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Plasmodium Falciparum and mosquito vector IgG patterns across suspected malaria cases in Ghana

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Abstract

Introduction Malaria, a widespread tropical disease, remains a significant global health issue, resulting in numerous deaths each year. In Ghana, malaria is a leading cause of illness, contributing to a large proportion of hospital outpatient visits. The study assessed the pattern of malaria and vector IgG antibody levels among suspected malaria patients seeking healthcare at selected health facilities across Ghana.

Methods Samples from a total of 823 participants aged 1 to 85 years with clinical malaria from the ten regions of Ghana were recruited into the study. Archived plasma obtained from each participant was used to assess antibody responses against MSP1 (19 k), MSP2 (FC27 & 3D7), MSP3, gSG6-P1, and GLURP-RO using ELISA. The data were categorized according to study site, age group, gender, and diagnostic tests. Data were analyzed using Kruskal–Wallis's statistics. The statistical significance was assessed at 0.05.

Results The mean ± standard error of the mean (S.E) of MSP3 IgG concentration for the different age groups were 16, 847 ± 3, 031 ng/mL for 0–4 years, 18, 973 ± 4,357 ng/mL for 5–10 years, 25,961 ± 5,436 ng/mL for 11–15 years and 76, 244 ± 8, 209 ng/mL for ≥ 16 years. A significant (Kruskal–Wallis statistic = 122.6, $p < 0.0001$) increase in *P. falciparum* MSP 3 ($p < 0.0001$) and gSG6-P1($p < 0.0001$) IgG concentration was observed with increasing age categories. There were significant differences in antibody responses against MSP2 (FC27) IgG (Kruskal–Wallis statistic = 29.63, $p = 0.0005$), MSP3 IgG (Kruskal–Wallis statistic = 32.53, $p = 0.0002$), GLURP-RO IgG (Kruskal–Wallis statistic = 52.8, $p < 0.0001$) and gSG6-P1 IgG (Kruskal–Wallis statistic = 152.8, $p < 0.0001$) across the study regions.

Conclusion The study reveals that IgG against merozoite surface proteins MSP3, GLURP-RO, and gSG6-P1 but not MSP1 and MSP2 antibodies increase with age. The mean IgG antibody concentrations varied in the selected regions of Ghana. A longitudinal study where confounding factors are controlled for is recommended to provide insights into the development of immunity and antibody efficacy, and to enhance the effectiveness of malaria prevention efforts in Ghana. This will help improve the overall understanding of malaria transmission.

Keywords *Plasmodium falciparum*, IgG patterns, MSP3 antibodies, GLURP-RO antibodies, GSG6-P1antibodies, MSP1 antibodies, MSP2 antibodies, Suspected malaria, Ghana

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Introduction

Malaria, a tropical disease affecting 249 million people globally, caused 608,000 deaths in 2020 [1, 2]. In 2022, the African region was home to 94% of malaria cases (233 million) and 95% (580,000) of malaria deaths [3]. Over 65% of global deaths in children under five in 2021 was a result of malaria [4]. Ghana is malaria endemic country and has malaria accounting for about 40% of hospital outpatient visits [5, 6].

Pregnant women and under five children face the highest risk of malaria due to their reduced immune status. This has prompted the introduction of enhanced interventions and preventive measures for pregnant women and young children, informed by the analysis of geographic patterns and the underlying factors that contribute to malaria transmission [7]. Antibody levels in children aged 6–36 months are generally low and increase with age [7, 8]. The increased exposure of older children to malaria results in higher levels of anti-malaria specific antibodies, some of which were associated with protection from clinical malaria and increased infected erythrocytes (IE) agglutination activity [8, 9].

Antibodies against gSG6-P1 salivary gland peptide from *Anopheles gambiae* has been associated with recent exposure to *An. gambiae* and *An. funestus* mosquitoes in Africa [10, 11]. Immune responses against gSG6-P1 is linked to human *P. falciparum* infection, likely resulting from recent mosquito bites [11–13].The Plasmodium merozoite membrane is composed of merozoite surface proteins (MSPs), including MSP1 (attached directly), MSP6 (joined via protein–protein interactions), and MSP9 (associated via protein–protein interactions) [14, 15]. MSPs interact with erythrocyte surfaces, playing a crucial role in invasion [16]. *P. falciparum* merozoites, when invading erythrocytes, were susceptible to circulating malaria antibodies resulting from naturally acquired immunity (NAI) [17]. MSPs have been linked to clinical malaria protection, with mechanisms involving antibody-dependent cellular inhibition (ADCI), opsonic phagocytosis (OP), and complement-mediated lysis [17]. Residents in malaria-endemic regions develop partial immunity against clinical malaria mediated by IgG that reduce parasitaemia and clinical symptoms [18].

Plasmodium falciparum infections maintain strain-specific anti-merozoite antibodies, with increased FC27 alleles causing higher antibody titers to MSP2-Dd2, but not MSP2-Ch150/9 [19]. An MSP1-C1 combination vaccine was developed but showed unacceptable reactogenicity [20–22]. On the contrary, GLRUP-RO antibodies are associated with a decrease in *P. falciparum* density [19]. Also, other studies have reported GLRUP-RO antibodies to offer antimalarial protective immunity to people living in malaria-endemic areas [19, 22]. Multiclonal infections, involving diverse *P. falciparum* clones, can benefit the host by increasing the breadth and diversity of antibodies the host produces. Such diverse antibodies would reduce the risk of clinical illness due to malaria infections caused by any of the previously encountered parasites.

The study assessed the pattern of malaria parasite and vector IgG responses in suspected malaria patients seeking healthcare at selected health facilities in Ghana.

Method

Study site and population

This study used archived plasma samples retrieved from a parent project 'Determining whether histidine-rich protein gene deletions causing negative histidine-rich protein II (HRP2) rapid diagnostic test (RDT) results among suspected patients with confirmed *P. falciparum* malaria have reached a threshold for change in diagnostic strategy'. All the samples used in this study had their parasitological data (Microscopy, RDT and PCR) published [23–26]. Samples used for this study had consent for future use.

The parent study collected samples between May and August 2021 from suspected malaria patients who received healthcare from ten health centres. The main study collected more than 19,000 malaria samples and this study is analyzing a small portion of the samples collected. Archived samples from 823 participants aged 1 to 85 years from ten regions of Ghana namely Central, Greater Accra, Western, Western North, Oti, Eastern, and Volta Region in the Southern zone and three regions thus Ahafo, Bono, and Bono East Region in the Middle zone was used for the study. (Fig. 1).

Sample size calculation

Yasmane sample size calculation was used;

n = N/(1 + (N*e^2); n = 19000/(1 + (19000*0.05^2) = 391.75 = 392 for each study zone. Hence, the total sample size = 784.

n = sample size, N = Sample collected by the parent study, e = margin of error.

We included a total of 823 samples for the two study zones.

Sample collection and processing

In the parent study, 1.0 ml of whole blood was collected from each participant and the plasma was separated from the red blood cells and both stored independently at −20℃. The 823 plasma samples used in this study were subsequently thawed and used to quantify antibody responses against MSP1 (19 k), MSP2 (FC27), MSP2 (3D7), MSP3, GLURP-RO and gSG6-P1 antigens.

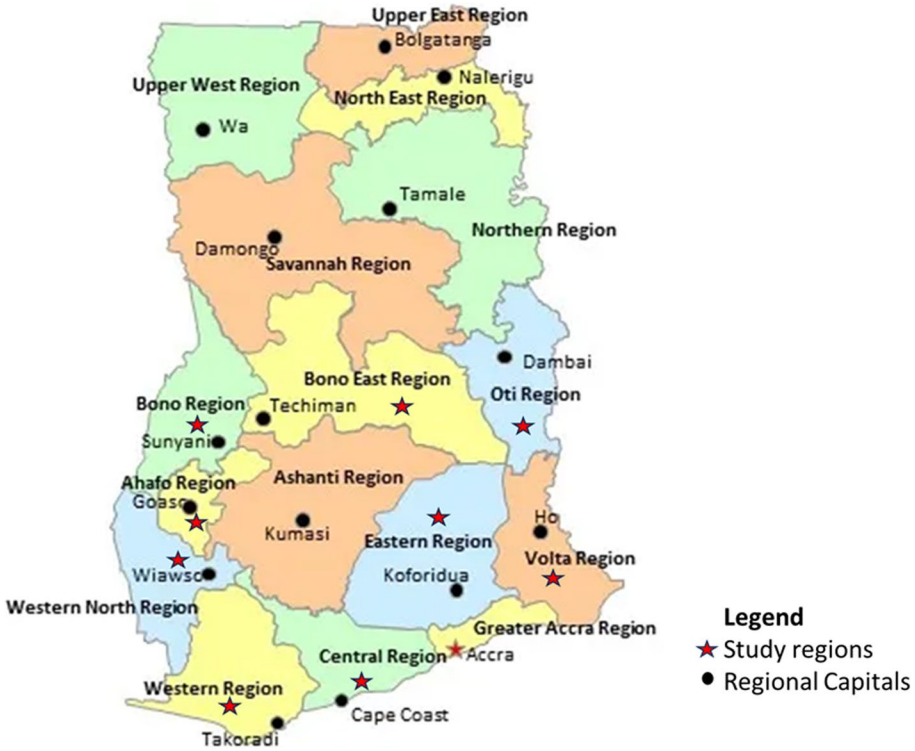

Fig. 1 Ghana Map indicating study regions

Determination of IgG Levels by Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

IgG antibody levels against MSP1 (19 k), MSP2 (FC27), MSP2 (3D7), MSP3, gSG6-P1, and GLURP-RO were quantified by indirect Enzyme-Linked Immunosorbent Assay [10, 11, 19]. A 96 well NUNC Maxisorp ELISA plate coated with recombinant antigens of MSP1 (19 k), MSP2 (FC27), MSP3, RO, and gSG6-P1 at 1 µg/well and 0.5 µg/well of MSP2 (3D7) in phosphate-buffered saline (PBS, pH 7.4) and incubated overnight at 4 °C. The plate was washed three times with 250 µl/well PBS-T (0.05% Tween-20) wash buffer. 200 µl of blocking buffer containing 3% skimmed milk in PBS was used to block the unbound regions in the wells and incubated for an hour. The plate was then washed three times and the diluted plasma, positive and negative samples obtained from a pool of seropositive and seronegative individuals respectively were incubated in duplicate at 100 µl/well for an hour at room temperature. The plates were washed three times and incubated for an hour with 100 µl/well of 1:3000 dilution of goat antihuman IgG-HRP (Thermo Scientific) at room temperature. The plates were finally washed and incubated with peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) for 12 min. 100 µl of 0.2 mM sulfuric acid was added to stop the enzymatic reactions and the optical densities (ODs) for the contents in the wells were read at 450 nm using a Multiskan FC Thermo Scientific ELISA plate reader.

Statistical analysis

The OD values obtained from the ELISA plate reader (Thermo Scientific Multiskan FC) were converted into weighted concentrations (wConc) using ADAMSEL (Ed Remarque, BPRC, Netherlands), a four-parameter fitting program. All data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA, USA), and the statistically analyzed with GraphPad Prism software, version 9.0.2 (GraphPad Software, San Diego, CA, USA). The data was categorized according to study sites, age groups, gender, and diagnostic tests. The Kruskal–Wallis statistics were used to assess statistical significance across the various categories and Mann–Whitney U statistics to compare statistical significance differences between two groups. All p-values less than 0.05 were considered statistically significant.

Table 1 Demographic characteristics of the study population

Characteristics	Overall (National)
Age, median (range, 95% CI)/yrs	11 (1–85, 9–12)
Temperature, median (range, 95% CI)/oC	37.60 (35.20–40.30, 37.50–37.70)

Results

Demographic characteristics of the study participants

All the 823 samples tested positive for *P. falciparum* malaria by the rapid diagnostic test. The median age (range) of the participants was 11 (1–85) years and the median body temperature (range) was 37.60 (35.20–40.30) ºC (Table 1). The mean (S.E of the mean) overall concentration of *P. falciparum* MSP1 (19 K), MSP2 (FC27), MSP2 (3D7), MSP3, and GLURP-RO IgG were 122,574 (7884) ng/mL, 31,234 (2477) ng/mL, 89,902 (6559) ng/mL, 44,613 (3635) ng/mL, and 19,277 (1051) ng/mL respectively. Also, the mean (S.E of the mean) of mosquito vector gSG6-P1 IgG was 3,430 (110.7) ng/mL (Table 2; Fig. 2).

P. falciparum MSP1 (19 k), MSP2 (FC27) and MSP2 (3D7) IgG concentration decreases with increasing age categories

The MSP 1 (19 k) IgG concentrations were observed to decrease gradually with increasing age. The 0–4 years age group had the highest concentration of 163, 266 ± 24, 779 ng/mL (mean ± S.E), which gradually decreased across the increasing age categories with the age group ≥ 16 years having the lowest MSP 1 (19 k) IgG concentration of 101,888 ± 9,715 ng/mL. The decrease in IgG concentrations however, was not statistically significant (Kruskal–Wallis statistic = 1.396, p value = 0.7064) (Fig. 3 a).

Table 2 The mean and median distribution of *Plasmodium falciparum* and mosquito vector IgG

Plasmodium falciparum IgG antibody	
MSP1 (19 K), n/N (%)	823/823 (100)
Mean, S.E (95% CI)	122,574, 7884 (3120–40431)
Median (Interquartile Range)	34,952 (11,038–117,926)
MSP2 (FC27), n/N (%)	817/823 (99.3)
Mean, S.E (95% CI)	31,234, 2477 (26,371–36,097)
Median (Interquartile Range)	13,267 (7197–26,964)
MSP2 (3D7), n/N (%)	820/823 (99.6)
Mean, S.E (95% CI)	89,902, 6559 (77,027–102777)
Median (Interquartile Range)	29,994 (10,920–85871)
MSP3, n/N (%)	818/823 (99.4)
Mean, S.E (95% CI)	44,613, 3635 (37,479–51,748)
Median (Interquartile Range)	10,704 (5530–31316)
GLURP (RO), n/N (%)	812/823 (98.7)
Mean, S.E (95% CI)	19,277, 1051 (17,215–21,339)
Median (Interquartile Range)	11,446 (6938–19,709)
Mosquito vector IgG antibody	
gSG6-P1, n/N (%)	805/823 (97.8)
Mean, S.E (95% CI)	3430, 110.7 (3212–3647)
Median (Interquartile Range)	2739 (1600–4313)

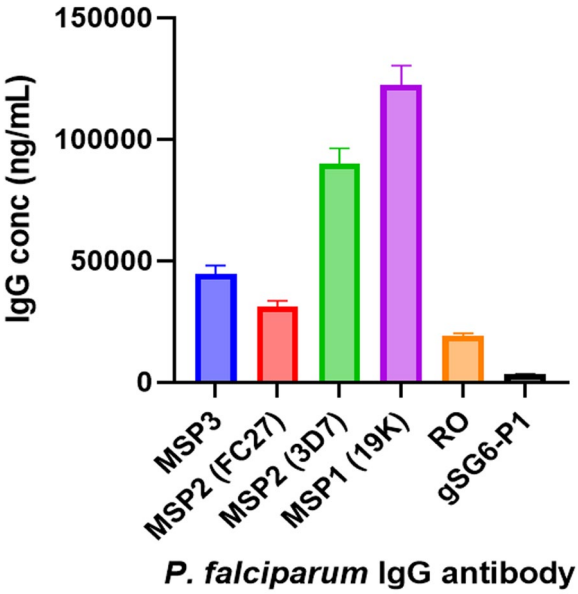

Fig. 2 The mean distribution of *P. falciparum* and mosquito vector merozoite IgG against merozoite surface antigens (MSP1, MSP2 (FC27), MSP2 (3D7), MSP3, and GLURP-RO) and mosquito vector antigen (gSG6-P1)

A similar observation was seen for MSP2 (FC27) IgG concentration which also decreased gradually from the 0–4 years age group (mean ± S.E) was 47,044 ± 9.552 ng/mL through to the age group 11–15 years, 22,445 ± 5, 283 ng/mL and a slight increase in the IgG concentration in the age group ≥ 16 years 30, 999 ± 3,744 ng/mL but there was no significant difference across the age groups (Kruskal–Wallis statistic = 5.468, *p*-value = 0.1406) (Fig. 3 b). Interestingly, MSP2 (3D7) IgG concentration remained fairly stable across the age groups (Fig. 3 c).

P. falciparum MSP3 GLURP-RO, and Salivary gland peptide (gSG6-P1) IgG concentration increases with increasing age

P. falciparum MSP3 IgG concentration increases with increasing age. The mean ± S.E of MSP3 IgG concentration for the age groups were 16, 847 ± 3, 031 ng/mL for 0–4 years, 18, 973 ± 4,357 ng/mL for 5–10 years, 25,961 ± 5,436 ng/mL for 11–15 years and 76, 244 ± 8, 209 ng/mL for ≥ 16 years. The Kruskal–Wallis statistic = 122.6, $p < 0.0001$ showed a significant increase in *P. falciparum* MSP3 IgG concentration in the increasing

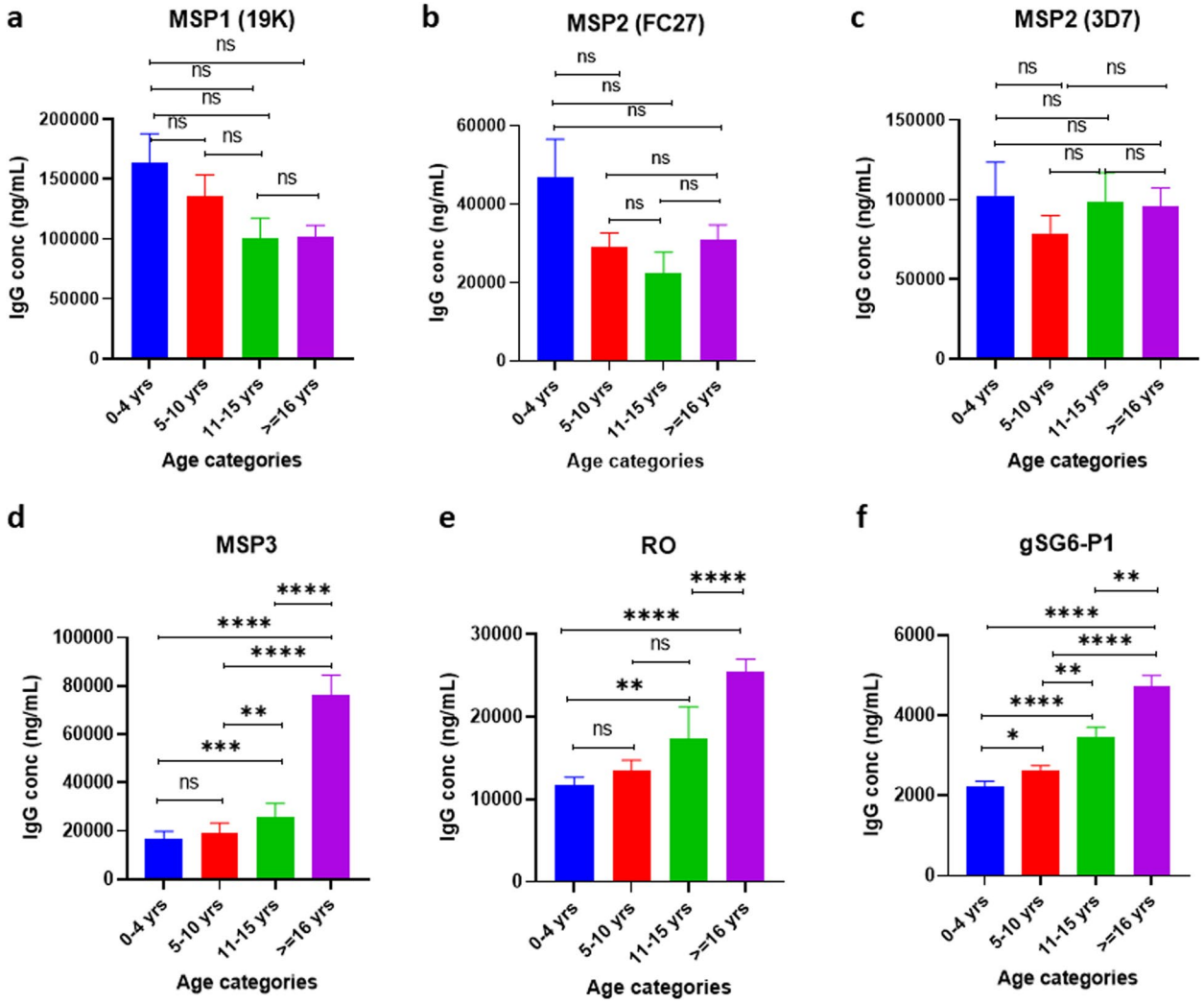

Fig. 3 *P. falciparum* and mosquito vector IgG antibody concentration across age categories. **a** The MSP 1 (19 k) IgG antibody concentration was observed to decrease gradually with increasing age. **b** MSP2 (FC27) IgG antibody concentration which also decreased gradually from the 0–4 years age group. **c** MSP2 (3D7) IgG antibody concentration remained fairly stable across the age groups. **d** MSP3 IgG antibody concentration increases with increasing age. **e** GLURP-ROIgG antibody concentration increases with increasing age. **f** The Salivary gland peptide (gSG6-P1) IgG antibody concentration increases with increasing age categories. The Kruskal–Wallis statistics were used to assess statistical significance across age categories and Mann–Whitney U statistics to compare statistical significance differences between age groups. > 0.05 (non-significant), < 0.05 (* = significant), > 0.01 (** = significant), < 0.001 (*** = highly significant), < 0.0001 (**** = highly significant)

age categories. The Mann–Whitney U showed a significant difference between the age group 0–4 years and 11–15 years ($p < 0.0001$) and between 0–4 years and ≥ 16 years ($p < 0.0001$), however, there was no significant difference between the IgG in the age group 0–4 years and 5–10 years ($p = 0.169$). There was also a significant difference between the other age categories (Fig. 3 d). *P. falciparum* GLURP-RO IgG concentration increases with increasing age (Kruskal–Wallis statistic = 104.1, $p < 0.0001$). Except for GLURP-RO IgG y concentrations in the 0–4 years and 5–10 years ($p = 0.078$), 5–10 years and 11–15 years ($p = 0.129$) age groups. There were highly significant differences in the IgG concentrations across the age groups (Fig. 3 e). The Salivary gland peptide (gSG6-P1) IgG concentration increases with increasing age categories (Kruskal–Wallis statistic = 114.8, $p < 0.0001$). The Mann–Whitney U showed a significant difference between the age group 0–4 years and 5–10 years (statistic = 11,426, $p = 0.016$), between 0–4 years and ≥ 16 years (statistics = 8705, $p < 0.0001$), between 5–10 years and 11–15 years (statistics = 6347, $p = 0.0015$) and between 11–15 years and ≥ 16 years (statistics = 9420, $p = 0.0019$) (Fig. 3 e).

Comparison of *P. falciparum* IgG in participants with and without fever

The concentrations of IgG against selected *P. falciparum* MSP1 (19 k), MSP2 (FC27), MSP2 (3D7), MSP3 and GLURP-RO antigens and mosquito salivary gland gSG6-P1 antigen was compared between participants with normal and high body temperatures to understand the modulatory role of the IgG. There was a high *P. falciparum* MSP1 IgG among participants with high body temperature ranging between ≥ 37.5 °C (mean ± S.E = 132,492 ± 11, 613 ng/mL) compared to participants with normal body temperature ranging between < 37.5 °C (mean ± S.E = 108,343 ± 9,499 ng/mL). However, there was no significant difference in MSP1 median IgG between the participants with normal and high body temperature (Mann–Whitney U = 79,883, $p = 0.535$) (Fig. 4 a). There was also no significant difference in the MSP2 (FC27) IgG among participants with high body temperature (mean ± S.E = 30,191 ± 2,915 ng/mL) compared to participants with normal body temperature (mean ± S.E = 32,690 ± 4,325 ng/mL), $p = 0.2519$ (Fig. 4 b). Similarly, there was no significant difference in the MSP2 (3D7) IgG among participants with high body temperature compared to participants with normal body temperature, $p = 0.1844$ (Fig. 4 c). Again, there was no significant difference between in *P. falciparum* MSP3 IgG among participants with high and low body temperature, Mann–Whitney U = 76,418, $p = 0.1577$ (Fig. 4 d). Also, there was no significant difference in the concentration of GRURP-RO IgG

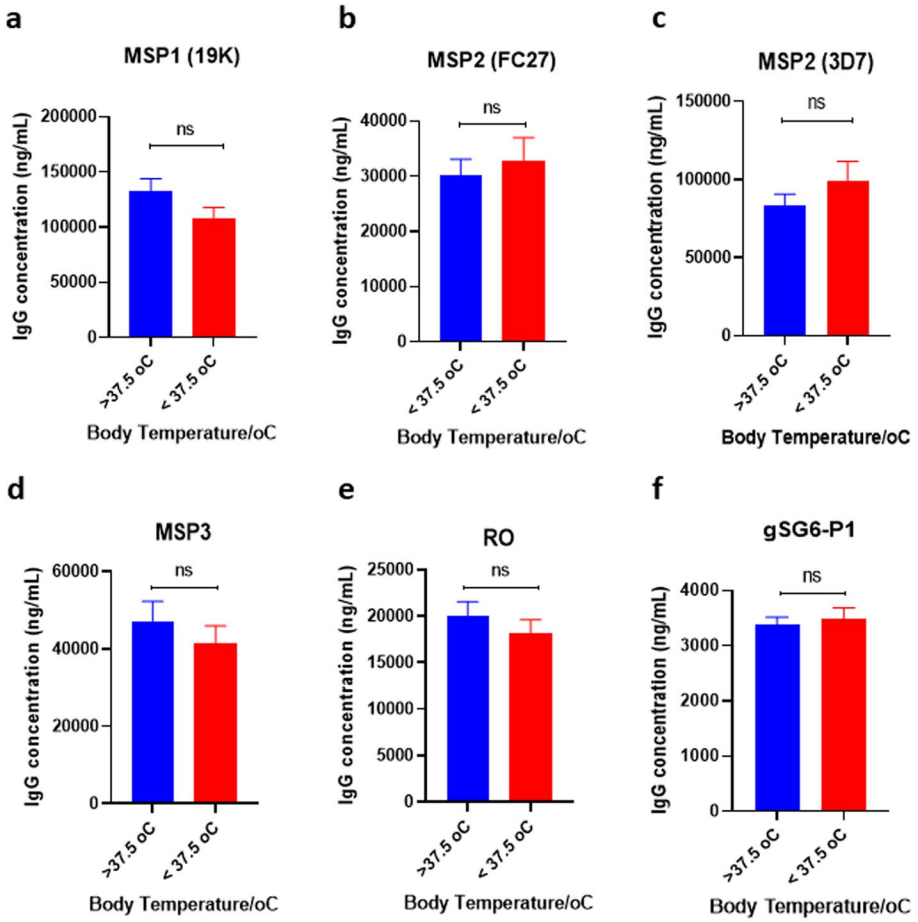

Fig. 4 IgG against selected *P. falciparum* antigens and mosquito salivary gland antigens between normal and high body temperature. **a** MSP1 median IgG antibody between the participants with normal and high body temperature. **b** MSP2 (FC27) IgG antibody among participants with high body temperature. **c** MSP2 (3D7) IgG antibody among participants with high body temperature compared to participants with normal body temperature. **d** MSP3 IgG antibody among participants with high body temperature compared to participants with normal body temperature. **e** GRURP (RO) IgG antibody between the participants with normal and high body temperature. **f** gSG6-P1IgG antibody between the participants with normal and high body temperature. Mann–Whitney U statistics was used to assess the statistical significance between normal and high body temperature. > 0.05 (non-significant), < 0.05 * = significant)

antibody between the participants with normal and high body temperature (Mann–Whitney U = 75,965, $p = 0.2492$) (Fig. 4 e) and the concentration of gSG6-P1 IgG between the participants with normal and high body temperature (Mann–Whitney U = 76,469, $p = 0.4879$) (Fig. 4 f).

Regional distribution of *Plasmodium falciparum* and mosquito vector IgG

The *P. falciparum* and mosquito vector IgG across the various study regions were compared for the antibody distribution. The result showed that there were significant differences in the distribution of MSP2 (FC27) IgG (Kruskal–Wallis statistic = 29.63, $p = 0.0005$), MSP3 IgG (Kruskal–Wallis statistic = 32.53, $p = 0.0002$), GLURP-RO IgG (Kruskal–Wallis statistic = 52.8, $p < 0.0001$) and gSG6-P1 IgG (Kruskal–Wallis statistic = 152.8, $p < 0.0001$) across the study regions (Table 3).

P. falciparum and mosquito salivary gland IgG antibody levels across the study regions

The overall mean distribution of *P. falciparum* and vector salivary gland IgG was compared to the individual regional mean distribution and the inter-regional of the antibodies. There was no significant difference in the overall *P. falciparum* MSP1 (19 K) compared to the individual regional distributions of the IgG, $p > 0.05$. However, there were significantly higher levels of *P. falciparum* MSP1 (19 K) IgG in the Western region compared to the Ahafo region (Mann–Whitney U = 1771, $p = 0.0152$) and the Central region (Mann–Whitney U = 1734, $p = 0.0077$) as well as the Eastern region compared to the Central region (Mann–Whitney U = 4120, $p = 0.0409$) (Fig. 5 a).

There was also a significant difference in the distribution of *P. falciparum* MSP2 (FC27) (Fig. 5 b), MSP3 (Fig. 5 d), GLURP-RO (Fig. 5 e), and gSG6-P1IgG across the study regions (Fig. 5 f). Interestingly, there was a similar distribution of *P. falciparum* MSP2 (3D7) IgG across all study regions (Fig. 5 c).

Discussion

In malaria-endemic regions, as individuals grow older, the prevalence of malaria tends to decrease, along with the burden of parasites and the incidence of anaemia [27, 28]. Thus, adults in malaria-endemic communities develop immunity that reduces parasite density leading to chronic malaria and asymptomatic infection [29, 30]. Malaria immunity develops slowly after repeated exposures, impacting clinical outcomes [31]. Age dependent naturally acquired antibodies has been evaluated based on past and present exposure [32]. Antibodies significantly enhance immunity against *P. falciparum* malaria, but their impact on naturally infected individuals and age-related increase remains unclear. Indeed, the protective immune responses against various merozoite antigens, including MSP1 (19 k), MSP2 (FC27 and 3D7), MSP3, GLURP-RO, and mosquito exposure antigen (gSG6-P1), remain unclear. High antibodies may reduce parasite burden and result in milder disease, but the mechanisms were complex [27, 32].

The study found that *P. falciparum* MSP1 IgG antibody concentration decreases with age, with the highest concentration in 0–4 years and gradually decreasing across older individuals. This suggests a potential decrease in the protective effect of MSP1 antibodies in malaria-endemic areas [33, 34] or that these antibodies are used up to clear early parasites infection. The absence of significant associations between variant-specific serology or functional antibodies and infection suggests other factors may contribute to malaria susceptibility in adults [35].

MSP1-42 is processed into MSP1-33 and MSP1-19 during merozoite invasion, facilitating invasion and targeting the host immune response [36]. However, recent trials in western Kenya showed no protective efficacy against malaria infection, highlighting the ineffectiveness of IgG for older age groups [37, 38]. Age increases malaria-specific antibody responses in children under 5 years old, and antibodies targeting MSP2 from FC27 and 3D7 parasite strains did not increase across age groups.

The study reveals a notable trend of increased concentrations of MSP3, GLURP-RO, and gSG6-P1IgG with increasing age groups. This observation suggests that older individuals tend to have higher levels of antibodies targeting these malaria antigens compared to younger age groups [19]. The increase in antibody concentrations with age may reflect cumulative exposure to malaria parasites over time, leading to the development of a more robust immune response [39, 40]. These findings are consistent with the concept of acquired immunity to malaria, wherein repeated exposure to the parasite results in the development of protective immune responses [40]. The merozoite surface proteins MSP3 and GLURP-RO, parasite antigens released when mature schizont-infected erythrocytes burst are also exposed to host immune defences. One of these antigens, GLURP-R0, is included in a current malaria vaccine candidate known as GMZ2. The GMZ2 is a chimeric vaccine composed of the R0 fragment of GLURP-R0 fused to MSP3 has been reported to offer anti-malaria protective immunity and is associated with low malaria density in Ghana [19, 22]. Antibodies targeting both GLURP-R0 and MSP3 have been implicated in the acquisition of protective immunity to malaria [19, 41].

The study reveals an increase in gSG6-P1IgG, which target the salivary protein produced by Anopheles mosquitoes, indicating exposure to mosquito bites and

Table 3 Regional distribution of *Plasmodium falciparum* and mosquito vector IgG

Characteristics	Regions										Kruskal–Wallis test	
	Ahafo	Bono	Bono East	Central	Eastern	Greater Accra	Oti	Volta	Western	Western North	Statistics	p
***Plasmodium falciparum* IgG antibody**												
MSP1 (19 K), n/N (%)	98/823 (11.9)	52/823 (6.3)	32/823 (3.9)	96/823 (11.7)	100/823 (12.2)	100/823 (12.2)	98/823 (11.9)	103/823 (12.5)	98/823 (11.9)	46/823 (5.6)	13.91	0.1772
Mean, S.E (95% CI)	112,679, 20,618 (71,758–153,600)	107,630, 32,695 (41,992–173,268)	105,464, 32,482 (39,217–171,711)	110,808, 16,556 (78,020–143756)	140,904, 22,298 (96,660–185147)	162,103, 31,814 (98,969–225,237)	94,617, 49,172 (69,654–119,580)	112,448, 19,667 (73,414–151,482)	157,215, 39,800 (77,147–237,282)	104,786, 26,337 (50,922–158651)		
Median (Interquartile Range)	20,599 (9167–76,981)	33,837 (7431–70,589	44,151 (15,512–123,655)	24,649 (9413–157,902)	50,118 (11,082–132529)	34,953 (13,256–159,117)	36,323 (14,676–123,448)	57,419 (17,497–124,017)	57,419 (17,497–124,017)	47,181 (13,104–140164)		
MSP2 (FC27), n/N (%)	98/817 (12)	52/817 (6.4)	32/817 (3.9)	98/817 (11.8)	98/817 (12)	95/817 (11.6)	98/817 (12)	104/817 (12.7)	94/817 (11.5)	48/817 (5.9)	29.63	0.0005
Mean, S.E (95% CI)	33,941, 7365 (19,322–48,559)	21,039, 2329 (16,363–25,714)	22,073, 5602 (10,647–33499)	36,550, 9666 (17,361–55,738)	20,857, 3135 (14,635–27,078)	32,848, 4121 (24,666–41,030)	33,773, 4588 (24,673–42,873)	23,962, 4588 (14,863–33,060)	44,272, 13,858 (16,760–71783)	34,721, 9672 (15,264–54,178)		
Median (Interquartile Range)	12,332 (5756–23,809)	16,125 (10,008–24180)	11,514 (6725–28,546)	12,355 (7393–32,440)	10,343 (4819–20,376)	19,252 (9606–34624)	15,442 (9404–40899)	11,268 (5007–23944)	11,164 (6813–23,667)	13,917 (6200–34406)		
MSP2 (3D7), n/N (%)	99/820 (12.1)	53/820 (6.5)	32/820 (3.9)	97/820 (11.8)	99/820 (12.1)	95/820 (11.6)	98/820 (12.0)	104/820 (12.7)	98/820 (12.0)	45/820 (5.5)	12.46	0.1886
Mean, S.E (95% CI)	83,168,14,823 (53,752–112,584)	72,670, 18,115 (36,320–109019)	56,627, 15,252 (25,317–87,937)	94,763, 19,644 (55,769–133,756)	59,177, 8593 (42,125–76,230)	98,728, 16,428 (66,109–131347)	120,658, 27,487 (66,104–175212)	100,783, 19,435 (62,238–139,327)	93,647, 26,174 (41,700–145594)	86,874, 21,298 (43,951–129,798)		
Median (Interquartile Range)	28,888 (8231–91,944)	31,859 (12,455–64,609)	26,770 (8676–57,953)	37,744 (10,195–85260)	20,895 (10,748–63780)	36,458 (13,761–105,528)	38,796 (17,360–114279)	29,946 (12,116–75,072)	20,770 (7286–96,184)	28,168 (7698–137,513)		
MSP3, n/N (%)	94/818 (11.5)	52/818 (6.4)	32/818 (3.9)	97/818 (11.9)	98/818 (12.0)	100/818 (12.2)	99/818 (12.0)	100/818 (12.2)	98/818 (12.0)	48/818 (5.9)	32.53	0.0002
Mean, S.E (95% CI)	21,049, 4993 (11,135–30,964)	52,886, 16,249 (20,264–85508)	28,956, 8106 (12,425–45,488)	37,080, 9880 (17,469–56,691)	36,560, 8875 (18,945–54,174)	52,708, 13,595 (25,732–79,684)	43,589, 8551 (26,620–60558)	53,711, 10,842 (32,198–75,224)	74,212, 15,088 (44,267–104,157)	29,763, 7717 (14,240–45287)		
Median (Interquartile Range)	6921 (3560–16580)	13,390 (7326–34,411)	11,793 (5344–30,512)	8223 (3355–28,384)	9149 (5306–24749)	9935 (6349–24,749)	11,284 (6723–37,157)	15,320 (6188–49,425)	12,914 (6188–46,758)	12,928 (6333–31,957)		
GLURP (RO), n/N (%)	97/812 (11.9)	51/812 (6.3)	30/812 (3.7)	96/812 (11.8)	99/812 (12.2)	99/812 (12.2)	99/812 (12.2)	97/812 (11.9)	97/812 (11.9)	47/812 (5.8)	52.8	< 0.0001

Table 3 (continued)

Characteristics	Regions										Kruskal–Wallis test	
	Ahafo	Bono	Bono East	Central	Eastern	Greater Accra	Oti	Volta	Western	Western North	Statistics	p
Mean, S.E (95% CI)	14,412, 1834 (10,772–18052)	30,036, 9965 (10,021–50051)	10,158, 1552 (6983–2515)	17,875, 3667 (10,595–25155)	15,575, 1947 (11,711–19,439)	21,855, 2380 (17,133–26,578)	15,118, 1739 (11,667–18,569)	22,514, 2064 (18,418–26,611)	26,710, 3653 (19,458–33,962)	15,424, 2235 (10,925–19923)		
Median (Interquartile Range)	10,141 (5779–15,822)	10,580 (7140–24098)	6961 (5183–13,044)	9910 (5755–18,244)	9486 (6217–16,647)	13,912 (7171–29,549)	10,327 (6946–16,622)	18,154 (12,004–24959)	12,552 (7685–30,474)	10,901 (7023–16287)		
Mosquito vector IgG antibody												
gSG6-P1, n/N (%)	83/805 (10.3)	52/805 (6.5)	32/805 (4.0)	96/805 (11.9)	97/805 (12.0)	100/805 (12.4)	99/805 (12.3)	103/805 (12.8)	96/805 (11.9)	47/805 (5.8)	152.8	< 0.0001
Mean, S.E (95% CI)	2259, 202.6 (1856–2662)	2786, 251.9 (2224–3348)	1994, 251.2 (2689–3595)	3142, 228.2 (2689–3595)	2017, 149.7 (1720–2314)	4775, 468.6 (3846–5705)	4570, 291.2 (3992–5148)	3966, 211.1 (3275–4112)	3966, 365.5 (3240–4692)	3751, 762 (2217–5285)		
Median (Interquartile Range)	1615 (897.4–3211)	2128 (1353–2617)	1620 (1042–2617)	2654 (1614–3866)	2656 (990.1–2656)	3739 (2417–2656)	3828 (2939–4916)	3299 (2208–4412)	3003 (2025–4623)	2261 (1514–4925)		

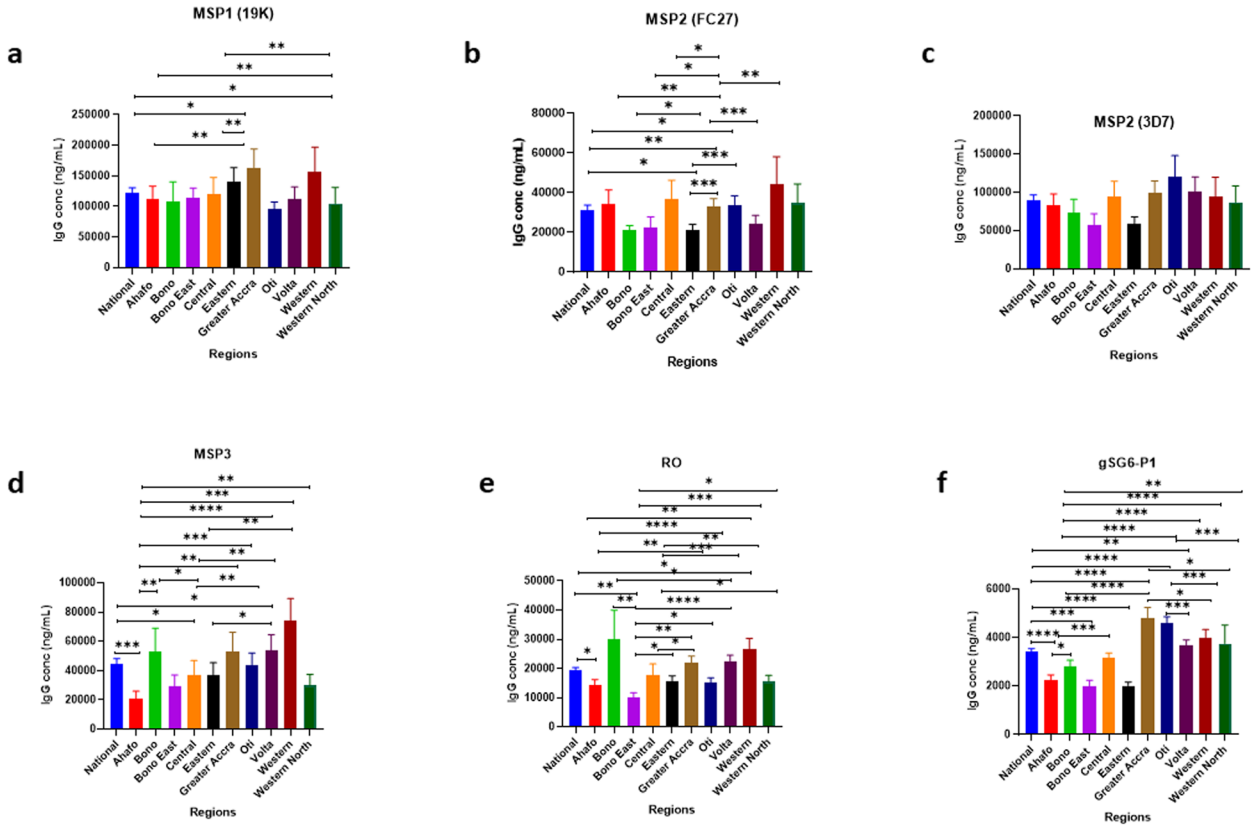

Fig. 5 *P. falciparum* and mosquito salivary gland IgG against selected antigens across the study regions. **a** MSP1 (19 K) IgG antibody, **b** MSP2 (FC27) IgG antibody, **c** MSP2 (3D7) IgG antibody, **d** MSP3 IgG antibody, **e** GRURP (RO) IgG antibody, **f** gSG6-P1IgG antibody levels across the study regions. Mann–Whitney U statistics was used to assess the statistical significance between study regions. > 0.05 (non-significant), < 0.05 (* = significant), > 0.01 (** = significant), < 0.001 (*** = highly significant), < 0.0001 (**** = highly significant)

malaria transmission. This suggests that older individuals have higher levels of antibodies compared to younger individuals. The increase in gSG6-P1IgG with age likely reflects cumulative exposure to mosquito bites over time [10, 11].

A study conducted in vitro using PvMSP1-19 recombinant protein found that the binding affinity of malarial and dengue antigens to monoclonal antibodies increased significantly at 40 °C and was further enhanced with thermal equilibration [42]. The study suggests that fever can enhance antibody-antigen binding and protein–protein affinity, particularly for limited samples, highlighting the adaptive nature of fever as a host defence mechanism against pathogens [42–44]. However, comparing the antibody concentration between study participants with and without fever showed that there was no significant association between the levels of body temperature and malaria or mosquito vector IgG. It implies that the effectiveness of high body temperature might not be solely dependent on the quantity of antibodies present in circulation. Instead, it suggests that elevated body temperature could enhance the quality of antibodies by improving their affinity to the antigen they're targeting [45, 46]. This could potentially lead to a more efficient immune response, even with a similar antibody concentration [42, 43].

The regional variation in *Plasmodium falciparum* and mosquito vector IgG concentrations across Ghana could be attributed to differences in malaria transmission intensity, environmental factors, socioeconomic and public health disparities, host immunity, and mosquito vector species. Regions with higher transmission rates experience more frequent exposure to *P. falciparum* and mosquito bites, leading to elevated antibody levels. Additionally, regions with better access to control interventions have lower exposure and antibody levels.One limitation of this study is the choice to use a cross-sectional design, as this will only provide information at a single time point. Secondly, the study did not account for potential confounding factors like previous differences in malaria control measures.

A longitudinal study is recommended to assess antibody effectiveness by controlling confounding factors, to provide insights into immunity development

and antibody effectiveness, and to enhance the effectiveness of malaria prevention efforts in Ghana. This will help improve the overall understanding of malaria transmission.

In conclusion, the study shows that the IgG against merozoites surface proteins MSP3 and GLURP-RO increase with increasing age groups contrarily MSP1 (19 k) and MSP2 (FC27 and 3D7) IgG did not increase with increasing age groups. Also, the IgG against the gSG6-P1salivary peptide increase with increasing age groups. Except for MSP2 (3D7), all the IgG antibody concentrations measured in the study showed variations across the different regions of Ghana.

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Authors' contributions
Conceptualization, LEA Formal analysis; KKA, LEA Methodology; SSK, PT, VS Supervision, SVN Funds acquisition, LEA Validation; KKA, LEA Visualization; SSK, PT, VS, KKA, LEA Writing – original draft, KKA, SSK, PT, VS, SVN, LEA Writing – review & editing; KKA, SSK, PT, VS, SVN, LEA.

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Data availability
All the data are available in the manuscript.

Declarations

Ethics approval and consent to participate
Ethical approval for this study was obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (CPN 068/17–18) before the commencement of the study. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki (as amended in 2013), ensuring the protection of the rights, safety, and well-being of the study participants. All participants provided written informed consent as well as consent for sample reuse prior to their inclusion in the study, and the confidentiality of their data was strictly maintained throughout the research process.
Written informed consent, with permission to reuse stored samples, including assent for adolescents aged 11 to 17, and parental consent for children aged 17 years and below, was obtained for all samples used in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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