deliberation N° 2007-48) and the university of Oxford. The study volunteers or their guardians gave written informed consent for participation.

Study site, samples and data collection

The study was carried out in four rural villages of Burkina Faso, Northeast (Barkoundouba Peulh and Barkoundouba Mossi) and East (Bassy and Zanga) located

50 kms from Ouagadougou, the capital city. The study site has been already described elsewhere [15]. The biological samples and clinical data were collected during a cross-sectional survey and a longitudinal follow-up.

Cross sectional survey

The blood samples were obtained during a cross-sectional study from 1113 individuals, adults and children belonging to three sympatric ethnic groups (Mossi, Fulani and Rimaibé). Plasma samples were used to measure the IgG antibody immune responses of the study population against a panel of *Plasmodium falciparum* specific antigens. Thick and thin blood smears were prepared to estimate the prevalence of malaria infection in the population at the time of samples collection. Malaria rapid diagnostic test (RDT) was performed in children with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) or history of fever during the 24 hours before the visit). Children whose RDT was positive received Coartem® (Artemether-lumefantrine) as antimalarial treatment against malaria.

Longitudinal follow-up

The longitudinal follow-up aimed to assess the clinical malaria incidence in young children (under 5 years old). A sub cohort of 218 children under 5 years old received home visit three times weekly for active detection of malaria episodes. During the home visit the clinical signs were recorded and a malaria rapid test was performed for the children with fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) or history of fever. Children with RDT positive result received an antimalarial treatment with Coartem® as first line treatment against uncomplicated malaria. The thick and thin blood smears were prepared from children with fever or history of fever during the previous 24 hours. The longitudinal follow-up of children lasted 4 months from august to November 2007. A malaria episode was defined as a parasitemia $\geq 5000/\mu\text{l}$ asexual stages of *Plasmodium falciparum* associated to a fever (axillary temperature $\geq 37.5^{\circ}\text{C}$) or history of fever during 24 hours before the visit.

Malaria diagnosis

Plasmodium falciparum infection was diagnosed using light microscopy. Thick and thin blood smears were prepared and stained with Giemsa according to standard operating procedures. Then, two skilled microscopists read slides independently. To establish parasitaemia, thick and thin were evaluated under 100x oil immersion and parasitaemia was calculated from the number of asexual parasites per 200 leukocytes, assuming mean leukocyte counts of 8000 $/\mu\text{l}$ and the mean density from two readings was used. A third reader was involved when the two readers disagreed about positivity or when estimated densities differed by $> 30\%$. In these cases, the mean of the two closest density readings was considered.

Plasmodium falciparum antigens

We used microarrays containing in total 92 *Plasmodium falciparum* synthetic peptides of which 88 were alpha-helical coiled coil segments and 4 well known synthetic malaria antigens (MSP3, MSP2-3D7, (NANP)10 and P27A) [7]. The bioinformatics screening and the chemical synthesis procedure are described elsewhere [7].

Protein microarray immunoassay

Manufacturing of the microarrays

For microarrays construction, the synthetic peptides were suspended in PBS 1x to a concentration of 300 µg/mL. Peptides were printed to aldehyde -derivatized glass microscope slides (CEL Associates, Houston, TX, USA) using computer-controlled high-speed robotics (Micro grid II Biorobotics®, Genomic Solutions Ltd, Huntingdon, Cambridge shire, UK). The solutions of antigens were transferred from 384-well microtiter plates (Greiner bio-one, USA) onto glass slides by using stainless steel solid pins of 200 µm of diameter. The microarrays consist of 16 x 16 matrices that included (i) the *P. falciparum* antigens printed in two replicates; (ii) Human Serum Albumin (HSA) as negative controls; (iii) an anti-mouse IgG and human purified IgG as signal controls and (iv) phosphate buffered saline (PBS) used as carry-over controls (blanks). The printing of the peptides was performed inside the printer cabinet at a temperature (22°-24 °C) and humidity (45-55%). Printed slides were kept overnight inside the printing cabinet before removal in order to optimize the peptides binding. Slides were subsequently kept in boxes at room temperature in the presence of silica gel bags.

Immunoassay

Printed slides were incubated for 1 hour at room temperature with a blocking buffer (PBS 1X, BSA 2%) in order to prevent no specific binding. A hydrophobic pen (ImmEdge™ PEN, Vector Laboratories Inc., Burlingame, USA) was used to delimit each array prior the blocking step in order to contain reagents and samples within arrays areas and prevent cross contamination. After incubation step, slides were washed with washing buffer (PBS 1X, Tween 20 at 0.01%) in order to remove unbound material. Plasma samples were diluted at 1: 100 in a dilution buffer (PBS 2X, BSA 2%, Tween 20 at 0.01 %) and 50 µl of diluted sample were allowed to react with each area for 1 hour at room temperature. Slides were washed with washing buffer to remove no specific material. To reveal IgG bound to the printed peptides, the slides were incubated 20 minutes at room temperature with Alexa 647-labelled goat anti-human IgG secondary antibody (Southern Biotech) at a concentration of 10 µg/mL. Unbound secondary antibody was removed by washing with the washing buffer. Then, the slides were dried by centrifugation at 3000 rpm for 1 minute prior to the detection of the fluorescence. All the plasma samples were tested in double. Plasma samples obtained from Italian malaria naive volunteers were used as negative controls.

Detection and quantification of array components fluorescence

The fluorescent signal from the slides were read at 633 nm by using a Scan ArrayGx Scanner (Perkin-Elmer, Cambridge, UK). The fluorescent signal from each array component was visualized in a pseudo color scale corresponding to increasing fluorescence (from dark-blue; blue; green; red to white). Images generated, saved as JPEG and TIF files. JPEG files were used for signal visualization. TIF files were used for signal quantification with the Scan Array Express TM software (Perkin-Elmer).

Treatment of microarray data and Statistical Analysis

Quantified array signals were analyzed using Excel (Microsoft). Measurements for each spot were corrected against the internal PBS negative control to identify signals above background. Four replicate measurements were calculated for each antigen. Negative or zero values after the background subtraction were assigned a net value of zero. The data were normalized before statistical analysis were performed. For each peptide, the subject mean of the negative control was subtracted. For each volunteer, the breadth of antibody response was calculated as the number of positive responses in the 92 *Plasmodium falciparum* peptides. Association between antibody responses against individual *P. falciparum* peptide at baseline and subsequent protection from symptomatic malaria were identified by logistic regression. Subject for which the reactivity value to a given peptide is above the upper limit of the confidence interval for the geometric mean (negative controls) was considered positive. A peptide is defined overall positive for a population if the SI is above the cutoff for more than 60% of the adult population. To determine the positive reactivity cut-off of a sample to a given antigen, we calculated the mean from the 20 Italian negative controls. A sample was reactive if the intensity of the antigen-bounded IgG was above the upper limit of the confidence interval of the mean of the negative control. The ratio of response intensity for each antigen was obtained by taking the ratio of the geometric mean of the samples to the geometric mean of the negative controls. We then selected the most reactive antigens (antigens for which the prevalence of IgG antibodies was greater than 50% within our study population and whose response intensity ratio was greater than 5). These antigens numbered 36 including well-known antigens (MSP3, MSP2, (NANP)10 and (P27A) used as controls in our study. Protein array data were analyzed using STATA (Version 11.0, College Station, TX: StataCorp; 2011). Classical t test of hypothesis and logistic regression were used. A p-value < 0.05 was considered as threshold for the statistical significance of hypothesis tests.

3. Results

Characteristics of study participants at baseline (cross sectional survey).

The study population during the cross- sectional survey was composed of children and adults belonging to three ethnic groups. Those communities live in sympatric and have different susceptibilities to malaria. In total 1113 individuals were enrolled during the cross- sectional study which aim was to estimate the prevalence of malaria infection and to collect biological samples to measure the immunological correlates of protection. Two hundred eighteen children under five enrolled at the cross sectional received a longitudinal follow-up to estimate clinical malaria incidence in that subpopulation. The study population was composed of 51.5% of female individuals and 48.5% of male individuals. The prevalence of malaria infection was 51.6%. The characteristics of the study participants at baseline are shown in [Table 1](#).

Table 1. Characteristics of study population at baseline

	n	n (%)
Sex		
Female	573	51.5
Male	540	48.5
Prevalence of malaria infection		51.6
Prevalence of <i>P. falciparum</i> infection by ethnic group		
Fulani		40.0
Mossi		60.3
Rimaibé		56.4
Clinical status of volunteers at baseline		
Symptomatic		7.2
Asymptomatic		92.8
Prevalence of clinical malaria by ethnic group		
Fulani		6.3
Mossi		6.1
Rimaibé		9.1

Relationship between antibody response and malaria infection at baseline

In order to evaluate the relationship between concomitant parasitemia and the level of the IgG antibody response, we compared the number of antigens recognized and the level of antibodies between volunteers in whom the microscopic diagnosis was positive and those whose microscopic diagnosis was negative. The average number of antigens recognized was respectively 28 (CI: 28-29) for uninfected volunteers and 26 (CI: 25-27) for those who were infected with a statistically significant difference ($p < 0.0001$). The intensity of the IgG antibody response was 16.14 (CI: 16.03-16.25) for volunteers who had a negative thick film and 15.89 (CI: 15.79-15.99) for those who had a positive thick film with a statistically significant difference ($p = 0.0012$). We also specifically compared the level of antibodies directed against each antigen between volunteers who had a negative microscopy and those who had a positive microscopy at the time of the cross-sectional survey. This comparison showed that 13 antigens were associated with a high-level of IgG response in people who were uninfected. The Table 2 shows the antigens associated with a higher IgG level in uninfected volunteers.

Relationship between antibody response and clinical malaria at baseline

In order to evaluate the relationship between antibody response and clinical malaria status of participants at the baseline survey, we classified volunteers in two groups. The volunteers who were asymptomatic at the time of sampling (positive microscopy with absence of fever and

history of fever) and symptomatic volunteers. The number of recognized antigens was respectively 26 (CI: 25-27) for asymptomatic individuals and 21 (CI: 19-24) for symptomatic volunteers with a statistically significant difference ($p = 0.0008$). There was also a statistically significant difference ($p = 0.0002$) between the mean IgG antibody level in asymptomatic (11.03; CI: 10.95-11.11) and symptomatic (10.47; CI: 10.18-10.77) volunteers. We also specifically compared the level of IgG directed to each antigen between volunteers who were symptomatic and those who were asymptomatic. The results showed that IgG antibodies against 8 antigens had a significantly higher level in individuals who were asymptomatic at the time of the survey transversal. The results of comparisons of IgG antibody level between asymptomatic and symptomatic volunteers are shown in the Table 3.

Characteristics of population (longitudinal follow-up)

The assessment of the association between the IgG antibody response and the incidence of clinical malaria involved 218 children under 5 years old. These children also belonged to the three communities (Fulani, Mossi and Rimaibé) and were enrolled at the time of the cross-sectional survey. This subpopulation composed of 46.7% girls and 53.3% boys. The number of episodes of clinical malaria recorded was higher among the Rimaibé (50.7%) compared to the Fulani (37.9%) and the Mossi (12.3%). The number of episodes per age group increased between 1 and 3 years and then decreased. The sociodemographic and clinical characteristics of the study subpopulation are summarized in the Table 4.

Clinical malaria incidence among children under five years old

Two hundred eighteen (218) children under 5 years old enrolled in the study benefited from a 4-month longitudinal follow-up between August and November 2007. Among them, 152 children were protected from clinical malaria during the follow-up period while 66 children were not protected. No episodes of clinical malaria were reported in children who were protected. Regarding children who were susceptible to clinical malaria, each of them had at least one episode of clinical malaria during the follow-up period. Seven children experienced at least 2 malaria episodes during the follow-up period. The total number of episodes of clinical malaria reported was 73. This number increased in children aged between 1 and 3 years and then decreased in those aged between 4 and 5 years. The Table 5 shows the results of malaria incidence during longitudinal follow-up according to age groups.

Table 2. Characteristics of antigens associated to high level of IgG in uninfected volunteers

Antigen	Gene ID	Description of gene product	Mean log IgG (95% CI)		
			Uninfected volunteers	Infected volunteers	p-value
LR149	PF3D7_0203000	repetitive organellar protein, putative	6.32 (6.19-6.46)	6.02 (5.88-6.17)	0.0029
LR163	PF3D7_1120000	conserved <i>Plasmodium</i> protein, unknown function	6.51 (6.29-6.74)	5.78 (5.54-6.01)	0.0000
MR198	PF3D7_1409200	conserved <i>Plasmodium</i> protein, unknown function	8.12 (7.98-8.26)	7.88 (7.75-8.01)	0.0145
MR214	PF3D7_1205000	conserved <i>Plasmodium</i> protein, unknown function	6.07 (5.92-6.22)	5.83 (5.66-5.99)	0.0314

MR231	PF3D7_0510500	topoisomerase I	6.30 (6.16- 6.43)	6.00 (5.89-6.16)	0.0059
MR232	PF3D7_0510500	topoisomerase I	7.33 (7.17-7.48)	7.06 (6.90-7.21)	0.0176
MR232A	PF3D7_0510500	topoisomerase I	8.07 (7.94- 8.20)	7.78 (7.64-7.92)	0.0029
MR233	PF3D7_0510500	topoisomerase I	7.34 (7.22-7.45)	7.09 (6.96-7.21)	0.0047
MR236	PF3D7_0820000	Snf2-related CBP activator, putative	6.85 (6.71-6.99)	6.56 (6.41-6.72)	0.0073
MR256	PF3D7_1319200	conserved <i>Plasmodium</i> protein, unknown function	7.16 (7.05-7.27)	6.98 (6.87-7.09)	0.0280
MR258	PF3D7_1348000	conserved <i>Plasmodium</i> protein, unknown function	6.63 (6.47- 6.80)	6.26 (6.08-6.44)	0.0026
MR259C	PF3D7_0603400	trophozoite exported protein 1	7.19 (7.03-7.35)	6.94 (6.77-7.11)	0.0324
(NANP)10		Circum Sporozoite Protein	7.71 (7.57-7.85)	6.93 (6.78-7.07)	0.0000

Table 3. Characteristics of antigens associated to high level of IgG in asymptomatic volunteers

Antigen	Gene ID	Description of the gene product	Mean log IgG (95% CI)		
			Asymptomatic volunteers	Symptomatic volunteers	p-value
LR162		reticulocyte binding protein homologue 1	6.79 (6.61-6.97)	5.72 (4.66-6.78)	0.0035
MR219	PF3D7_1120000	conserved <i>Plasmodium</i> protein, unknown function	6.28 (6.06- 6.50)	5.23 (3.88-6.57)	0.0382
MR232	PF3D7_0510500	topoisomerase I	7.10 (6.93-7.27)	6.14 (5.09-7.19)	0.0083
MR232A	PF3D7_0510500	topoisomerase I	7.79 (7.64-7.94)	7.09 (6.35-7.82)	0.0274
MR233	PF3D7_0510500	topoisomerase I	7.13 (7.00-7.27)	6.44 (5.81-7.06)	0.0092
MR256	PF3D7_1319200	Conserved <i>Plasmodium</i> protein, unknown function	6.99 (6.86-7.11)	6.39 (5.93-6.85)	0.0095
MR261A	PF3D7_0603400	trophozoite exported protein 1	6.91 (6.72- 7.09)	5.95 (5.20-6.71)	0.0084
(NANP)10		Circum sporozoite protein	6.91 (6.76-7.07)	6.28 (5.49-7.07)	0.0448

Table 4. Sociodemographic and clinical characteristics of children under 5 years old

	n	n (%)
Sex		
Female	102	46.7
Male	116	53.3
Ethny		
Fulani	83	37.9
Mossi	61	28.1
Rimaibé	74	34.0
Clinical malaria cases by ethnic group		
Fulani	27	37.0
Mossi	9	12.3
Rimaibé	37	50.7
Clinical malaria episodes by age group		
0-1	15	20.6
1-2	13	17.8
2-3	25	34.2
3-4	15	20.6
4-5	5	6.8

Table 5. Clinical malaria incidence by age group

Age group (years)	Number of children	Episode number	Total person-year of risk	Incidence rate (95% CI Per person-year at risk)
0-1	38	15	5.63	2.63 (1.59-4.37)
1-2	32	13	7.40	1.75 (1.01-3.02)
2-3	59	25	10.11	2.47 (1.67-3.65)
3-4	38	15	8.63	1.73 (1.04-2.88)
4-5	51	5	11.99	0.17 (0.38-1.00)
Total	218	73	43.82	1.66 (1.32-2.09)

Table 6. Characteristics of antigens associated to a low risk of clinical malaria incidence

Antigen	Gene ID	Description of gene product	Odd-ratio	P-value
LR150B	PF3D7_0203000	repetitive organellar protein, putative	0.28 (0.08-.091)	0.035
LR163	PF3D7_1120000	conserved <i>Plasmodium</i> protein, unknown function	0.09 (0.01-0.69)	0.021
MR232	PF3D7_0510500	topoisomerase I	0.25 (0.07-0.80)	0.021
MR259C	PF3D7_0603400	trophozoite exported protein 1	0.14 (0.03-0.061)	0.008
MR261A	PF3D7_0603400	trophozoite exported protein 1	0.16 (0.04-0.69)	0.014
MR282 (P 27A)	PF3D7_0603400	trophozoite exported protein 1	0.24 (0.07-0.78)	0.018

Association between antibody response and clinical malaria incidence among children under five years old

The children were divided into two groups based on the level of antibodies measured against each of the antigens in order to evaluate the effect of the level of antibodies on the incidence of clinical malaria during the longitudinal follow-up. For each antigen a threshold corresponding to a value equal to 2 times the average of the intensity of the response obtained from the samples tested was considered. Children whose antibody level for a given antigen was

greater than 2 times the average of all samples tested were considered those who had a strong response against that antigen. Children whose IgG antibody level was less than or equal to 2 times the average of the samples were considered as those who had a weak response to this antigen. We carried out a logistic regression (controlling the effect of the variables age and ethnicity) to predict the probability of having at least one episode of clinical malaria during the longitudinal follow-up according to the level of IgG antibody response of children directed against each antigen at the time of the cross-sectional survey. Children who had a high concentration of IgG antibodies directed against 6 antigens (LR150B, LR163, MR232, MR259C, MR261A and P27A) had a lower risk of having an episode of clinical malaria compared to those who had a low level of IgG. The Table 6 shows the antigens associated to protection against clinical malaria.

4. Discussion

We have previously shown that these novel synthetic peptides were immunogenic in population naturally exposed to *P. falciparum* malaria [8]. The antibodies specific against the vast majority of these peptides were age dependent and different patterns were reported in ethnic groups with different susceptibility to malaria.

In this study, we have investigated the influence of concurrent parasitaemia on the IgG antibody response to *P. falciparum* alpha-helical coiled coil protein segments in populations living in a malaria endemic area in Burkina Faso. Moreover, we have assessed the influence of the IgG antibody response at baseline on the infection and the clinical status of study population. We have also investigated the influence of the antibody response measured at baseline on the subsequent clinical malaria incidence in children under 5 years old, looking for protective malaria antigens. The knowledge of the influence of those factors on the immunity against malaria is essential in the selection of *Plasmodium* antigens as potential malaria vaccine candidates. We have used protein microarrays technology, a high throughput immunoassay, which gives the possibility to screen in parallel many antigens in a single experiment. The protein microarray has been used in previous studies exploring the NAI with aim to contribute to identify novel malaria protective antigens [16,17,18,19].

Regarding the relationship between the concurrent parasitemia and the IgG antibody response, the findings of the study showed that the antibody response was influenced by parasitaemia. Indeed, breadth and magnitude of the IgG antibody responses were higher in individuals without malaria parasite compared to those with malaria parasites. These findings concerned both children and adults. As the samples were collected from individuals from high and seasonal malaria transmission setting, people may develop antiparasite immunity to the studied antigens. Alpha helical coils coiled segments, appear to be antigens that elicit antiparasite immunity in exposed individuals living in malaria endemic area. Similar findings were obtained by other authors in other settings with other malaria antigens [20]. In malaria endemic area, the submicroscopic infections are quite

common, particularly in adults who have developed a strong but no sterile immunity against malaria. Antiparasitic immunity appears to be primarily mediated by antibody responses against blood stage antigens [9,10]. The study showed that, in general, people with low parasitemia recognized more antigens with higher signal intensity compared to people with high parasitemia, demonstrating that some of the antigens were likely associated to antiparasite immunity. In malaria endemic area, antiparasite immunity is acquired in populations over time. Indeed, adults show lower parasite density compared to adolescents and children, due to the cumulative antimalarial immunity acquired over time. In Chiu and al study, authors founded that anti-PfRh5 antibody were associated with high parasitemia and reduce incidence of malaria clinical episodes [21].

We also investigated whether the antibody response level at baseline influences the clinical status of the study participants. For this purpose, we compared the breadth and magnitude of the IgG antibody response in people with parasitaemia and clinical symptoms and people with parasitaemia without clinical symptoms. We found that the breadth and the magnitude of IgG influenced the clinical status at baseline. The asymptomatic volunteers had higher IgG intensity and recognized more antigens compared to the symptomatic volunteers. In a study conducted in India, authors found that asymptomatic adults infected with *P. falciparum* had higher breadth and magnitude of IgG antibody response compared with symptomatic adults [20].

In order to identify protective antigens, we investigated, through a longitudinal follow-up, the influence of the IgG antibody level measured at baseline in children under 5 years old on subsequent occurrence of clinical malaria.

The study findings showed that Fulani children had a high level of protection compared to Rimaibé but were more susceptible than Mossi. Some previous studies conducted in the same communities have shown that the Fulani ethnic group was better protected against malaria with low parasite density and low clinical malaria episodes compared to the other communities [22,23]. Six peptides were associated to a lower risk of clinical malaria in children during the longitudinal follow-up, and were likely associated to anti-disease immunity in children.

5. Conclusion

The study allowed the assessment of the relationship between the antibody response to *Plasmodium falciparum* and the protection against malaria among people living in a malaria endemic area. The findings from this study showed that the antibody response to *P. falciparum* alpha-helical coiled coil proteins in populations living in malaria endemic area is influenced by the concurrent parasitaemia. However, the clinical status of people at the time of blood sample collection was not influenced by the presence of the parasite. Six (6) *Plasmodium falciparum* blood-stage antigens were found to be associated with the reduction of clinical malaria incidence in children under 5 years old. These findings should be considered in any strategy directed to the clinical development of new potential malaria vaccines. These antigens can be assessed as future

malaria vaccine candidates either as subunit vaccines or in combination with the existing malaria vaccines in a strategy of multistage vaccines.

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Conflict of Interest Statement

The authors have no conflict of interest to declare.

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