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Association between Malaria Prevalence and Seropositivity of Immunoglobulin G Subtypes Directed to *Plasmodium falciparum* Merozoite Surface Protein 1-$_{19}$

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EA and AN developed the proposal, collected samples and did laboratory analyses of samples and participated in writing the manuscript. Author LM performed statistical analyses. Author JC conceived the study idea, supervised the whole project and wrote the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To determine Immunoglobulin G (IgG) subtypes (IgG1, IgG2, IgG3 and IgG4) responses to PfMSP1-$_{19}$ antigens and their associations with malaria across different age groups.
Study Design: A community based cross sectional study.
Place and Duration of Study: Bondo Ward, in Handeni district of Tanga Region between January and May 2016.
Methodology: We included 331 participants; 216 females, 115 males aged between 1 and 82 years, with a median age of 10 years and an inter-quartile range 5 -30 years. Two milliliters of blood was collected from each participant in EDTA coated tubes for detection of malaria and serology.

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Anti-MSP1-19 IgG subtypes were measured by indirect ELISA based on a protocol developed by Afro Immuno-Assay Consortium. Demographic data were collected using designed record form.
Results: Out of 331 participants, 68 (20.5%) were malaria positive. We report malaria prevalence to be highest in the age category of between 6 and 15 years, compared to individuals above 15 years (OR= 4.5; 95% CI = 2.2–8.9). Most participants were seropositive for total IgG (87.0%), IgG1 (78.5%) and IgG3 (52.9%). Concentration (optical densities) of total IgG, IgG1 and IgG3 was generally lower in the 1-5 year age category. There was no clear pattern for IgG 2 and IgG4 seropositivity across age categories. After adjusting for age, only IgG1 seropositivity was significantly associated with lower malaria prevalence in all age categories (OR=0.4; 95% CI = 0.2 – 0.8).
Conclusion: IgG1subtype to MSP1-$_{19}$ is associated with lower malaria prevalence which may imply its possible suitability a target of a prospective malaria vaccine.

Keywords: MSP1-$_{19}$ antigen; IgG subtypes; malaria; immunity; falciparum.

1. INTRODUCTION

Despite all efforts to control malaria, the disease remains the major cause of morbidity and mortality especially to children in endemic areas. The current disease control methods are only partially effective. Several previous studies have generally shown that continuous exposure to *Plasmodium falciparum* infection enables acquisition of natural acquired immunity to falciparum malaria as infected adults rarely experience overt disease [4,7,10,18,22]. However, a consensus on the protective immune responses and their target parasite epitopes has not been reached.

Anti- *P. falciparum* IgG antibodies have been implicated as important component of the natural acquired immunity gained following exposure to malaria infection in endemic areas but the protective IgG subtypes specific to various *P. falciparum* blood stage antigens have not yet conclusively defined. The need to provide protective immunity against malaria in the form of vaccination especially in children remains of utmost urgency. Before this can be realized, the protective components of the natural acquired immunity against malaria must be defined and protection parameters enumerated [5,8,9,13,21].

Subtypes of antibodies produced against antigens are known to play an important role in protection against malaria [12,15]. Immunoglobulin G (IgG) subtypes differ in their structures and mediate different immune effector functions [6]. Knowledge of subtype responses associated with protection against malaria is important for understanding immunity and guiding vaccine development. IgG1 and IgG3 are the predominant subtypes produced in response to merozoite antigens [26]. IgG1 and IgG3 are cytophilic and T cell dependent, have high affinity for Fc receptors, and mediate phagocyte activation and complement fixation [6]. It has been suggested that IgG3 is more efficient at mediating these processes [17]. For reasons that are not well understood, different merozoite antigens induce different relative levels of IgG1 and IgG3 [23]. At an era when the burden of malaria is known to shift from young children to school age children [19], this study was designed to determine immunoglobulin G (IgG) subtypes (IgG1, IgG2, IgG3 and IgG4) responses to PfMSP1-19 antigens and their association with malaria across different age groups.

2. MATERIALS AND METHODS

This study was conducted in a site called Bondo, located in the North–Eastern Coastal region of Tanzania called Tanga. The region is endemic to malaria with a perennial transmission. The study area has two rainy seasons per year which denotes the peaks of malaria transmission. The prevalence of malaria in the present study is 20.5%. A total of 331 participants from 1 year and above were enrolled in the study. Community sensitization to participate in the study was done through community leaders prior to sampling. Sensitization included description of the study. Parents or guardians consented for children less than 18 years of age. A structured questionnaire was used to collect demographic data such as age and gender. A blood sample of 0.5-1 mL was taken by venipuncture from all consenting participants. Rapid diagnostic tests for malaria (SD BIOLINE Malaria Ag P. f/Pan) were used to screen for malaria positivity. Participants who were found to be malaria positive by rapid diagnostic test were treated with anti-malarial according to national guidelines. Whole blood samples were immediately

separated and plasma stored at -20C freezer. Serology was performed immediately after sample collection was completed. All samples collected had no personal identifiers on labels.

2.1 Parasitological Diagnosis

Thick and thin blood films were air dried, thin blood films were fixed with methanol, and both thin and thick films were stained with Giemsa 3% solution. One hundred high-power fields (HPF) were examined, and the number of malaria parasites in each field was recorded. The number of parasites per microliter of blood was calculated with the assumption of 20 white blood cells (WBC) per high-power field and a fixed white cell count of 8,000/µl.

2.2 Merozoite Surface Protein 1 (PfMSP1-19)

PfMSP1-19 is a surface protein in several Plasmodium species which is expressed on the surface of trophozoites and schizonts. It is considered as a promising antigen for the development of a vaccine against asexual blood stage parasites. MSP1 used in this study was a Baculovirus antigen of the C-terminal region of merozoite surface protein 1, produced in insect cells infected with a recombinant Baculovirus containing a synthetic G-C enriched PfMSP1 gene (Palo Alto allele), coding for 43 N-terminal MSP1 precursor residues and 16 amino acid residues upstream of the "classical" MSP1-19 (Bonnet et al. 2006) (Donated by Ed Remarque, BPRC, Rijswijk, The Netherlands).

2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

Antibodies to PfMSP1-19 were measured by ELISA based on a protocol developed by Afro Immunoassay Program [6]. Serum titres of total IgG were measured by using indirect ELISA for PfMSP1-19 antigens. During the ELISA procedure, microtiter plates (NUNC) were coated with PfMSP1-19 at 1 µg/ml in PBS (1%), overnight at 4ºC. Plates were then blocked with 3% dry, nonfat skim milk powder in PBS -Tween 20 (PBS/T) for three hours. Plates were washed three times with washing buffer (PBS with 0.5% Tween 20) in each step. Washing buffer was left for one minute before it was emptied.

Plasma samples were diluted in blocking buffer (1% skimmed milk in PBS/T) at 1: 1000 (MSP1 19: blocking buffer). Diluted plasma samples were then added into the microtiter wells of ELISA plates in duplicates. ELISA plates were incubated at 4°C overnight. For detection of IgG subtypes, each plate was developed with peroxides-conjugate goat anti-human IgG diluted 1:8000, horseradish peroxidase-conjugated, rabbit anti-human IgG (DAKO) diluted 1:5000, Peroxidase-conjugated sheep anti-human IgG1/HRPO diluted 1:20,000, Peroxidase-conjugated sheep anti-human IgG2/HRPO diluted 1:5,000, Peroxidase-conjugated sheep anti-human IgG3/HRPO diluted 1:8,000 and Peroxidase-conjugated sheep anti-human IgG4/HRPO diluted 1:2,000 in dilution bufferand incubated for 3 hrs at room temperature on a plate shaker. Plates were washed six times with washing buffer (PBS with 0.5% Tween 20) for each washing step. Washing buffer was left for one minute before it was discarded. Antibody (IgG) subtypes were quantified by staining with ready to use 50 µl TMB (3, 3', 5, 5'-tetra-methybenzidine) and incubated at room temperature for 20 minutes in the dark. To stop the reaction, 50 µl of 1M H_2SO_4 was added into each well, and then plates read at 450 nm. Cut-off value optical density (OD) were determined by fitting the OD readings as the sum of two Gaussian distributions (assuming a narrow distribution of seronegatives and a broader distribution of seropositives) using maximum likelihood methods. The mean OD of the Gaussian corresponding to the seronegative population plus three standard deviations was used as the cut-off for seropositivity. The normalized optical density in the different ELISA assays was considered as an indicator of the magnitude of antibody response in the analyses on a plate reader.

2.4 Statistical Analyses

Data obtained were characterized into demographic (age, sex), malaria (microscopy) and immunological (IgG subtypes to MSP-119) data. The age was characterized into three groups of 1-5, 6-15 and above 15 years. Data were collected, recorded then entered into the database created and analyzed using SPSS version 22.0 (IBM SPSS, Chicago). Analysis was done to examine the relationship between immunological variables and parasite measurement. A chi square test was used to explore relationship between presence of malaria positivity and IgG subtypes. IgG levels were not normally distributed, therefore Mann-Whitney U test was used to investigate the relationship between IgG levels and malaria status in

stratified age groups. Multivariate logistic regression was used to identify factors that independently associate with malaria status. A p value of <0.05 was considered significant.

Table 1. Descriptive statistics and general characteristics of study participants (n=331)

Variables	n (%)
Sex	
Male	115 (34.7)
Female	216 (65.3)
*Age (years)**	10 (5 – 30)
Age groups (years)	
1– 5	101 (30.5)
6 – 15	100 (30.2)
> 15	130 (39.3)
IgG0 status	
Negative	43 (13.0)
Positive	288 (87.0)
IgG1 status	
Negative	71 (21.5)
Positive	260 (78.5)
IgG2 status	
Negative	247 (74.6)
Positive	84 (25.4)
IgG3 status	
Negative	156 (47.1)
Positive	175 (52.9)
IgG4 status	
Negative	246 (74.3)
Positive	85 (25.7)

** Age expressed in median (inter quartile range)*

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 General characteristics of study participants and malaria positivity

The study population involved 331 participants aged between 1 to 82 years with media age of 10 years and IQR of 5 to 30 years. Out of the 331 participants, 216 (65.3%) were females, most participants were Seropositive for IgG0 (87.0%) and IgG1 (78.5%) and IgG3 (52.9%). Table 2 summarizes participant's data on malaria positivity while Table 3 presents IgG subtype seropositivity across age categories of individuals. In agreement with previous studies, IgG seropositivity to $MSP1_{-19}$ was predominant for IgG0, IgG1 and IgG3, with a clear age dependence pattern. Seropositivity was highest in the >15 years category and lowest in the 1-5 years category except for IgG3 which was highest among 6-15 years old individuals. There was little IgG2 and IgG4 reactivity, and thus seropositivity to $MSP1_{-19}$, with irregular trends across age categories (Table 4 and Fig. 1). The proportion of IgG3 seropositive individuals was lower than that of IgG0 and IgG1.

Table 2. Prevalence of malaria by age groups and gender (n=331)

Category	Total	Malaria prevalence n (%)*
Overall	331	68 (20.5)
Sex		
Male	115	33 (28.7)
Female	216	35 (16.2)
Age		
1 – 5	101	19 (18.8)
6 – 15	100	35 (35.0)
> 15	130	14 (10.8)

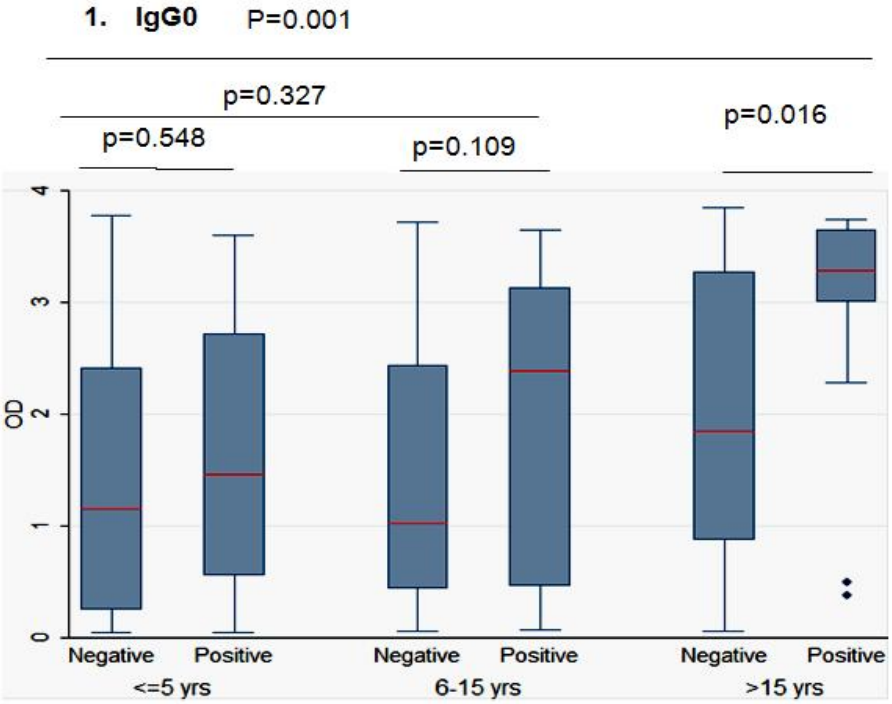
**The total number of participants tested for malaria is presented with the number (percentage) of those positive for malaria by microscopy*

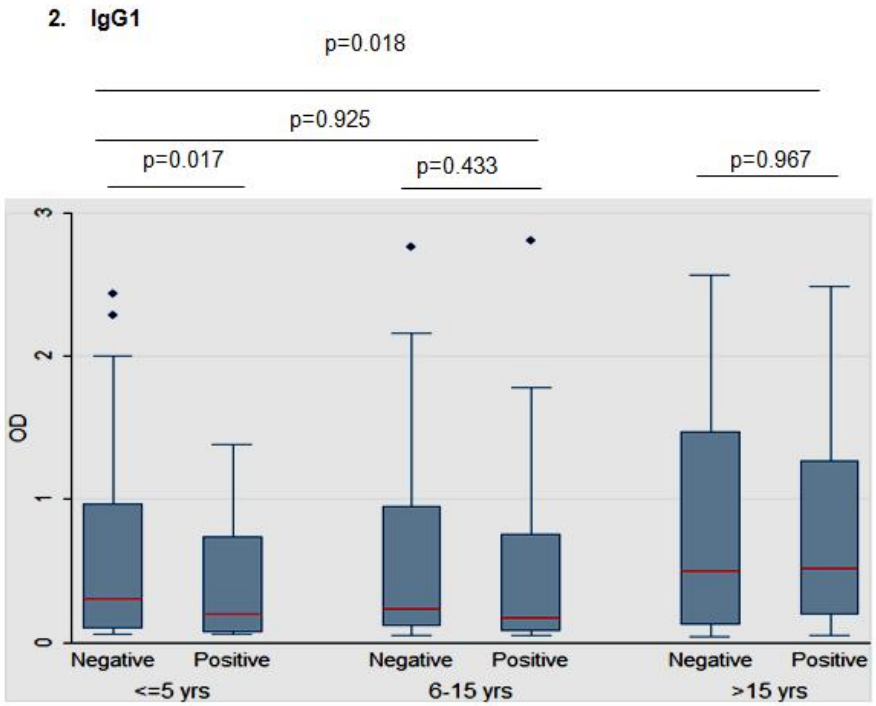
3.1.2 Logistic regression analyses

3.1.2.1 Univariate regression

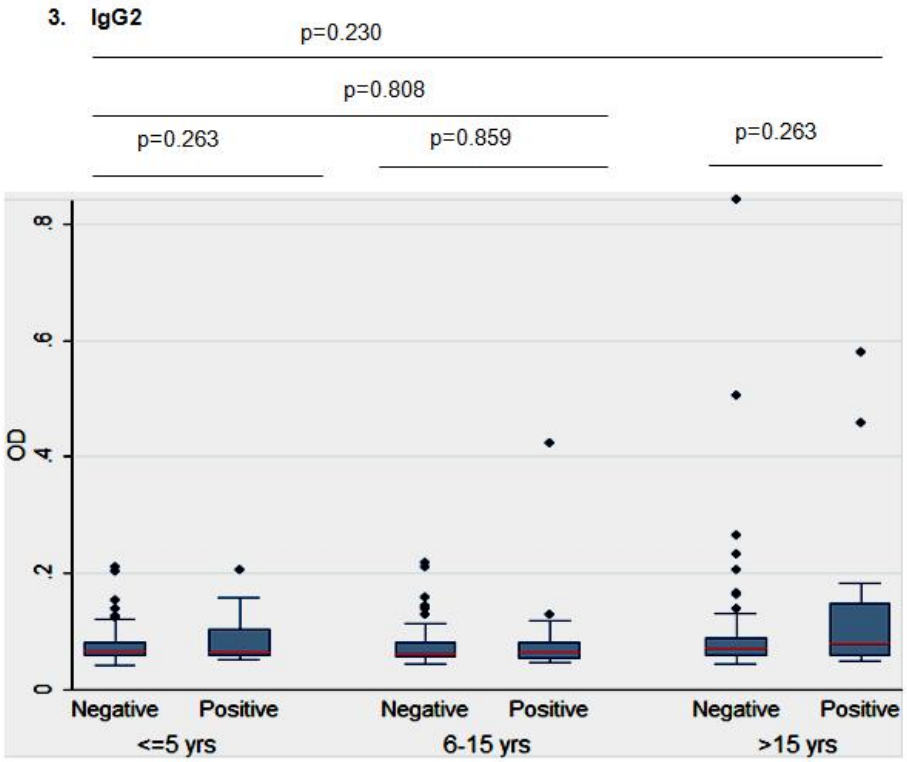
We performed univariate analysis to determine the effect of individual IgG subtype seropositivity, age and participant sex with malaria positivity. Results presented in Table 4 indicate that, being 6-15 years old was associated with higher odds of being positive for malaria parasites for up to 4.5 [(2.2-8.9, p<0.001]compared to being older than 15 years. Children of age 1-5 years had 1.9 higher odds of being malaria positive but this association did not reach statistical significance (OR=1.9; 95% CI=0.9-4.0, p=0.087]. Being male was associated with 2.1 times higher odds of being malaria positive (OR=2.1; 95% CI=0.3-1.5, p=0.007). Seropositivity to IgG1 was associated with lower malaria positivity (OR=0.5; 95%CI=0.3-0.9, p=0.014) implying that the IgG1 Seropositive group was less likely to be malaria positive by 50% compared to the group of seronegative individuals. We show in the same table that IgG3 Seropositivity has an insignificant association with higher malaria positivity, whereas seropositivity to IgG0, IgG2 and IgG4 subtypes were negatively associated with positivity to malaria although the association was not statistically significant. When additional analysis were done to determine associations of individual IgG subtypes stratified by age with malaria positivity, only IgG1 seropositivity in the

6-15 year category showed marginal association with malaria positivity. None of the other IgG subtypes across all age categories showed any association with malaria positivity (Table 5).





3. IgG2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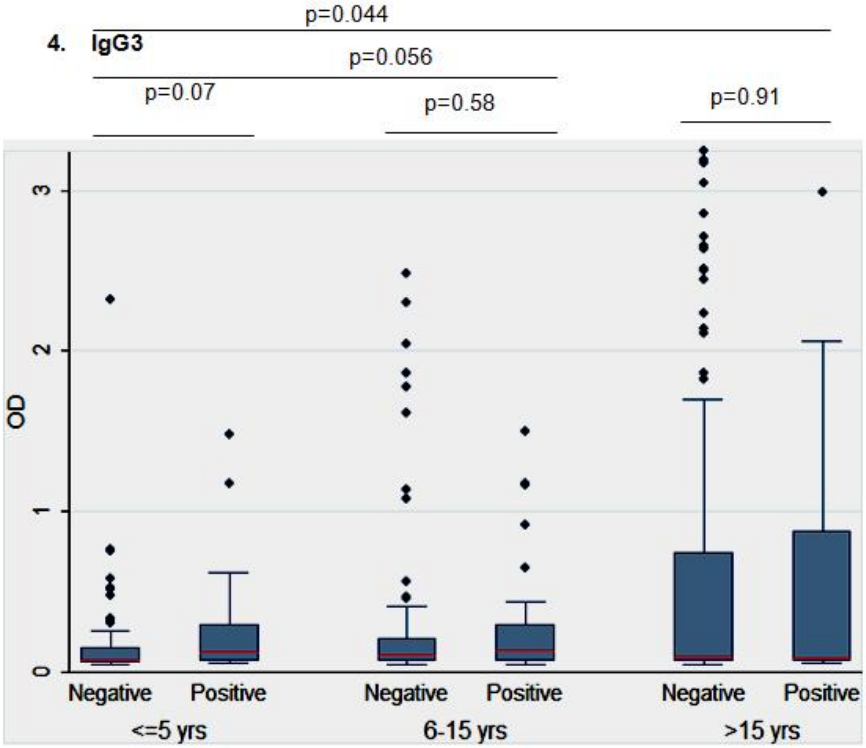
p=0.808
p=0.263
p=0.859
p=0.263
OD
0
.2
.4
.6
.8
Negative
Positive
Negative
Positive
Negative
Positive
<=5 yrs
6-15 yrs
>15 yrs

4. IgG3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p=0.056
p=0.07
p=0.58
p=0.91
OD
0
1
2
3
Negative
Positive
Negative
Positive
Negative
Positive
<=5 yrs
6-15 yrs
>15 yrs

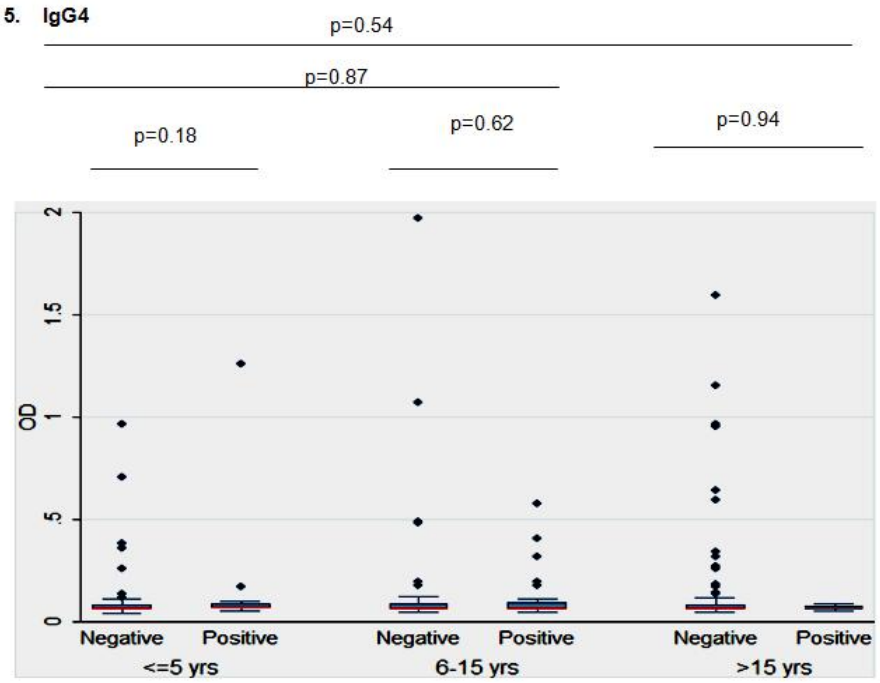

Fig. 1. Associations between IgG subtypes responses (optical densities) with malaria status (positive/negative) across age groups. Data are plotted as Optical Densities (OD) values against malaria positivity and presented as whisker and box plots

Table 3. Age-specific seropositivity of MSP1 specific IgG subtypes among participants (n=331)

		IgG subtype seropositivity* n (%)				
Age (years)	**N**	**IgG0@**	**IgG1**	**IgG2**	**IgG3**	**IgG4**
1 – 5	101	79 (78.2)	72 (71.3)	24 (23.8)	46 (45.5)	25 (24.7)
6 – 15	100	87 (87.0)	78 (78.0)	21 (21.0)	59 (59.0)	28 (28.0)
> 15	130	122 (93.8)	110 (84.6)	39 (30.0)	70 (53.8)	32 (24.6)

**Cut-off value optical density (OD) were determined by fitting the OD readings as the sum of two Gaussian distributions (assuming a narrow distribution of sero negatives and a broader distribution of seropositives) using maximum likelihood methods. The mean OD of the Gaussian corresponding to the seronegative population plus three standard deviations was used as the cut-off for seropositivity. @Total IgG*

3.1.2.2 Multiple logistic regressions

The odds of the 6-15 year category being positive for malaria were 3.7 [(2.2-8.9), p<0.001] times higher compared in participants older than15 years. The IgG1 Seropositive group was 60% less likely to be malaria positive [OR0.4 (0.2-0.8), p=0.009]. We observed a marginal association between seropositivity to IgG3 and malaria positivity [OR =1.8 (1.0-3.3)].

3.1.2.3 Antibody responses to MSP1-19 (Optical density)

IgG1 levels, measured as optical density readings, were significantly higher among malaria negative participants who were 5 years old and below compared to malaria positive participants of the same age (Fig. 1-IgG1). IgG1 levels were also higher among participants older than 15 years compared to children of 5 years and below (median IgG1 OD of 0.295 for ≤ 5 years participants versus median IgG1 OD of 0.496 for >15 years participants [p=0.018]). IgG3 levels were also significantly higher among older participants (>15 years) than among younger children (≤ 5 years).

3.2 Discussion

The invention of immunogenic malaria parasite antigens was a big step towards development of protective molecules against malaria infection in

form of vaccination. Studies on various surface antigens have widened the knowledge on the antibodies associated with malaria infection and further more to antibodies associated with protection against malaria positivity. In the present study, we have explored how IgG antibody subtypes specific to one of the potential malaria vaccine candidates, MSP1-19, are associated with protection against malaria.

In this study, most participants were seropositive for total IgG (IgG0), IgG1 and IgG3 against MSP1-19 whereas the seropositivity for IgG2 and IgG4 was only about a quarter of all the participants. This is consistent with the hypothesis that the two antibodies (IgG2, IgG4) are non cytophilic and thought to decrease in the increase of cytophilic antibodies [11] and also interact with the protective effects of cytophilic antibodies (IgG1 and IgG3) hence considered not to be important in protective immunity to malaria [14]. Similar results were reported in previous studies in which the response of IgG2 and IgG4 antibodies to three antigens (MSP1, MSP2 and AMA1) was extremely low [6,27]. However in some studies, IgG2 to MSP2 was found to increase with age and correlated with low risk of malaria attacks [2] suggesting that it plays a role in protection to malaria infection. These conflicting results were thought to arise from polymorphisms of Fc receptors in which IgG2 does not bind to FcγRIIA receptor on monocytes but shows affinity to the variant FcγRIIα receptor carrying the H131 allele [3] hence thought to be only protective in individuals with the variant FcγRIIα receptor.

The levels of total IgG and IgG subtypes were lower in the youngest age group of between 1and 5 years than older age groups with the exception of IgG2 and IgG4 that had an irregular pattern. There were more seropositive individuals in the older age groups than the younger ones. This may explain the age-dependence development of malaria immunity which is considered to be an accumulation of immune responses against poorly immunogenic conserved determinants [24]. Previous studies had reported similar findings on the age-dependent protective roles of IgG subtypes to several *P. falciparum* asexual blood stage [6,11]. Findings in this study showed sex difference in

Table 4. Factors associated with malaria prevalence

	Malaria n (%)		Total	OR (95% CI)	p-value
Variables	**Yes**	**No**			
Age (years)					
1 – 5	19 (18.8)	82 (81.2)	101	1.9 (0.9 – 4.0)	0.087
6 – 15	35 (35.0)	65 (65.0)	100	4.5 (2.2 – 8.9)	<0.001**
> 15	14 (10.8)	116 (89.2)	130		
Sex					
Female	35 (16.2)	181 (83.8)	216		
Male	33 (28.7)	82 (71.3)	115	2.1 (1.2 – 3.6)	0.007**
Antibodies					
IgG0					
Negative	11 (25.6)	32 (74.4)	43		
Positive	57 (19.8)	231 (80.2)	288	0.7 (0.3 – 1.5)	0.381
IgG1					
Negative	22 (31.0)	49 (69.0)	71		
Positive	46 (17.7)	214 (82.3)	260	0.5 (0.3 – 0.9)	0.014*
IgG2					
Negative	54 (21.7)	193 (78.1)	247		
Positive	14 (16.7)	70 (83.3)	84	0.7 (0.4 – 1.4)	0.309
IgG3 status					
Negative	27 (17.3)	129 (82.7)	156		
Positive	41 (23.4)	134 (76.6)	175	1.5 (0.8 – 2.5)	0.169
IgG4					
Negative	51 (20.7)	195 (79.3)	246		
Positive	17 (20.0)	68 (80.0)	85	0.9 (0.5 – 1.8)	0.886

Malaria positivity was based on microscopy

Table 5. Age-specific distribution of different subtypes of anti-MSP1 IgG antibodies stratified by malaria positivity

	Malaria n (%)		Total	OR (95% CI)	p-value
Seropositivity	**Yes**	**No**			
1 – 5 years (n=101)					
IgG0					
Negative	4 (18.2)	18 (81.8)	22		
Positive	15 (19.0)	64 (81.0)	79	1.1 (0.3 – 3.6)	0.932
IgG1					
Negative	8 (27.6)	21 (72.4)	29		
Positive	11 (15.2)	61 (84.7)	72	0.5 (0.2 – 1.4)	0.154
IgG2					
Negative	16 (20.8)	61 (79.2)	77		
Positive	3 (12.5)	21 (87.5)	24	0.5 (0.1 – 2.1)	0.367
IgG3					
Negative	8 (14.5)	47 (85.5)	55		
Positive	11 (23.9)	35 (76.1)	46	1.8 (0.6 – 5.1)	0.233
IgG4					
Negative	14 (18.3)	62 (81.6)	76		
Positive	5 (20.0)	20 (80.0)	25	1.1 (0.4 – 3.5)	0.861
6 – 15 years (n=100)					
IgG0					
Negative	6 (46.2)	7 (53.8)	13		
Positive	29 (33.3)	58 (66.7)	87	0.6 (0.2 – 1.9)	0.368
IgG1					
Negative	11 (50.0)	11 (50.0)	22		
Positive	24 (30.8)	54 (69.2)	78	0.4 (0.2 – 1.2)	0.097
IgG2					
Negative	29 (36.7)	50 (63.3)	79		
Positive	6 (28.6)	15 (71.4)	21	0.7 (0.2 – 2.0)	0.489
IgG3					
Negative	12 (29.3)	29 (70.7)	41		
Positive	23 (39.0)	36 (61.0)	59	1.5 (0.6 – 3.6)	0.318
IgG4					
Negative	25 (34.7)	47 (65.3)	72		
Positive	10 (35.7)	18 (64.3)	28	1.0 (0.4 – 2.6)	0.926
> 15 years (n=130)					
IgG0					
Negative	1 (12.5)	7 (87.5)	8		
Positive	13 (10.7)	109 (89.3)	122	0.8 (0.1 – 7.4)	0.871
IgG1					
Negative	3 (15.0)	17 (85.0)	20		
Positive	11 (10.0)	99 (90.0)	110	0.6 (0.1 – 2.5)	0.508
IgG2					
Negative	9 (9.9)	82 (90.1)	91		
Positive	5 (12.8)	34 (87.2)	39	1.3 (0.4 – 4.3)	0.622
IgG3					
Negative	7 (11.7)	53 (88.3)	60		
Positive	7 (10.0)	63 (90.0)	70	0.8 (0.3 – 2.6)	0.761
IgG4					
Negative	12 (12.2)	86 (87.8)	98		
Positive	2 (6.3)	30 (93.7)	32	0.5 (0.1 – 2.3)	0.886

parasite prevalence, males had higher prevalence than females. Although it may appear difficult to provide scientific proof on this finding, a recent study reported slightly higher levels of antibodies in women than men [16]. Whether such antibodies are associated with protection to

malaria or not, it remains to be confirmed by carefully designed clinical studies. A quick explanation on the scenario could be due to differences in exposure to malaria infection between males and females whereby males are usually considered to be more active outdoors and stay late.

Table 6. Multiple logistic regression analysis of factors associated with malaria

Variables	OR[1](95%CI)	p-value
Age (in years)		
1 – 5	1.4 (0.9 – 4.0)	0.372
6 – 15	3.7 (2.2 – 8.9)	<0.001**
>15	Reference	
Sex		
Female	Reference	
Male	1.9 (1.1 – 3.6)	0.027*
Antibodies		
IgG1 status		
Negative	Reference	
Positive	0.4 (0.2 – 0.8)	0.009**
IgG3 status		
Negative	Reference	
Positive	1.8 (1.0 – 3.3)	0.051

[1] Odds ratios are adjusted for age, sex, IgG1 status and IgG3 status

***Significant at p<0.01; *Significant at p<0.05*

We found the highest IgG3 seroprevalence in the group of individuals aged between 6 and 15 years, the same age group with the highest malaria prevalence. This seemingly predisposing effect of IgG3 may be explained as a result of prolonged exposure to infection that leads to IgG3 class switching [25,27]. This argument is in agreement with findings from a study conducted in Senegal that the level of parasite specific IgG3 is a critical factor and explain its efficient functional activity following repeated exposure to *P. falciparum* [1]. Analysis of data on age-specific distribution of IgG subtypes to MSP1-19 against malaria positivity showed no significant association between malaria and the seropositivity of IgG subtypes. However, IgG1 seropositivity showed a consistent association with low malaria prevalence in all age groups. After adjusting for potential confounding factors such as age and IgG subclasses, only seropositivity for IgG1 antibody was significantly associated with reduction of malaria prevalence by about two thirds. Similar findings were reported by other studies [6,20].

One fifth of the study participants had malaria. When adjusted for age, one third of those positive for malaria were in individuals aged between 6 and 15 years. Over the years, malaria has been most prevalent in children below 5 years of age. Our finding suggests an apparent shift in extent of exposure to infection from young children to the older, school age group of children. This shift may have complex explanations ranging from biological (host and parasite), policy, climatical, ecological and social factors. Subsidies in malaria medications and ITNS have mainly been targeting under fives and pregnant mothers and less so to the other groups. What we observe could partly be attributed to such concerted efforts implemented by the government of Tanzania. A previous study conducted in Korogwe district, North-Eastern Tanzania reported a similar observation where highest malaria prevalence was recorded in the 6-9 years old children [19]. Similarly, a study in Malawi reported highest malaria prevalence in children between 6 and 15 years [27]. More studies that investigate host and parasite biology, changes in climate and whether such changes have in any way modified vector mosquito structures and competence, impact of malaria control interventions and community knowledge on malaria control could provide clues to the possible shift in malaria prevalence.

Participants in the present study developed antibodies to MSP1-19, mainly total IgG, IgG1 and IgG3 and less so for IgG2 and IgG4. Among those IgG subtypes produced, only IgG1 antibody to MSP1-19 was associated with reduced malaria prevalence. It is known that, different types of blood stage *P. falciparum* antigens may elicit different IgG antibody subtypes which are protective to malaria. Extensive investigations should be done to define each protective anti- *P. falciparum* IgG subtypes antibody produced in response to specific antigen preferably in longitudinal studies. Although our study is limited by its cross sectional design when it comes to measuring protection, our findings are still valuable and provide important clues regarding IgG subtype responses against an important malaria vaccine candidate molecule, MSP1-19.

4. CONCLUSION

We report a high prevalence of malaria in the study area, with highest malaria prevalence recorded in older children of 6-15 years of age. Our findings show that only IgG1 antibody to

MSP1-19 is associated with low malaria prevalence, suggesting a possible protective role of the subtype against malaria. We report very low responses and seropositivity of IgG2 and IgG4 subtypes. Based on our present findings, IgG1 to MSP1-19 could be an important target of a prospective malaria vaccine.

CONSENT

Written informed consent was obtained from the study participants for participation in the research.

ETHICAL APPROVAL

All authors hereby declare that this study has been examined and approved by the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (CRERC) with approval certificate # 882 and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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