ORIGINAL ARTICLE

# Natural IgG Antibody Responses to *Plasmodium spp* Merozoite Surface Protein-1 in Forest Malaria Endemic Areas of Tanah Bumbu, South Kalimantan, Indonesia

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## ABSTRACT

**Introduction:** Merozoite surface protein-1 (MSP-1) is a protein that is frequently observed on the surface of *Plasmodium spp*. merozoites, and hence, it is suggested as a potential candidate for a malaria vaccine. The immune response of the host to MSP-1 is known to vary depending on host genetics, geographical distribution, and regional malaria endemicity. This study evaluated the production of IgG antibodies targeting MSP-1 *Plasmodium spp*. among individuals residing in the malaria-endemic region of Tanah Bumbu Regency, South Kalimantan, Indonesia. **Methods**: This cross-sectional study involved 155 participants aged 15-60 years, randomly selected from a forest malaria-endemic region. Malaria infection was confirmed through microscopic evaluation and real-time PCR. Serum anti-MSP-1 IgG concentrations were measured by a commercial ELISA kit targeting full-length MSP-1. Demographic and behavioral information was obtained through structured interviews using pre-validated questionnaires. **Results:** The findings demonstrate that 67.74% of participants possessed naturally acquired IgG antibodies specific to MSP-1. Statistically significant differences in antibody levels were observed for gender ($p = 0.022$), a history of malaria ($p = 0.001$), use of long sleeves at night ($p = 0.023$), use of mosquito nets ($p = 0.007$), and regular use of malaria drugs ($p = 0.015$). Antibody levels were also strongly correlated with body temperature ($p = 0.001$), parasitemia percentage ($p = 0.030$), and the duration of exposure to malaria ($p = 0.011$). **Conclusion:** High anti-MSP-1 IgG prevalence indicates prior exposure and possible partial immunity. However, due to the cross-sectional design and lack of functional assays, protection cannot be inferred. Longitudinal studies are needed to confirm the protective role of MSP-1–specific responses.
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## INTRODUCTION

Malaria has existed for a long time and is still a problem in the world. *Plasmodium spp.*, a member of the apicomplexan parasite family, causes malaria through the bite of the Anopheles mosquito. Annually, over 200,000 cases are reported globally, with 95% occurring in sub-Saharan Africa. The global number of cases of malaria climbed from 227 million in 2019 to 241 million in 2020 and further to 249 million by 2022 (1). The COVID-19 pandemic disrupted malaria elimination programs, resulting in a rise in cases (2).

In Asia, Indonesia ranks second for the number of malaria cases, following India. Although the majority of regions in Indonesia are categorised as malaria-free, specific transmission hotspots persist, particularly in remote forested areas, often referred to as forest malaria zones. Despite various beneficial efforts, such as the provision of insecticidal mosquito nets and spraying, forest malaria remains a residually transmitted disease, where *Plasmodium spp*. transmission persists (3, 4). Therefore, assessing malaria transmission in such a setting by evaluating host antibody responses to *Plasmodium spp*. antigens is essential. Targeted interventions are required to address residual transmission, which may differ significantly from strategies used in non-forest areas (3). A thorough understanding of the immune response to *Plasmodium spp*. antigens such as MSP-1 is crucial for designing effective interventions, particularly in forest

malaria zones with unique transmission characteristics.

Merozoite surface protein-1 (MSP-1) is a protein located on the surface of *Plasmodium spp*. merozoites, and remains a leading candidate for malaria vaccine development (5). This protein has a molecular weight of 180 kDa and undergoes a proteolytic process. MSP-1 is a protein made up of four different parts: an 83 kDa piece at the start (MSP-183), two internal pieces weighing 30 and 38 kDa (MSP-130 and MSP-138), and a 42 kDa piece at the end (MSP-142) (5, 6) Residents of endemic regions frequently have antibodies to MSP-1. In light of the immune reaction, scientists created vaccinations against MSP-1. The body's reaction to MSP-1 is connected to both preventing malaria symptoms and the seriousness of the illness, but this connection seems to change in different areas where malaria is common (6–8).

Despite extensive studies from Africa and parts of Asia, data from Indonesia's forest populations, where malaria transmission dynamics and Plasmodium species composition are highly heterogeneous, remain limited. This study characterises the anti-MSP-1 IgG antibody profile among adults and younger individuals residing in the forested areas of Tanah Bumbu, South Kalimantan, and examines its association with parasitaemia levels, body temperature, and preventive practices. The findings may serve as indicators of parasite transmission intensity and the presence of protective immunity within the region.

## MATERIALS AND METHODS

### Study area and study design

Some of the village in Tanah Bumbu Regency remains classified as forest malaria-endemic areas. Several location in this regions are still covered by primary rain forest, situated between coordinates 20° 57' 0" to 30° 38' 24" South Lattitude and 115° 24' 0" to 116° 49' 12" East Longitude (1, 9). An observational, cross-sectional study was conducted to analyze the IgG antibody response to *Plasmodium spp*. MSP-1 antigen. Data collection took place from September 2020 to July 2021. All study participants were residents of forest-based villages with an annual parasite index (API) exceeding 5 in 2018.

The required sample size was estimated following the approach proposed by Charan & Biswas (10) indicating a minimum of 107 participants. The number of subjects obtained was constrained by the small population size of the study village, which comprised roughly 385 inhabitants, 68.95% of whom were of working age. The sampling framework was derived from official household and resident registers, applying inclusion criteria that covered men and non-pregnant women aged 15–60 years who were permanent residents. Participants were selected through a simple random sampling technique from the established list, and written informed consent was obtained prior to participation. Reasons for refusal among selected candidates were carefully recorded. Individuals presenting with severe acute illness that could interfere with interviews or venepuncture were excluded. In total, 155 participants were recruited. Data collection was undertaken through household visits by the research team in collaboration with local health staff, involving structured interviews, venous blood collection, and brief environmental assessments. Blood was drawn from the median cubital vein into EDTA tubes, and serum separated from these samples was subsequently utilised for IgG antibody measurement.

### Variables

The variables of this study were levels of IgG antibody of anti-Plasmodium MSP-1, per cent parasitemia, body temperature, prevalence of forest malaria and all demographic and behavioural factors, including malaria history (having been diagnosed with malaria (and received treatment) by a local health worker in the past; verified from medical records where available).

### Malaria diagnosis

Malaria infection was confirmed through microscopic evaluation of both thick and thin blood smears, in accordance with WHO procedure (11) Blood smears were read by a certified parasitology laboratory analyst from the Faculty of Medicine, ULM. For quality control purposes, a second experienced microscopist randomly selected 5% of the slides for re-examination. Additionally, molecular confirmation was performed using real-time polymerase chain reaction (RT-PCR), as outlined in the methodology described by Kamau et al (12). Rapid diagnostic tests were not performed, diagnosis was only based on microscopic and PCR examinations.

### Parasitemia Measurement

To determine the percentage of parasitemia, a thin blood smear was examined by calculating the proportion of red blood cells infected with *Plasmodium spp*. using a denominator of 1.000 red blood cells as the counting baseline (11).

### The level of *Plasmodium spp* MSP-1 IgG antibodies

The serum IgG levels against whole-length *Plasmodium spp* MSP-1 were determine by competitive ELISA using a commercial kit (Human Merozoite Surface Protein 1 ELISA kit) from Mybiosource. All samples were tested in duplicate, and mean values were used for subsequent analyses. The choice of this assay was justified by the availability of the kit and its broad epitope coverage across different MSP-1 domains. A noted limitation is that the use of the full-length antigen does not allow distinction between specific domains (e.g., MSP-119 and MSP-142). The primary outcome variable was antibody concentration (pg/mL), treated as a continuous measure. Seropositivity was determined according to the manufacturer's calibrator-based criteria, while the main analysis relied on continuous data values. Work

procedures were in accordance with the factory manual (13).

### Data analysis

Statistical analysis was performed using the licensed SPSS software version 28.0 for windows. The Mann-Whitney U test was applied to compare mean antibody levels between two independent groups, while the Kruskal-Wallis test was employed to examine differences among three or more groups. Analysis correlation between IgG antibodies levels with parasitemia and body temperature was performed with Spearman rank. P value <0.05 is considered to be significantly different.

### Ethical Clearance

This research is affiliated with the study conducted by Istiana et al (1), and hence possesses the identical ethical clearance number as that study. Ethical clearance for this research was obtained from the Health Research Ethics Committee of the 'Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia, under approval number 603/KEPK- FK ULM/EC/V/2021'. Written ethical approval and informed consent were obtained from all participants. For individuals aged 15–17 years, consent was provided by a parent or legal guardian, accompanied by the participant's own assent. Participation in the study was entirely voluntary, and respondents retained the right to withdraw at any stage without any consequences.

## RESULTS

This study enrolled 155 individuals who reside in region with forest malaria. The majority of participants were employed in occupations related to forest activities. Microscopic examination identified malaria parasites in 19 participants (12.25%) while real-time PCR detected infection in 26 individuals (16.77%). Participant aged varied from 16 to 60 years and a mean age of 32 years. Body temperature measurements ranged between 36.1°C and 37.8°C with an average of 36.6°C. Further details are presented in Table I.

In this study, behavioural factors were also examined, including going out at night, routinely wearing long-sleeved clothing, sleeping under insecticide-treated mosquito nets, seeking treatment when unwell, adhering to prescribed antimalarial medicines, and cleaning water storage containers. The findings are presented in Table II.

### Concentration of MSP-1-specific IgG antibodies

The concentration of IgG antibodies specific to MSP-1 was determined using the ELISA method, with values ranging from a minimum of 562.22 pg/mL to a maximum of 3987.23 pg/mL. Distribution of antibody levels according to various risk factors is presented in Table III.

**Table I. Prevalence and risk factors of malaria infection**

| Variables | Total (N=155) |
|---|---|
| **Age (years) (median, range)** | 32y (16-60) |
| 15– 45 | 132 (85.16%) |
| >45 | 23 (14.84%) |
| **Gender** | |
| Male | 99 (63.87%) |
| Female | 56 (36.13%) |
| **Ethnicity** | |
| Banjar | 115 (74.19%) |
| Dayak | 40 (25.80%) |
| **Education** | |
| No school | 66 (42. 58%) |
| Elementary | 74 (47. 74%) |
| Middle school | 10 (6.45%) |
| High school | 5 (3. 23%) |
| **Occupation** | |
| Forest workers | 116 (74. 83%) |
| Non-Forest workers | 39 (25. 17%) |
| **Microscopic examination** | |
| Positive | 19 (12. 25%) |
| Negative | 136 (87. 75%) |
| **Real Time PCR examination** | |
| Positive | 26 (16. 77%) |
| Negative | 129 (83. 23%) |
| **Temperature (°C)** | |
| (mean, range) | 36.66 (36.1 –37.8) |
| **Parasitemia (%)** | |
| (mean, range) | 0.877 (0.505 – 2. 039) |
| **History of malaria** | |
| Yes | 104 (66.7) |
| Not | 51 (33.3) |
| **Duration of previous malaria illness (month)** | |
| (mean, range) | 10.58 (3-50) |

**Table II. Behavioral factors of respondent in forest malaria endemic areas**

| Variables | Total (N=155) |
|---|---|
| **Night out** | |
| Always | 26 (16.77%) |
| Sometimes | 84 (54.19%) |
| Never | 45 (29.04%) |
| **Wearing long sleeves at night** | |
| Always | 11 (7.09%) |
| Sometimes | 43 (27.74%) |
| Never | 111(71.61%) |
| **The use of insecticidal nets** | |
| Always | 95 (61.29%) |
| Sometimes | 32 (20.64%) |
| Never | 28 (18.06%) |
| **Seek treatment** | |
| Always | 61 (39.35%) |
| Sometimes | 86 (55.48%) |
| Never | 8 (5.17%) |
| **Taking malaria medications** | |
| Always | 25 (16.13%) |
| Sometimes | 77 (49.67%) |
| Never | 53 (34.19%) |
| **Cleaning water reservoir** | |
| Always | 58 (37.41%) |
| Sometimes | 37 (23.87%) |
| Never | 60 (38.72%) |

### Association between MSP-1 specific IgG levels with Parasitemia

The association between IgG antibody concentrations specific to MSP-1 and parasitemia can be seen in

**Table III. Average levels of MSP-1 specific IgG antibodies**

| Group | Average | SD | P value |
|---|---|---|---|
| **RT PCR examination** | | | 0.056 |
| Positive(n=26) | 2402.45 | 333.52 | |
| Negative (n = 129) | 1754.28 | 123.70 | |
| **Microscopic** | | | 0. 068 |
| Positive (n=19) | 2465.03 | 257.10 | |
| Negative (n=136) | 1698.71 | 125.46 | |
| **Gender** | | | 0. 022 |
| Male (n=99) | 2220.63 | 149.95 | |
| Female (n=56) | 562.23 | 72.46 | |
| **Ethnicity** | | | 0. 051 |
| Banjar (n=115) | 2320.33 | 123.13 | |
| Dayak (n=40) | 1962.22 | 162.14 | |
| **Occupation** | | | 0. 728 |
| Forest worker (n=116) | 1996.09 | 134.17 | |
| Non forest worker (n=39) | 1782.41 | 154.37 | |
| **Education** | | | 0. 821 |
| No school | 1761.43 | 162.19 | |
| Elementary | 1818.36 | 164.32 | |
| Middle school | 1676,45 | 105.67 | |
| High school | 1288,23 | 124,56 | |
| **History of malaria** | | | 0. 001 |
| Yes (n=104) | 2862.45 | 134.72 | |
| No (n=51) | 754.28 | 132.09 | |
| **Age group** | | | 0. 491 |
| 15-45y(n=132) | 1767.46 | 131.37 | |
| >45y (n=23) | 1893.40 | 249.25 | |
| **Night out** | | | 0. 110 |
| Never (n=45) | 1324.13 | 225.68 | |
| Sometimes (n=84) | 2382.32 | 243.34 | |
| Always (n=26) | 1244.61 | 322.28 | |
| **Wearing Long sleeves** | | | 0. 001 |
| Never (n=111) | 2657.80 | 247.82 | |
| Sometimes (n=43) | 1726.37 | 243.73 | |
| Always (n=11) | 924.30 | 134.61 | |
| **Using insecticidal nets** | | | 0. 023 |
| Never (n=28) | 2987.23 | 301.67 | |
| Sometimes (n=35) | 1862.22 | 269.11 | |
| Always (n=95) | 1612.63 | 139.09 | |
| **Seek Treatment** | | | 0. 203 |
| Never (n=8) | 2465.18 | 568.29 | |
| Sometimes (n=86) | 1596.42 | 147.00 | |
| Always (n=61) | 1981.10 | 192.90 | |
| **Taking malaria medication** | | | 0. 015 |
| Never (n=53) | 2117.54 | 206.43 | |
| Sometimes (n=77) | 1786.63 | 159.32 | |
| Always (n=25) | 732.33 | 93.51 | |
| **Cleaning water reservoirs** | | | 0. 239 |
| Never (n=60) | 1964.68 | 180.31 | |
| Sometimes (n=37) | 1988.84 | 254.54 | |
| Always (n=58) | 1489.52 | 172.49 | |

Figure 1. Figure 1 illustrates that elevated antibody levels correlate with a lower percentage of parasitemia. Statistical analysis using the Spearman rank correlation test produced a p-value of 0.030, indicating a significant correlation between MSP-1 specific IgG levels and the degree of parasitemia.

### Association between MSP-1 specific IgG levels and body temperature

Figure 2. illustrates the association between IgG antibody levels targetting MSP-1and the body temperature of individuals residing in forest malaria-endemic zones of

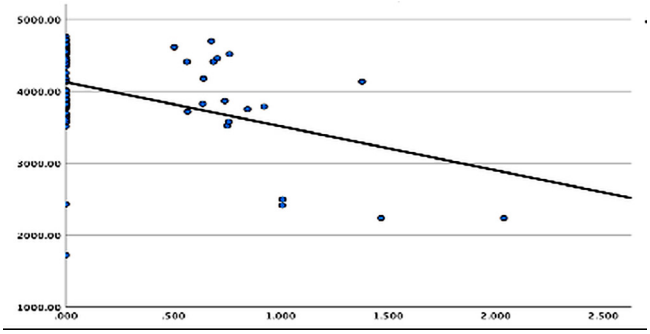


**Figure 1: The relationship of MSP-1 specific IgG antibody levels and parasitaemia**

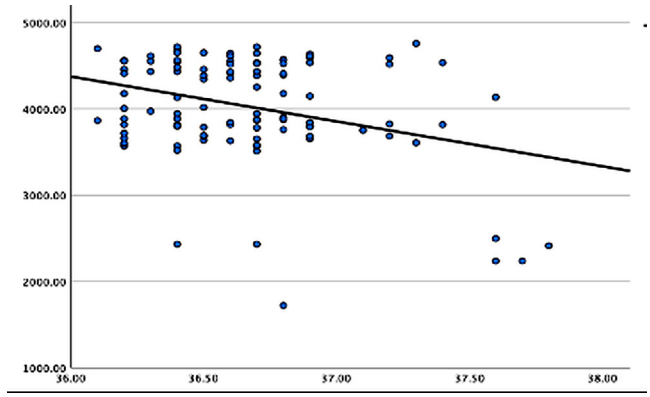


**Figure 2: The relationship of MSP-1 specific IgG antibody levels with body temperature**

Tanah Bumbu Regency. Figure 2 shows that a rise in anti-MSP-1 antibody levels is associated with a reduction in body temperature. Statistical evaluation revealed a p-value of 0.001, confirming a significant correlation between anti-MSP-1 IgG levels and body temperature in the study population.

## DISCUSSION

MSP-1 is recognized as a highly immunogenic antigen located on the surface of *Plasmodium spp.* merozoites. Individuals living in malaria-endemic areas frequently develop antibodies again this antigen (7, 8, 14, 15). In region with intense malaria transmission, immune response to MSP-1 has been linked to both protective immunity and markers of disease severity (16, 17). Even in areas with unstable or low transmission intensity, individuals infected with malaria tend to produce antibodies against merozoite-stage antigens. Nevertheless, the role of MSP-1 specific antibodies in providing protection remains controversial and appears to differ across various endemic regions (8, 15, 18, 19).

In the current study, a large proportion of participants were found to have IgG antibodies specific to MSP-1, suggesting the presence of naturally acquired immunity likely stemming from prior exposure to the parasite. Repeated exposure to malaria is known to activate memory B cells (MBCs), which in turn can elicit a strong

humoral immune response. Interestingly, the detection of MSP-1 specific IgG in malaria-negative individuals at the time of sampling support the idea that MBCs specific to *Plasmodium spp*. antigens may persist over a long time, even in without further infection or in location where infection has substantially declined (17, 19). These findings are important for assessing community-level malaria risk in low transmission regions and may help guide future vaccine development strategies targetting population in elimination pr pre-elimination phases.

The high prevalence of anti-MSP-1 IgG in this study is consistent with cumulative exposure to vectors and parasites in areas of sustained transmission, which typically drives the maturation of merozoite-specific antibody responses over time. This finding aligns with reports from Asia (e.g., the China–Myanmar border, Thailand and the Greater Mekong region, including sero-surveys in Indonesia) and Africa (Kenya, both highland and lakeside areas, Mozambique, Uganda and Ghana), which likewise document high seroprevalence/titres of IgG to MSP-1 antigens (including the C-terminal MSP-119 domain) and their association with transmission intensity and exposure history (18-23). Biologically, this pattern is consistent with anti-MSP-1 antibodies acting as markers of exposure as well as contributors to functional immunity against the merozoite stage.

Evidence from multiple settings shows that IgG responses to MSP-1 increase with transmission intensity. Comparative surveys in East Africa and the Greater Lakes region, for example, report higher seroprevalence and higher median optical-density or arbitrary-unit readouts to MSP-119 in high-transmission communities than in neighbouring areas with moderate or low transmission, including micro-gradients such as valley bottoms versus uphill villages. Multi-site studies likewise demonstrate stepwise rises in antibody levels across sites classified as low, moderate and high transmission, and longitudinal work shows that when transmission declines, both the magnitude and avidity profiles of anti-MSP-1 antibodies fall accordingly. Notably, most studies report titres as OD or assay units rather than absolute concentrations (pg/mL), but the direction of effect is consistent: higher endemicity is associated with higher anti-MSP-1 IgG levels across age groups (24-25).

The presence of IgG antibodies in healthy individuals living in areas with high transmission can be attributed to the persistence of long-lasting antibodies. These antibodies are produced by long-lived plasma cells that originate from germinal centers (27–30). The presence of IgG antibodies in the negative malaria group suggests a significant prior exposure to *Plasmodium spp*. Prior exposure to the malaria parasite led to the development of antibodies against MSP-1 during the initial phase of infection, and these antibodies were present even after the individual recovered (31). This phenomenon is observable in patients who have undergone recovery for a period beyond 12 months, as their antibody levels remain elevated. These findings suggest that residents in forest malaria-endemic regions are capable of mounting and maintaining a long-lasting humoral immune response (32, 33). Ayieko et al, found that antibodies and memory B cells can remain present for over 12 months even without any detectable infection in adults (34). Nevertheless, the efficacy of these antibodies in providing protection still requires additional validation through cohort studies and investigation using alternative approaches.

In this study, males exhibited substantially higher anti-MSP-1 IgG concentrations than females. This pattern makes biological sense in forest-exposed communities, where men are more likely to engage in night time or outdoor work that increases vector contact, and may differ in the consistency of personal protective measures. This association remains credible after accounting for sex-specific immunobiology, but needs further investigation with adjustments for age, occupation, nocturnal behavior, bed net use, length of stay, and recent malaria history.

The variation in MSP-1-specific IgG antibody levels observed among respondents may partially attributed to behavioural factors that affect the risk of malaria transmission. Individuals who consistently used insecticide-treated mosquito nets, wore long-sleeved clothing when outdoors at night, and routinely took antimalarial medication demonstrated higher levels of MSP-1 specific IgG antibodies. These protective behaviours may contribute to repeated low-level exposure to parasite, which could stimulate and maintain humoral immune responses without resulting in overt clinical illness (1, 33). Similar findings have been reported in previous studies, where behavioural interventions not only reduces clinical malaria incidence but also influenced the development of naturally acquired immunity (35, 36) This suggest that behavioural factors should be considered in the interpretation of immunological data, especially in areas with ongoing but reduced transmission.

An higher IgG response against MSP-1 appears to be associated with reduced parasite densities and a decreased probability of developing clinical malaria, indicating that these antibodies contribute to anti parasitic immunity. The decrease in parasite density is likely attributable to the capacity of MSP-1-specific IgG antibodies to directly suppress the proliferation of parasites during the blood stage of infection (6). Antibodies to MSP-1 are known to induce several effector mechanisms to control parasitaemia. These include direct inhibition of erythrocyte invasion by merozoites and/or intra-erythrocytic development, activation of classical complements pathway, monocyte-mediated opsonization, and neutrophil-mediated ROS

release (6, 37). According to in vitro evidence, ROS are implicated in parasite destruction and phagocytosis, and has been associated with parasite clearance and protective immunity in various immunological studies. Opsonization facilitated by MSP-1-specific antibodies is essential for regulating parasitaemia during the blood stage (6). Beyond neutrophil activation, these antibodies can also stimulate natural killer (NK) cells and monocytes, promoting parasite clearance through phagocytosis or via the release of TNF and other soluble immune mediators (13, 38). Additionally, in vitro investigations have shown that antibodies specific to MSP-1 might the growth of parasites, although such effects are often modest and variable in consistency (6, 39).

Individuals living in endemic areas developed natural immunity as a protection against both infection and severe clinical symptoms. Some study have found that IgG antibodies against MSP-1 can protect against the development of clinical symptoms and the severity of the disease (anti-disease immunity) and can also supress the number of parasites in the body (anti-parasite immunity) (40–42).

This study showed a significant relation between an increase in anti-MSP-1IgG antibodies and a decrease in body temperature. Clinically, protection against malaria is characterized by the absence of fever in malaria infections, which antibodies can mediate through various ways, including direct inhibition and opsonization (6). Fowkes et al reported 19% reduction in malaria risk in individuals who had the antibody MSP-119 (43,44).

This study has several limitations. First, its cross-sectional design and relatively small sample size limit causal inference and statistical power. Second, we did not quantify MSP-1-specific IgG subclasses (e.g., IgG1/IgG3) or perform functional assays, both of which are important for assessing protective activity given the distinct effector functions of each subclass. Third, we also lacked a direct measure of mosquito bite intensity, an important proxy for transmission intensity and relied on a whole-length MSP-1 antigen, which constrains domain-specific interpretation (e.g., MSP-119 vs MSP-142). In addition, multiple comparisons may increase the risk of type I error. These factors should be considered when interpreting the findings and addressed in future longitudinal studies with larger samples, domain-specific antigens, subclass profiling, functional readouts, and entomological exposure measurements.

## CONCLUSION

This study revealed interesting findings, particularly the high prevalence of MSP-1-specific IgG antibodies among the majority of participants. This suggests a well-developed immune system that may protect against infection or the development of severe clinical symptoms, as demonstrated by the association with body temperature and parasitemia. Previously exposed individuals had higher antibody levels, suggesting that these antibodies may persist for a longer period. Furthermore, the observed increase in antibody levels in the population may also be a useful indicator of the intensity of malaria transmission in the study area, as well as the maturity of the local population's immunological adaptation to malaria. Future longitudinal studies incorporating IgG subclass profiling, functional assays, and entomological measures are needed to clarify the protective significance and epidemiological utility of these antibodies.

The local health office needs to implement various kinds of strategies to reduce transmission and evaluate the effectiveness of insecticide nets. The local health office can increase the effort by routinely testing residents in the region and enhancing public awareness of malaria through regular education. The number of asymptomatic malaria cases allows them to spread the disease to others. Therefore, it is crucial to regularly conduct mass blood surveys to identify individuals who are infected but not symptomatic, yet have the potential to transmit the disease. Treating them enables to break the chain of transmission.

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