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Predictive markers of transmission in areas with different malaria endemicity in north-eastern Tanzania based on seroprevalence of antibodies against *Plasmodium falciparum*

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Abstract

Objective: A community-based cross-sectional study was done to assess *Plasmodium falciparum* exposure in areas with different malaria endemicity in north-eastern Tanzania using serological markers; *PfAMA-1* and *PfMSP-1*$_{19}$.

Results: Bondo had a higher seroprevalence 36.6% (188) for *PfAMA-1* as compared to Hai 13.8% (33), $\chi^2 = 34.66$, $p < 0.01$. Likewise, Bondo had a higher seroprevalence 201(36.6%) for *PfMSP-1* as compared to Hai 41 (17.2%), $\chi^2 = 29.62$, $p < 0.01$. Anti-*PfAMA-1* titters were higher in malaria positive individuals (n = 47) than in malaria negative individuals (n = 741) (p = 0.07). Anti-*PfMSP*-1 antibody concentrations were significantly higher in malaria-positive individuals (n = 47) than in malaria-negative individuals (n = 741) (p = 0.003). Antibody response against *PfAMA-1* was significantly different between the three age groups; < 5 years, 5 to 15 years and > 15 years in both sites of Bondo and Hai. Likewise, antibody response against *PfMSP-1*$_{19}$ was significantly different between the three age groups in the two sites ($p < 0.001$). We also found significant differences in the anti-*PfAMA-1*and anti-*PfMSP-1*$_{19}$ antibody concentrations among the three age groups in the two sites (p = 0.004 and 0.005) respectively. Immunological indicators of *P. falciparum* exposure have proven to be useful in explaining long-term changes in the transmission dynamics, especially in low transmission settings.

Keywords: Malaria, *Plasmodium falciparum*, Seroprevalence, Transmission, Tanzania

Introduction

Africa carries the highest burden of malaria with more than 70% of all malaria cases and deaths [1]. Each year, 10 to 12 million people contract malaria and more than 80,000 dies [2, 3]. *Plasmodium falciparum* is mainly responsible for 99.7% of estimated malaria cases [4].

In many countries, local malaria transmission has decreased due to the extensive efforts being devoted to malaria control and elimination [5]. *P. falciparum* accounts for 96 percent of cases [6], malaria prevalence varies from < 1 percent in the highlands of Arusha to as high as 15 percent in the Southern Zone and 24 percent along the Lake and Western Zones. Immunity to *P. falciparum* malaria is poorly understood, however, evidence shows that antibody-dependent cellular mechanisms play a key role in immunity against *P. falciparum* malaria

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parasite [7, 8]. The rate of its development is believed to be associated with transmission intensity which is stage-specific and is rarely sterile [6]. In many epidemiological studies, the determination of malaria transmission has been based on the antibody levels against *P. falciparum* antigens [9]. Recent immunological studies revealed that antibodies against merozoite antigens act as biomarkers of malaria exposure and that, with increasing exposure and responses of higher levels, antibodies may act as biomarkers of protective immunity [10].

Apical membrane antigen 1 (AMA-1) is expressed on merozoites and sporozoites of *P. falciparum* as a type I integral membrane protein [11] while Merozoite surface protein1 (MSP-1), is a highly conserved protein among *Plasmodium* species as well as the most abundant protein expressed on the surface of merozoites [12]. Antibodies against MSP-1 and AMA-1 antigens are potential markers of both exposure to *P. falciparum* and protection against the disease [7, 13] and have proven to be informative, in areas where transmission has dropped to low sustained levels, for monitoring the timing and magnitude of transmission reduction [13] as well as in obtaining epidemiological information in malaria control programmes [14].

In areas with low malaria transmission, it has become extremely difficult to detect changes in transmission intensity using conventional methods such as the entomologic inoculation rate (EIR) or malaria prevalence rates. Low transmission areas (low endemicity) sometimes have low mosquito density, below the detection limits of common mosquito trapping methods [15, 16] and the parasite prevalence also becomes less reliable [17–19]. Malaria serological markers may aid in estimating malaria transmission intensity [20–22]. Seroconversion rates may provide insight into recent changes in malaria transmission [23]. Due to the fact antibodies can persist for months or years after infection, seroconversion rates are less affected by the effects of unstable or seasonal transmission [20, 21]. We investigated the antibody response to recombinant AMA-1 and MSP-1 in individuals living in two regionally distinct malaria-endemic zones.

Main text

Materials and methods

Study area

The study was conducted during April and December 2014 in two different areas of the Tanzanian mainland. The first site was Bondo in the Tanga region, inhabited by 7970 people [24]. The second study site was Hai in Kilimanjaro region [14]. Participant recruitment procedures and study design have been previously described [25], (Additional file 1: Fig. S1).

Participant enrolment and sample collection

Participants 2 years of age and above who reside in the study areas were enrolled in the study. A blood sample was obtained by finger prick, a portion of blood was used for malaria rapid test, which was performed on-site. A blood spot was prepared for each participant, then dried and stored for further analysis. A 3.0 mm diameter circle of dried blood spot (equivalent to 2 µl whole blood/1 µl serum) was reconstituted in 200 µl of sodium azide-phosphate buffered saline-tween (0.05%) (PBST/0.1% Azide).

Enzyme-linked immuno-sorbent assay (ELISA)

Indirect immunosorbent Assay (ELISA)was performed using two *P. falciparum* surface antigens, *P. falciparum MSP* 1_{19} (Pf*MSP* 1_{19}) and *P. falciparum AMA-1*(*PfAMA-1*) [21].

Malaria parasite detection by polymerase chain reaction (PCR)

Parasite DNA was extracted using the simple Chelex–Saponin method, *Plasmodium* nucleic acid amplification was conducted using genus-specific reverse and forward primers (rPLU6-5′TTAAAATTGTTGCAG TTAAAACG3′ and rPLU5-5′CCTGTTGTTGCCTTA AACTCC3′) targeting small sub-unit ribosomal RNA (ssurRNA) of the parasite. A reaction mix of 20 µl per sample was used, 5 µl of template DNA extracted from participants whole blood plus 15 µl of nuclease-free water, dNTPs, Taq enzyme, buffers and salts. Amplification conditions were, 95 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 58 °C for 2 min and 72 °C for 5 min then one final extension cycle at 72 °C for 10 min. Amplification products were run in Ethidium bromide agarose gel (2%) electrophoresis at 120 V, 50 watts and 120 mA. The amplified bands were visualized under ultra-violet light trans-illuminator [26, 27].

Data analysis

All data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism8 software (San Diego, CA). After verifying that Optical density (OD) values were not normally distributed ($p < 0.0001$; Anderson–Darling test), non-parametric tests were performed to compare the OD. The Mann–Whitney test was used for the comparison of Antibody levels of two independent groups. The non-parametric Kruskal–Wallis test was used for the comparison of more than two groups. Pearson's Chi-squared (χ^2) test was used to compare two proportions.

Results

Population characteristics and malaria prevalence

The study enrolled a total of 788 participants, 239 (30.3%) from Hai and 549 (69.7%) from Bondo. Males were 283 (35.9%) and females were 505 (64.1%). About 405 (51.4%) participants had more than 15 years of age, 212 (26.9%) were between 5 and 15 years and 171 (21.7%) were below 5 years. The malaria prevalence by mRDT was 8.6% (47) in Bondo and 0% in Hai (Fisher exact test *$p < 0.001$). By PCR, malaria prevalence was 20.4% (161), with Bondo having a higher prevalence 28.1% (n = 154) than Hai 2.9%, (n = 7), $\chi^2 = 64.64$, $p < 0.01$ (Additional file 2: Table S1).

Seroprevalence of anti-PfAMA-1 and PfMSP-119 antibodies

Bondo had a higher seroprevalence 36.6% (188) for *PfAMA-1* as compared to Hai 13.8% (33), $\chi^2 = 34.66$, $p < 0.01$. Likewise, Bondo had a higher seroprevalence 201(36.6%) for *PfMSP-1* as compared to Hai 41 (17.2%) ($\chi^2 = 29.62$, $p < 0.01$). In Bondo, participants with more than 15 years had a significantly higher seroprevalence of *PfAMA-1* 61.7% (116) ($\chi^2 = 58.69$, $p < 0.001$) and *PfMSP-1*$_{19}$ 63.7 (128) ($\chi^2 = 65.36$, $p < 0.001$) as compared to other age groups. Likewise, participants with 5–15 years and < 5 years had a higher prevalence of malaria as measured by mDRT ($\chi^2 = 30.76$, $p < 0.001$) (Table 1).

Anti-PfAMA-1 and PfMSP-119 antibody concentrations

Anti-*PfAMA-1* titters were higher in malaria positive individuals (n = 47) than in malaria negative individuals (n = 741) (Mann–Whitney U test, p = 0.07) (Additional file 3: Fig. S2A). Anti-*PfMSP*-1 antibody concentrations were significantly higher in malaria-positive individuals (n = 47) than in malaria-negative individuals (n = 741) (Mann–Whitney U test, p = 0.003) (Additional file 3: Fig. S2B).

We determined whether the two sites differed in antibody concentration and found that anti-*PfAMA-1* antibody concentrations, were higher among participants in

Table 1 Age-specific prevalence of malaria by serology, mRDT, microscopy and PCR

Study site	Age group	PfAMA-1 % (n)		PfMSP-1$_{19}$ % (n)		mRDT % (n)		PCR % (n)	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Bondo	< 5 years	9.0 (17)	32.7 (118)	14.4 (29)	30.5 (106)	46.8 (22)	22.5 (113)	18.8 (29)	26.8 (106)
	5–15 years	29.3 (55)	36.6 (132)	21.9 (44)	41.1 (143)	48.9 (23)	32.7 (164)	36.4 (56)	33.2 (131)
	> 15 years	61.7 (116)	30.7 (111)	63.7 (128)	28.4 (99)	4.3 (2)	44.8 (225)	44.8 (69)	40.0 (158)
		$\chi^2 = 58.69$, $p < 0.001$		$\chi^2 = 65.36$, $p < 0.001$		$\chi^2 = 30.76$, $p < 0.001$		$\chi^2 = 3.8$, $p = 0.1$	
Hai	< 5 years	18.2 (6)	14.6 (30)	9.8 (4)	16.2 (32)	0.0 (0)	15.1 (36)	14.3 (1)	15.1 (35)
	5–15 years	6.1 (2)	11.2 (23)	4.9 (2)	11.6 (23)	0.0 (0)	10.5 (25)	28.6 (2)	9.9 (23)
	> 15 years	75.8 (25)	74.3 (153)	85.4 (35)	72.2 (143)	0.0 (0)	74.5 (178)	57.1 (4)	75.0 (174)
		*p = 0.6		*p = 0.2		–		*p = 0.2	

*Computed by Fisher exact test

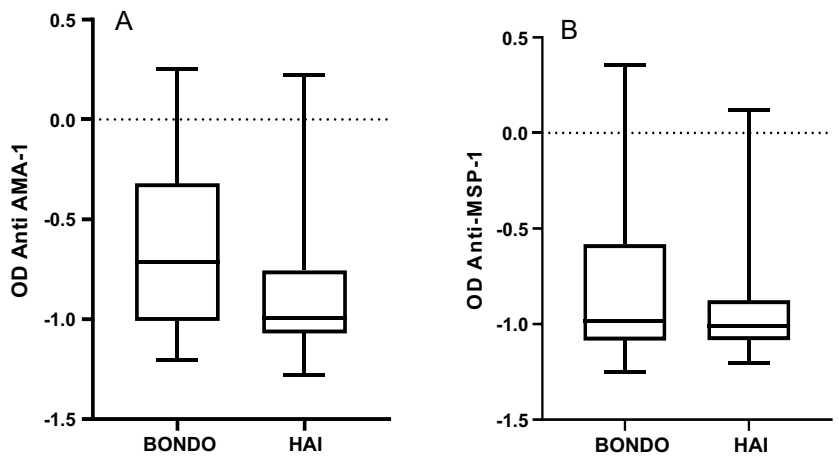

Fig. 1 A graph showing mean OD values for *PfAMA-1* (**A**) and *PfMSP-1*$_{19}$ (**B**) at Bondo and Hai sites. Presented in the Y-axis is the Log_{10} transformed OD values in two sites (X-axis)

Bondo (n = 549) as compared in Hai (n = 239), (Mann–Whitney U test, p < 0.001) (Fig. 1A). Anti-*PfMSP-1* antibody concentrations were higher among participants in Bondo (n = 549) than those of Hai (n = 239), (Mann–Whitney U test, p = 0.01) (Fig. 1B).

In assessing whether these differences were influenced by age, we calculated the differences among < 5 years, 5 to 15 years and > 15 years per site. Antibody response against *PfAMA-1* was significantly different between the three age groups in both sites. (Kruskal–Wallis test, p < 0.001) (Table 1). Likewise, antibody response against *PfMSP-1*$_{19}$ was significantly different between the three-age groups in the two sites (Kruskal–Wallis test, p < 0.001) (Table 1). We also found significant differences in the anti-*PfAMA-1*antibody concentrations among the groups (Kruskal–Wallis test, p = 0.004), as indicated in Fig. 2A, B. Lastly, we also noted significant differences in the anti-*PfMSP-1*$_{19}$ antibody concentrations among the age groups (Kruskal–Wallis test, p = 0.005) (Fig. 2C, D).

Discussion

The purpose of this study was to use immunological markers to investigate malaria transmission patterns in areas with diverse malaria endemicities.

In this study, malaria prevalence by PCR in Bondo was 28.1%. Since Bondo is a malaria-endemic area, malaria transmission occurs nearly all year long with a peak period from April to June. No significant difference was observed in malaria prevalence among age groups in the present study. This is contrary to the study conducted

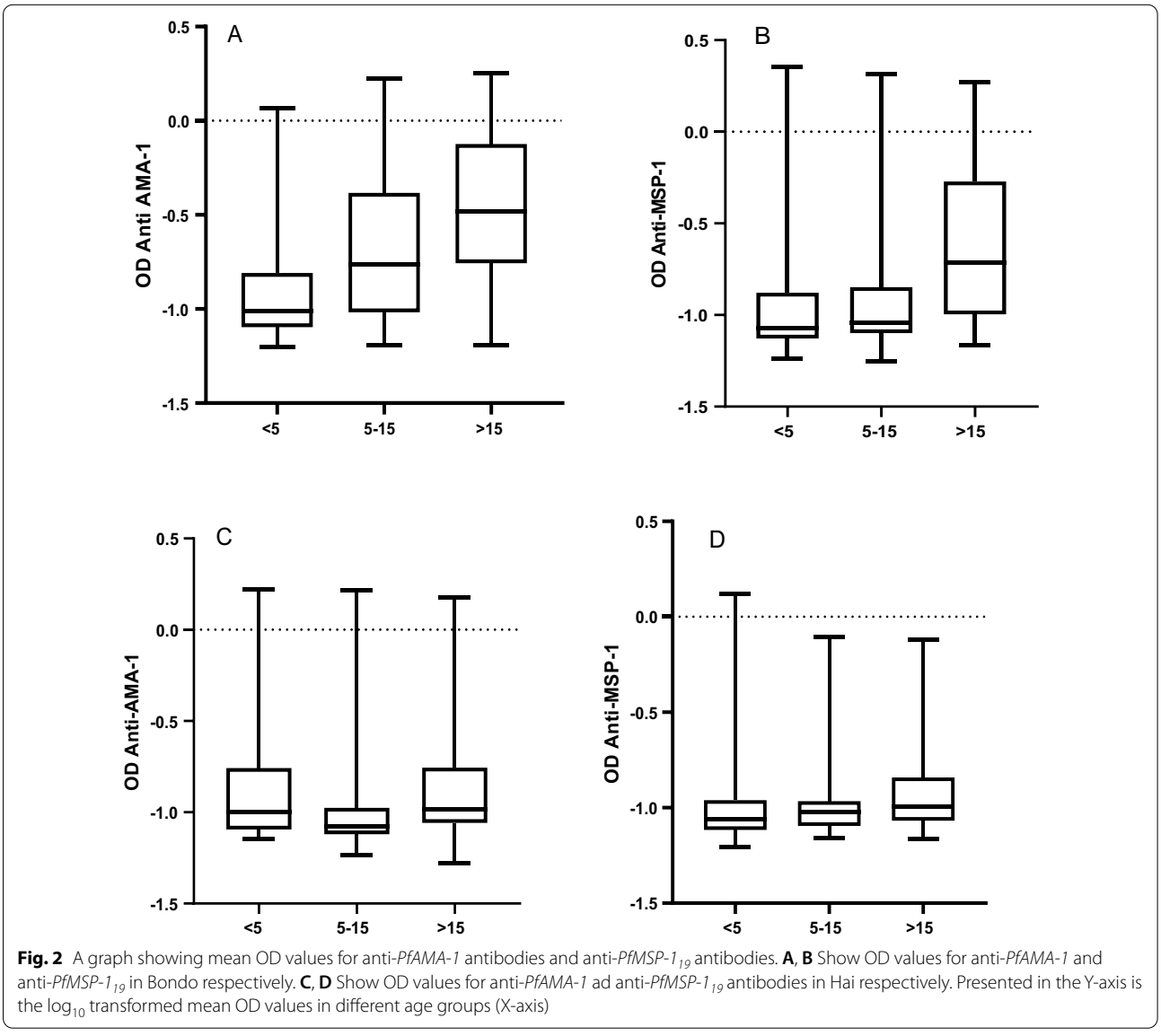

Fig. 2 A graph showing mean OD values for anti-*PfAMA-1* antibodies and anti-*PfMSP-1*$_{19}$ antibodies. **A**, **B** Show OD values for anti-*PfAMA-1* and anti-*PfMSP-1*$_{19}$ in Bondo respectively. **C**, **D** Show OD values for anti-*PfAMA-1* ad anti-*PfMSP-1*$_{19}$ antibodies in Hai respectively. Presented in the Y-axis is the $\log_{10}$ transformed mean OD values in different age groups (X-axis)

in 2011 which suggested a widening of the age group at risk for malaria infection to older children of 5–15 years [28]. A previous study conducted in two villages in the same region about 70 km from the current study found a re-emergence of malaria despite previous reports of a decline in malaria [29]. It is estimated that parasite prevalence at that time was 25% and it stayed there throughout 2016 [30]. Hai site had a very low malaria prevalence, thus remaining an area of low transmission and The mRDT tests did not detect any active infections, which suggests low-density parasite circulating in the population, similar to earlier findings [31]. There is, however, some evidence that individuals harbouring submicroscopic parasites could be sources of new infections since mosquitoes can carry parasites with very low density (< 5 parasites/µl) [26, 32, 33], and hence, the use of a more sensitive diagnostic tool like PCR in clinical malaria diagnosis is necessary. Consequently, scientific evidence from these findings is consistent with the notion of mass drug therapy for individuals with microscopic parasites considering efforts to eliminate malaria.

In our study, Interestingly, when the age-dependent analysis was done, older children (5–15 years) had a relatively low seroprevalence to *PfAMA-1* antigens as compared to younger children and Adults. Antibodies to malaria antigens can explain long-term changes in malaria transmission dynamics [21]. In 2009 a survey conducted in Moshi district, an area bordering Hai found a low seroprevalence in younger children suggesting very low exposure to malaria parasites [34]. In populations with low immunity, such as young children, antibodies to MSP-1 act as a significant biomarker of malaria exposure and with increasing exposure the antibodies may contribute to protective immunity [10].

Seroprevalence in moderate malaria transmission setting such as Bondo can play a small role in determining malaria transmission patterns although seroprevalence is almost two folds higher than Hai. A slight decline in seroprevalence was observed in the study area when compared with previous studies [21, 31], indicating a long-term reduction in malaria parasite exposure**,** which may be attributed to intense malaria interventions in Tanzania [35, 36].

Study results showed that the overall concentration of *PfMSP-1*$_{19}$ was significantly higher in participants with positive malaria tests than in non-positive participants. As expected Bondo had higher antibody concentrations against both antigens as compared to Hai. Children with < 5 years present with low antibody titters suggesting a lack of recent malaria exposure and this makes the group vulnerable to the symptomatic manifestation of the disease. Earlier findings revealed that more than half of the participants reported being symptomatic and were malaria positive by mRDT [21]. There is evidence of malaria transmission in low malaria-endemic areas, where traditional malaria indicators like prevalence and sporozoite levels may underestimate the burden of the disease.

Conclusion

In this study, immunological markers were found to be a useful indicator of ongoing malaria transmission, especially in low-endemic areas. Routine malaria surveillance can be made more effective by using these immunological markers to highlight the importance of customized and targeted control interventions.

Study limitation

This study might not explain the recent changes in malaria transmission since it was a cross-sectional survey. A longitudinal study would have been appropriate in explaining seasonal variations in malaria infection rates across the study areas.

Abbreviations

CRERC: College Research and Ethics Review Committee; OD: Optical density; AMA-1: Apical membrane antigen1; PCR: Polymerase chain reaction; ELISA: Enzyme-Linked Immuno-Sorbent Assay; ssurRNA: Small sub-unit ribosomal RNA; *PfMSP 1*$_{19}$: *Plasmodium falciparum Merozoite Surface Protein 1*; *PfAMA-1*: *Plasmodium falciparum Apical Membrane Antigen 1*.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-021-05818-y.

Additional file 1: Figure S1. Map of Tanzania showing the study sites, the map was produced using ArcGIS version 10.3 software.

Additional file 2: Table S1. Prevalence of Malaria by serology, mRDT, Microscopy and PCR.

Additional file 3: Figure S2. A graph showing mean OD values for *PfAMA-1* (A) and *PfMSP-1*$_{19}$ (B) among malaria positive and negative individuals. Presented in the Y-axis is the Log_{10} transformed OD values among malaria positives and negatives (X-axis).

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Patients and public involvement

Participants were not involved in the design of this study. Community leaders were involved during participant's recruitment. There is a plan to disseminate results to the participating sites.

Authors' contributions

RDK: conceptualization of the study, data analysis, and writing the original draft of the manuscript; DCK: funding acquisition, investigation, data analysis and review of the manuscript; JJM, AJN, FWM and JOC: Interpretation of data and critical review of the manuscript; RAK: overall study design and review of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Kilimanjaro Christian Medical University College Research and Ethics Review Committee (CRERC) with certificate number 658. Permission to conduct the study was sought from Handeni/Bondo and Hai district authorities. Written informed consent was obtained from all participants and from parents or guardians for children under 18 years of age who agreed to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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