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Quantities of *total serum IgG* antibodies against Circumsporozoite Protein among Postpartum Mothers attending Kawempe National Referral Hospital. A cross-sectional study.

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ABSTRACT

Background:

The study aimed to determine the quantities of *total serum IgG* antibodies against CSP among Postpartum Mothers attending Kawempe National Referral Hospital.

Methodology:

A cross-sectional nested study was conducted using 194 archived serum samples randomly selected from 400 postpartum women who participated in a parent study at Kawempe National Referral Hospital. Samples stored at −80°C were analyzed at Makerere University College of Health Sciences Medical Biochemistry Laboratory. Anti-CSP IgG levels were quantified using ELISA, while IgG affinity was assessed using an ammonium thiocyanate avidity assay. Data were analyzed in Stata 14.2 using descriptive and inferential statistics, with statistical significance set at $p \leq 0.05$.

Results:

Among 194 postpartum mothers, 100.00% attended ANC; married/cohabiting mothers comprised 90.72%, while 9.28% were single/separated. With 51.03% having <4 visits and 48.97% having ≥4 visits. Fansidar was received by 64.25%, while 35.75% did not receive it. HIV status was negative in 97.94%, positive in 0.52%, and unknown in 1.55%. Mosquito-net ownership was reported by 96.37%, while 3.63% did not own a net. Overall median serum IgG antibody titre was 0.09875 OD (IQR: 0.044–0.370). Median titres were 0.0840 OD (IQR: 0.0415–0.3875) among mothers with <4 ANC visits and 0.1050 OD (IQR: 0.04735–0.3545) among those with ≥4 visits (p = 0.9297). Fansidar recipients had a median titre of 0.1160 OD (IQR: 0.049175–0.3725) versus 0.0670 OD (IQR: 0.0345–0.370) among non-recipients (p = 0.2191). Net owners had 0.09875 OD (IQR: 0.04465–0.370) versus 0.1325 OD (IQR: 0.026–0.765) among non-owners (p = 0.7643).

Conclusion:

IgG antibody levels were modest, reflecting low recent malaria exposure likely due to effective malaria control interventions.

Recommendation:

The Ministry of Health should strengthen malaria surveillance and research to monitor serum IgG levels against CSP among postpartum mothers.

Keywords: *Malaria, Plasmodium falciparum, Circumsporozoite protein, IgG antibodies, Postpartum mothers, Antibody titres.*
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BACKGROUND OF THE STUDY

The quantity of IgG antibodies against *Plasmodium falciparum* circumsporozoite protein (CSP) is an important measure of humoral immune response against the pre-erythrocytic stage of malaria. CSP is the major surface protein of malaria sporozoites and plays an essential role in sporozoite movement, hepatocyte recognition, and invasion. Antibodies targeting CSP can interfere with these processes and potentially prevent the parasite from establishing infection in the liver (Kayatani et al., 2022; Zavala, 2022).

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Consequently, measuring the amount of anti-CSP IgG provides an indication of the magnitude of malaria-specific humoral immunity.

Pregnancy is associated with substantial immunological adaptations that may influence the development and persistence of malaria-specific antibodies. Repeated exposure to *P. falciparum* in endemic settings can result in the development of naturally acquired immunity, characterized partly by the production of antibodies against several parasite antigens (Opi et al., 2021). IgG is particularly important in malaria immunity, as evidence from passive-transfer studies has demonstrated that malaria-specific IgG can contribute to the clearance of parasitemia and reduction of clinical disease (Oludada et al., 2023). The magnitude of antibody responses may also increase with repeated exposure to malaria parasites, leading to broader recognition of parasite antigens (Kusi et al., 2017; Watson et al., 2022).

Among postpartum women, the quantity of anti-CSP IgG may reflect immune responses developed during previous malaria exposure, including exposure occurring during pregnancy. However, antibody responses may vary according to the intensity and frequency of malaria exposure, previous infections, and individual immune characteristics. Although high antibody levels have been associated with protection against malaria in some studies, antibody quantity alone does not necessarily indicate functional protection because the effectiveness of antibodies also depends on their ability to recognize and bind their target antigen (Opi et al., 2021; Mugo et al., 2023). Therefore, determining the serum quantity of IgG against CSP among postpartum mothers attending Kawempe National Referral Hospital is important for characterizing the magnitude of malaria-specific humoral immunity in this population.

The study aimed to determine the quantities of *total serum IgG* antibodies against CSP among Postpartum Mothers attending Kawempe National Referral Hospital.

METHODOLOGY

Study design

This was a cross-sectional study involving serum samples collected from Postpartum Women at Kawempe National Referral Hospital (KNRH)

Study setting:

The Parent study from which the archived serum samples were obtained was conducted at KNRH, a public hospital located about 10 km from the city center and serves as a National Referral for the entire country. It also serves as a teaching hospital for Makerere University College of Health Sciences.

The parent study was titled 'Barriers, knowledge and antimalarial antibodies in relation to intermittent preventive treatment in pregnancy use in Kawempe National Referral Hospital' 400 mothers were recruited and serum samples obtained and stored in Makerere University College of Health Sciences Biochemistry laboratory at -80 degrees Celsius, the study was conducted between 4th September 2020 and 4th September 2021.

Consent was obtained from study participants for future use of samples; the principal investigator also gave consent for this particular study to be nested.

Serum samples were analyzed at the Makerere University College of Health Sciences Medical Biochemistry laboratory.

Study population

The population consisted of 400 archived serum samples that were collected from postpartum women who attended and delivered at KNRH during the study period of the parent study.

Inclusion and exclusion criteria

Inclusion criteria

Archived serum samples of postpartum mothers who were aged between 18-49 years with a singleton pregnancy and received postnatal services at Kawempe National Referral Hospital and should have delivered at Kawempe National Referral Hospital or delivered at home and reported to the health unit within 48 hours and, consented to participate in the study as per parent study protocol.

Archived serum samples stored at -80°C with undamaged, clearly labeled vials

Exclusion criteria

Archived serum samples of postpartum mothers with known chronic disease conditions, such as; Human Immune Virus, Diabetes Mellitus, chronic kidney, sickle cell, antimalarial treatment within 2weeks were excluded from the study.

Participants with chronic illnesses (e.g., HIV infection, diabetes mellitus, chronic kidney disease, and sickle cell disease) were excluded because these conditions can alter immune function and antibody responses, potentially confounding the measurement of malaria-specific IgG levels. Mothers who had received recent antimalarial treatment were excluded to avoid transient changes in antibody levels and avidity associated with recent infection or treatment. Additionally, samples with compromised

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integrity (e.g., inadequate volume, contamination, or improper storage) were excluded to ensure the accuracy and reliability of laboratory analyses.

Variables

The dependent variables in this study were the quantities of *total serum IgG* antibodies against CSP among Postpartum Mothers.

The independent variables included maternal demographic and malaria prevention factors such as age, education level, IPTp-SP use (Fansidar), antenatal care (ANC) attendance, and bed net ownership. These variables were considered potential determinants of malaria exposure and immune response.

Sample size determination

Using the formula for calculating the sample size for a two-sample t-test (Charan & Biswas, 2013), here's the formula**;**

$$n = \frac{2\cdot(\sigma)^2\cdot(Z_{\alpha/2}+Z_{\beta})^2}{(\mu_1-\mu_2)^2}$$

Where**;**

n=required sample size per group

σ = estimated standard deviation of the outcome measure

$Z\alpha/2$ = Z-score corresponding to the desired significance level (α)

$Z\beta$ = Z-score corresponding to the desired power ($1-1-\beta$)

$\mu1-\mu2$ = expected difference in means between the two groups

Given;

σ = 1.0 (standard deviation of affinity scores, hypothetical)

$Z\alpha/2$ = 2.807 (Z-score for α =0.005)

$Z\beta$ = 0.842 (Z-score for 1- β = *0.80)*

$\mu1$ - $\mu2$ = 0.5 (expected difference in means for affinity, hypothetical)

Using the formula;

$$n = \frac{2\cdot(\sigma)^2\cdot(Z_{\alpha/2}+Z_{\beta})^2}{(\mu_1-\mu_2)^2}$$

Substituting the values;

$$n = \frac{2\cdot(1.0)^2\cdot(2.807+0.842)^2}{(0.5)^2}$$

Calculating this gives;

n=193.77

Rounding up to the nearest whole number, the adjusted required sample size is approximately 194

The sample size was estimated using the two-sample t-test formula for comparing two independent means, as the primary outcome variable (IgG antibody levels measured as optical density values) is continuous. This formula is appropriate for determining the number of samples required to detect a meaningful difference in mean antibody levels between comparison groups with adequate statistical power. The calculation assumed a 95% confidence level ($Z\alpha/2$ = 1.96) and 80% power ($Z\beta$ = 0.84)**,** which are standard parameters in biomedical research (Lwanga & Lemeshow, 1991; Charan & Biswas, 2013).

Sampling procedure

A total of 400 serum samples were initially collected from postpartum women (6 – 8 weeks following labour) in the parent study, processed, and archived for future analysis. From these, 194 samples were selected using a simple random sampling technique. Each sample in the archive was assigned a unique identifier ranging from 1 to 400, and a random number generator was used to produce 194 random numbers within this range. The samples corresponding to these randomly generated numbers were then selected from the sampling frame for inclusion in the current study.

Study procedures.

Sample processing

This was a nested study, thus in the previous study, blood was, by venipuncture collected in a Serum Separator Tube (SST) vaccutainer; the vaccutainer was inverted gently no more than eight times. Further inversion could cause alterations in sample integrity. The stopper was not removed at any time.

Blood was allowed to clot in an upright position for at least 30 minutes but not longer than 1 hour before centrifugation. All samples (3 mls per sample) were transported under cold chain to Makerere University College of Health Sciences Medical Biochemistry laboratory for downstream processes. Centrifugation was done for at least 15 minutes at 2200-2500 RPM within one hour of collection to collect serum.

Serum obtained from the same separation procedure was distributed into aliquots and stored at -80^0C (Wu et al., 2019).

Following clearance from the Principal Investigator (PI) of the primary study and IRB, 1 ml per selected archived samples was retrieved methodically for analysis, that is; checking for labels, storage temperatures, and while excluding any compromise in the sample integrity.

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Laboratory procedures

Laboratory protocol

The laboratory protocol used in this study was adapted from established enzyme-linked immunosorbent assay (ELISA) and antibody avidity assay methods described in previous malaria immunology studies. Minor adjustments were made to suit the laboratory conditions and available reagents while maintaining the core methodological procedures. The assay was conducted in accordance with the standard operating procedures (SOPs) of the biochemistry laboratory to ensure consistency and reliability of results. (Kusi et al., 2017)

Laboratory analysis of the samples was conducted using an ELISA machine (Thermo Fisher scientific, USA) at the Makerere University College of Health Sciences Biomedical Laboratory, The Enzyme-Linked Immunosorbent Assay (ELISA) was employed to assess antibody responses, specifically targeting *Plasmodium falciparum* recombinant proteins CSP, as previously described (Hisaeda, Saul et al., 2002). Stock solutions of the antigen were diluted in phosphate-buffered saline (PBS), pH 7.2, to achieve optimal concentrations ranging from 0.1 to 4 µg/mL. ELISA tests were performed on archived serum samples as previously described by Spring, Cummings et al. (2009), using quadruplicates of serum diluted two-fold from 1:40 to 1:5120. The endpoint titre of antibodies was defined as the serum dilution that yields an optical density (OD) of 0.5 in the assay. The laboratory procedure for ELISA analysis of total serum IgG antibodies against the malaria vaccine candidate CSP involved the use of postpartum mothers' archived serum samples, recombinant CSP antigen, and various buffers including coating buffer (carbonate-bicarbonate buffer, pH 9.6), blocking buffer (5% non-fat dry milk in PBS with Tween-20), wash buffer (PBST), and serum diluent buffer. A secondary antibody specific to human IgG, conjugated with an enzyme such as horseradish peroxidase (HRP), was used for detection. Substrate solution, typically tetramethylbenzidine (TMB), and a stop solution that is 2 molar sulfuric acid were added to facilitate colorimetric detection. The assays were conducted on ELISA plates using both single-channel and multichannel pipettes, and absorbance readings were measured with the Thermo Fisher microplate reader.

ELISA Procedure

To prepare the ELISA plate, CSP antigen was diluted in the coating buffer to a working concentration (e.g., 1–19.1 µg/mL), and 100 µL of the diluted antigen was added to each well. The plate was incubated overnight at 4°C to allow antigen coating. After coating, the wells were aspirated, washed three times with wash buffer, and then filled with 200 µL of blocking buffer per well. The plate was again incubated overnight at 4°C to block nonspecific binding sites.

For serum sample dilution, archived serum samples from postpartum mothers were diluted in serum dilution buffer, typically at a ratio of 1 µL:499 mL, or as optimized. Dilutions were prepared to ensure IgG concentrations fell within the linear range of the standard curve. After blocking, 100 µL of the diluted serum was added to each well. Known IgG standards were also included for reference. The plate was incubated for 2 hours at room temperature.

Next, the wells were aspirated and washed three times with wash buffer. Then, 100 µL of enzyme-conjugated secondary antibody (anti-human IgG) was added to each well and incubated for 1 hour at room temperature. After incubation, the wells were washed three times again, and 100 µL of TMB substrate solution was added to each well. The plate was incubated for 30 minutes to allow for color development.

Finally, 100 µL of stop solution (2 molar sulfuric acid) was added to each well to stop the enzyme-substrate reaction. The plate was gently mixed to ensure even color development across wells, and the results were ready for reading.

Reading Absorbance and Data Interpretation

The absorbance of each ELISA well was measured using a Thermo Fisher microplate reader at 450 nm, with a reference wavelength of 620 nm. These optical density (OD) values were recorded for all wells. Control sera from known unexposed individuals were included to help establish thresholds. Any samples with OD readings exceeding 1.4 were further diluted, up to 1:1000, to ensure that absorbance values fell within the linear range of detection. Final OD values were multiplied by the dilution factor, and each specimen was tested in duplicate or triplicate. The average OD was used in the final analysis to improve accuracy.

Determination of Avidity

This technique measures the strength of IgG antibody binding to CSP antigens in postpartum serum samples. Essential materials included a Thermo Fisher plate reader, ELISA plates coated with CSP antigen, archived serum samples, washing and blocking buffers, secondary antibodies conjugated with an enzyme, and TMB substrate solution. Positive and negative controls were used.

To prepare the ELISA plate, CSP antigen was diluted in coating buffer to a concentration between 1–19.1 µg/mL. Then, 100 µL of the solution was added to each well and

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incubated overnight at 4°C to allow coating. Serum/plasma samples were diluted 1:500 in serum dilution buffer. After antigen coating, the plate was washed three times, and 200 µL of blocking buffer was added per well and incubated overnight at 4°C.

Following blocking, the plates were washed, and 100 µL of diluted serum samples was added to each well. The plates were incubated for 2 hours at room temperature to allow IgG antibodies to bind to the antigen. After incubation, the plates were washed four times with washing buffer. Then, 100 µL of 3 M ammonium thiocyanate was added to each well and incubated for 30 minutes at room temperature to challenge antibody-antigen binding. After a final wash, 100 µL of peroxidase-conjugated goat anti-human IgG (diluted 1:20,000 in dilution buffer) was added to each well and incubated for 60 minutes at room temperature.

The plates were then washed four more times, and 100 µL of TMB substrate was added to each well. Plates were incubated in the dark at room temperature for 30 minutes to allow color development. Afterward, 100 µL of stop solution was added to terminate the reaction. Absorbance was immediately read at 450 nm with a 620 nm reference using the Thermo Fisher plate reader. The signal intensity indicated the strength of antibody-antigen interaction. The molarity of ammonium thiocyanate causing a 50% reduction in maximum OD was interpreted as the relative antibody affinity, with higher molarity indicating stronger binding (Rajasekariah, Kay et al., 2003).

Quality Assurance

Data were analyzed using statistical methods to calculate IgG concentrations by constructing standard curves based on known IgG standards and their corresponding absorbance values. Concentrations for the test samples were calculated from these curves, and final results were expressed as total serum IgG antibody levels against CSP in postpartum mothers.

Laboratory procedures were conducted by the principal investigator**,** who was trained in the relevant laboratory techniques prior to sample analysis. The training and procedural guidance were provided by the Principal Laboratory Technologist and the In-Charge of the Biochemistry Laboratory, with additional oversight from the study supervisor, a biochemist**.** All laboratory procedures were performed according to established standard operating procedures **(**SOPs) of the biochemistry laboratory. The technologist and laboratory in-charge provided continuous technical guidance during the laboratory work to ensure adherence to established laboratory quality standards.

Data were securely stored in protected databases and managed according to confidentiality protocols. Finally, all research activities were carried out in compliance with ethical guidelines, with prior written consent obtained from the principal investigator of the parent study and adherence to ethical standards for human sample use.

Statistical analysis

Data were imported from Microsoft Excel 2016 into Stata version 14.2 for analysis. Descriptive statistics were computed for both categorical and continuous variables. For categorical variables (such as age group, IPTp use, and ANC attendance), frequencies and proportions were presented. For continuous variables (such as antibody optical density [OD] values), either the median and interquartile range (IQR: Q1–Q3) or the mean and standard deviation (SD) were reported, depending on the distribution of the data. Since the distribution of OD values was not normal, the median (Q1, Q3) was used.

To assess associations between the quantities of IgG antibodies and covariates at the bivariate level, non-parametric tests were employed: the Mann–Whitney U test for comparisons between two groups and the Kruskal–Wallis test for comparisons across more than two groups. A p-value of ≤0.05 was considered statistically significant.

The antibody avidity index was categorized into two groups: low affinity (<40%) and high affinity (≥40%). Given that the prevalence of high-avidity antibodies was greater than 10%, a modified Poisson regression model with robust standard errors was used instead of binary logistic regression to determine factors associated with high antibody affinity.

Variables with p-values less than 0.25 at the bivariate level, as well as those with known associations with antibody affinity based on previous literature, were included in the multivariable model. Multicollinearity was assessed using Pearson's correlation coefficients, and where $r > 0.4$ between any two variables, one was excluded from the model. Model adequacy and parsimony were assessed using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). The overall goodness-of-fit of the model was evaluated to ensure the robustness and reliability of the final results.

Ethical consideration

This was a nested study; thus, the principal investigator secured administrative clearance from the original study to access the samples for analysis.

Similarly, being a nested study, the researcher sought for and obtained waiver of consent from the Makerere University School of Biomedical Sciences Research and Ethics Committee (SBSREC) under SBS-2023-416 approval letter; and used archived serum samples stored at

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temperatures of -80^0 C, which were obtained from the study "Barriers, knowledge and antimalarial antibodies in relation to Intermittent preventive treatment in pregnancy use in at KNRH.

The primary study was approved by the Makerere University School of Medicine Research and Ethics Committee, and the Uganda National Council for Science and Technology under registration number HS620ES. Informed consent had been obtained from study participants for future use of the samples from the original study, and the current analyses of archived samples collected under renewed approvals from the ethical body mentioned above.

CSP, a synthetic peptide, was obtained from GenScript Biotechnology Corporation based in the United States of America, New Jersey; the corporation is certified and specializes in life-science services and products, biologics contract development and manufacturing organization (CDMO), industrial synthetic products, and an integrated global cell therapy company.

RESULTS

Background characteristics of Postpartum Mothers attending Kawempe National Referral Hospital

A total of 194 postpartum mothers participated in the study at Kawempe National Referral Hospital. Their mean age was 26 years (SD = 5), with most aged between 20–30 years (69.1%) and 12.4% aged 19 years or below. The majority were married or cohabiting (90.7%) and had a median of two living children (IQR: 1–3). In terms of education, 38.9% had attained secondary education, while 17.1% had no formal education. All participants had attended antenatal care (ANC), although only 48.97% completed four or more visits. A majority (64.3%) received Fansidar during pregnancy for malaria prevention, and 96.4% reported owning a mosquito net. HIV prevalence was low, with 97.9% testing negative and only one known positive case (Table 1).

Table 1: Background characteristics of postpartum mothers attending Kawempe National Referral Hospital (N = 194).

Characteristics	Summary measure
Age, Mean (SD)	26 (5)
Age category, n (%)	
≤ 19 years	24 (12.37)
20-30 years	134 (69.07)
> 30 years	36 (18.56)
Marital status, n (%)	
Married/cohabiting	176 (90.72)
Single/separated	18 (9.28)
Number of children alive, Median (Q1, Q3)	2 (1,3)
Education level, n (%)	
No formal education	33 (17.10)
Primary	64 (33.16)
Secondary	75 (38.86)
Tertiary	21 (10.88)
Attended ANC, n (%)	
Yes	194 (100.00)
Number of ANC visits, n (%)	
< 4 visits	99 (51.03)
≥ 4 visits	95 (48.97)
Given Fansidar during malaria, n (%)	
No	69 (35.75)
Yes	124 (64.25)
HIV status, n (%)	
Positive	1 (0.52)
Negative	190 (97.94)

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Don't know	3 (1.55)
Has a mosquito net, n (%)	
No	7 (3.63)
Yes	186 (96.37)

Quantities of Total Serum IgG Antibodies Against CSP Among Postpartum Mothers

A total of 194 postpartum mothers attending Kawempe National Referral Hospital were analyzed for total serum IgG antibody responses against the circumsporozoite protein (CSP). The overall median antibody titre was 0.09875 OD units (IQR: 0.044–0.370).

Mothers aged ≤19 years had the lowest median titre of 0.0540 OD (IQR: 0.02975–0.37175), compared to 0.1095 OD (IQR: 0.047–0.370) among those aged 20–30 years and 0.1165 OD (IQR: 0.05175–0.370) among those aged >30 years. The difference across the three age groups was not statistically significant (p = 0.3705). Interpretation: Although younger mothers tended to have lower antibody titres compared to older mothers, the differences were not statistically significant, suggesting that age may not strongly influence CSP-specific IgG responses.

Mothers who were married/cohabiting had a median titre of 0.09875 OD (IQR: 0.04275–0.370), while single/separated mothers had 0.0905 OD (IQR: 0.0495–0.410). The difference in antibody levels between the two groups was not statistically significant (p = 0.5877). Interpretation: Marital status did not appear to influence IgG antibody titres among postpartum mothers.

Antibody titres varied with education level. Mothers with secondary education recorded the highest titres (0.1275 OD, IQR: 0.047–0.4097), followed by those with tertiary education (0.1165 OD, IQR: 0.0535–0.3205). In contrast, lower titres were observed among mothers with primary education (0.0599 OD, IQR: 0.04075–0.21525) and no formal education (0.0920 OD, IQR: 0.04465–0.432). However, these differences were not statistically significant (p = 0.1606).

Mothers who attended <4 antenatal visits had a median titre of 0.0840 OD (IQR: 0.0415–0.3875), while those with ≥4 visits recorded 0.1050 OD (IQR: 0.04735–0.3545). The difference was not statistically significant (p = 0.9297). Interpretation: The number of antenatal visits did not have a significant effect on CSP-specific IgG antibody titres.

Mothers who received Fansidar during pregnancy had slightly higher antibody titres (0.1160 OD, IQR: 0.049175–0.3725) compared to those who did not (0.0670 OD, IQR: 0.0345–0.370). The difference was not statistically significant (p = 0.2191). Interpretation: Fansidar use was associated with a modest increase in IgG titres, but this effect was not statistically significant.

Ownership of mosquito nets was not associated with antibody levels. Mothers who owned mosquito nets had a median titre of 0.09875 OD (IQR: 0.04465–0.370), while those who did not own nets had 0.1325 OD (IQR: 0.026–0.765). This difference was not statistically significant (p = 0.7643).

Table 2: Median Serum IgG Antibody Titres Against CSP Among Postpartum Mothers

Variable	Category	Median OD	IQR	p-value
Overall	All mothers (N=194)	0.09875	0.044 – 0.370	–
Age group	≤19 years	0.0540	0.02975 – 0.37175	
	20–30 years	0.1095	0.047 – 0.370	
	>30 years	0.1165	0.05175 – 0.370	0.3705
Marital status	Married/Cohabiting	0.09875	0.04275 – 0.370	
	Single/Separated	0.0905	0.0495 – 0.410	0.5877
Education level	No formal education	0.0920	0.04465 – 0.432	
	Primary	0.0599	0.04075 – 0.21525	
	Secondary	0.1275	0.047 – 0.4097	

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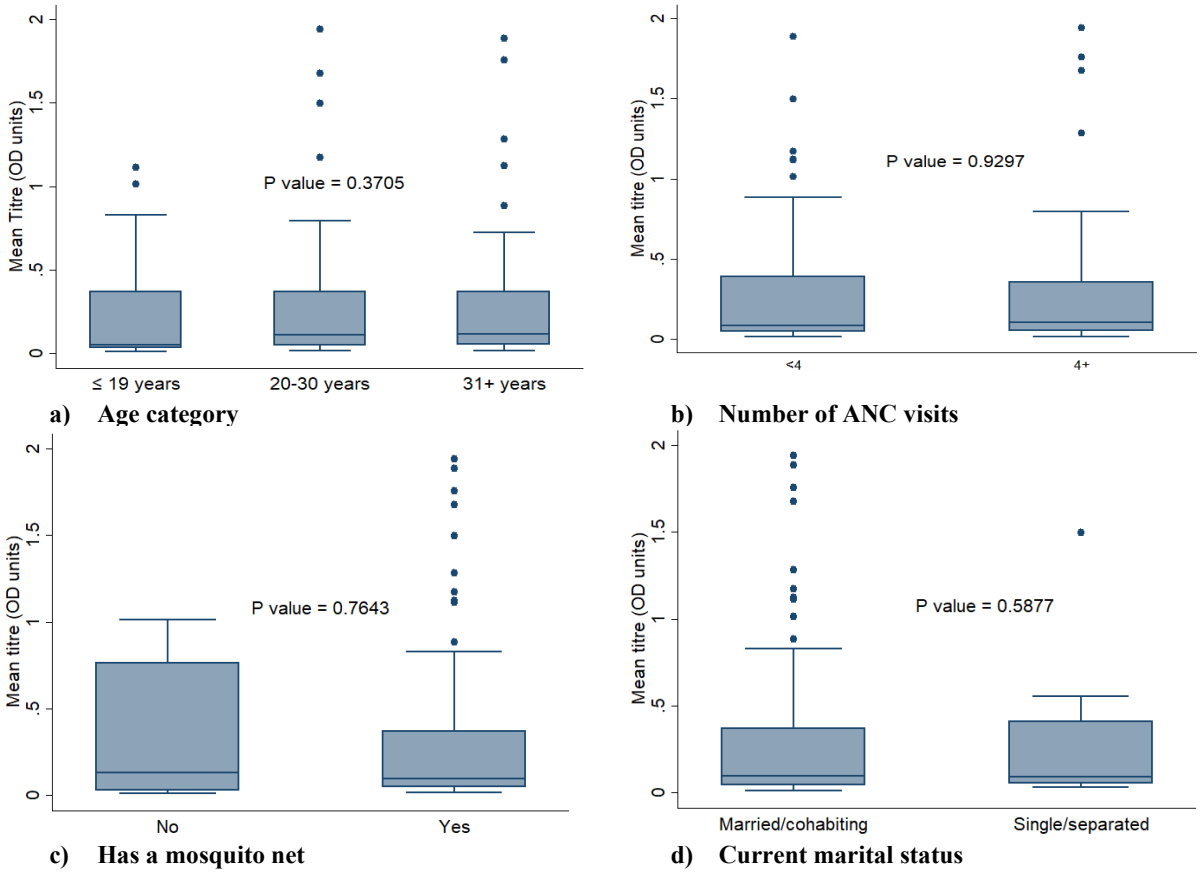
Variable	Category	Median OD	IQR	p-value
	Tertiary	0.1165	0.0535 – 0.3205	0.1606
Antenatal visits	<4 visits	0.0840	0.0415 – 0.3875	
	≥4 visits	0.1050	0.04735 – 0.3545	0.9297
Fansidar use	Yes	0.1160	0.049175 – 0.3725	
	No	0.0670	0.0345 – 0.370	0.2191
Mosquito net ownership	Yes	0.09875	0.04465 – 0.370	
	No	0.1325	0.026 – 0.765	0.7643

Figure 1: Distribution of total serum IgG antibody titres against CSP stratified by age, marital status, number of ANC visits, having a mosquito net, and marital status



DISCUSSION

The first objective of this study was to determine the quantities of total serum IgG antibodies against the circumsporozoite protein (CSP) among postpartum mothers at Kawempe National Referral Hospital. The median antibody level was 0.09875 OD units (interquartile range: 0.044–0.370). No statistically significant differences in antibody titres were observed across maternal age, marital status, education level, antenatal care (ANC) attendance, Fansidar use, or mosquito net ownership (all p-values > 0.05).

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These modest titres suggest relatively low recent exposure to *Plasmodium falciparum* or reduced malaria transmission intensity in this urban setting. Similar patterns have been reported in previous studies from Kampala and other urban areas, where malaria prevalence has declined due to strong control measures such as insecticide-treated nets (ITNs), indoor residual spraying (IRS), and improved ANC services (Arinaitwe et al., 2020; Kamya et al., 2020; Akello et al., 2022).

Lower titres in postpartum women may also result from reduced immunological stimulation during pregnancy, particularly among those who received intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (Fansidar). This observation is consistent with findings from Staalsoe et al. (2004) and Stephens et al. (2017), who reported lower CSP-specific IgG levels in women adhering to IPTp compared to those without prophylaxis.

Conclusion

Overall, IgG antibody levels were modest, reflecting low recent malaria exposure likely due to effective malaria control interventions such as IPTp, ITN use, and consistent ANC attendance.

Limitations

First, IPTp use and ANC attendance were self-reported, which may have introduced recall or social desirability bias. Second, the study was conducted in an urban referral hospital where malaria transmission is lower than in rural settings, limiting generalizability. Third, although the archived samples were stored under optimal conditions, there remains a possibility of minor degradation over time, which could influence antibody measurements.

Recommendation

The Ministry of Health should strengthen malaria surveillance and research to monitor serum IgG levels against CSP among postpartum mothers.

The Ministry of Health should support studies assessing factors associated with variations in anti-CSP IgG levels among women of reproductive age.

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List of abbreviations

ANC - Antenatal Care
AIC - Akaike Information Criterion
BIC - Bayesian Information Criterion
CSP - Circumsporozoite Protein
ELISA - Enzyme-Linked Immunosorbent Assay
HIV - Human Immunodeficiency Virus
IgG - Immunoglobulin G
IQR - Interquartile Range
IPTp - Intermittent Preventive Treatment in Pregnancy
IRS - Indoor Residual Spraying
ITN - Insecticide-Treated Net
KNRH - Kawempe National Referral Hospital
OD - Optical Density
PBS - Phosphate-Buffered Saline
PBST - Phosphate-Buffered Saline with Tween-20
PI - Principal Investigator
SOP - Standard Operating Procedure
SBSREC - School of Biomedical Sciences Research and Ethics Committee
SP - Sulfadoxine-Pyrimethamine
TMB - Tetramethylbenzidine
UNCST - Uganda National Council for Science and Technology

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Conflict of interest

The authors declare no conflict of interest

Data availability

Data are available upon request from the author

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Author contributions

MAO: conceptualization, methodology, investigation, writing original draft.
AL: data curation, formal analysis, Supervision.
IK: data curation, formal analysis, visualization.
FN: supervision, validation

Informed consent

Written informed consent was obtained from all participants prior to their inclusion in the study. Participants were informed about the purpose of the study, procedures involved, potential risks and benefits, and their right to withdraw at any time without penalty.

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