







RESEARCH ARTICLE

REVISED

Impact of malaria on haematological parameters of urban, peri-urban and rural residents in the Ashanti region of Ghana: a cross-sectional study [version 3; peer review: 2 approved]

Previously titled: Impact of malaria on haematological parameters of urban, peri-urban and rural patients in the Ashanti region of Ghana: a cross-sectional study

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Abstract

Background: We aimed at investigating the impact of malaria on the haematological parameters of residents from different demographic settlements in the Ashanti Region of Ghana. Malaria parasites trigger changes in certain haematological parameters, which may result in a number of clinical manifestations. Differences in demographic settlements, such as rural, peri-urban and urban settlements may also influence these changes, but this has not been extensively studied in Ghana.


Methods: We conducted a hospital-based, cross-sectional study from January to December 2018 in three different settlements. A total of 598 participants were recruited. Blood smears were examined to detect and quantify malaria parasitaemia, while haematological parameters were measured using a haematology analyser.


Results: Participants from the rural settlement had the highest malaria prevalence (21.3%) compared to urban (11.8%) and peri-urban areas (13.3%); however, the peri-urban area had the highest median parasite density (568; IQR=190.0-1312.0). Age was significantly associated with the odds of malaria positivity (OR: 0.97; CI:0.96 — 0.99; $p=4.96 \times 10^{-4}$). When haematological parameters of the malaria-infected study participants were compared to the parameters of uninfected participants, red blood cell count ($p=0.017$), haemoglobin ($p=0.0165$), haematocrit ($p=0.0015$), mean corpuscular volume ($p=0.0014$), plateletcrit ($p<0.0001$) and platelet count ($p<0.0001$) were all significantly lower in the malaria infected group. In addition to age, haemoglobin and plateletcrit levels were also inversely correlated with the odds of testing positive for malaria, suggesting that

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children who were anaemic and/or thrombocytopaenic were likely to be infected. After fitting the data to a logistic regression model comprising the three variables, the model correctly categorised 78% of uninfected study participants, but only 50% of the malaria-positive participants.

Conclusions: Study participants who were positive for malaria were younger and had low haemoglobin and plateletcrit levels compared to uninfected individuals. Further studies are needed to more precisely elucidate the relationship between malaria infection, demographic and haematological parameters.

Keywords

malaria, anaemia, parasitaemia, WBC count, thrombocytopaenia,

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REVISED Amendments from Version 2

We have reorganised [Table 1](#) to show the statistical comparison amongst the three sites for each parameter.
We have included the [Figure 1](#) x-axis title.

Any further responses from the reviewers can be found at the end of the article

Introduction

Malaria remains the most important protozoan infection of humans and continues to have an immense impact on the health and quality of life of people across the world. Despite the decrease in incidence of mortality due to malaria in the last decade, the most recent World Malaria Report revealed that 228 million clinical cases of malaria were reported, resulting in no less than 405,000 deaths, the majority of which were in sub-Saharan Africa¹.

The introduction of malaria parasites into the host peripheral blood by an infected female *Anopheles* mosquito triggers changes in several host haematological parameters, many of which play a role in malaria pathogenesis. These changes may subsequently affect the general physiology of the host, resulting in a number of clinical manifestations, with anaemia and thrombocytopaenia being the most common. Haematological parameters that are often affected include the relative numbers of circulating cell types such as erythrocytes, platelets, granulocytes and lymphocytes, as well as parameters like haemoglobin concentration²⁻⁴. While erythrocyte and platelet levels are consistently decreased in malaria-infected individuals, there have been conflicting reports on the effect of malaria on leukocyte counts. A recent study showed a significant reduction in leukocyte, as well as platelet and erythrocyte levels in malaria-infected study participants compared with their uninfected counterparts⁵. Moreover, Kotepui *et al.* (2014) reported that low platelet, white blood cell (WBC) and lymphocyte counts were important predictors of malaria infection and, when used with other clinical methods, could improve malaria diagnosis and treatment⁶. However, another study reported elevated leukocyte levels in malaria-infected study participants compared to their uninfected counterparts, suggesting that the relationship between malaria and certain immunohaematological parameters may be more complex than previously thought⁷.

While haematological changes associated with malaria have been well-characterized^{6,8,9}, it is possible that factors such as differences in demographic settlements could also influence observed changes. However, there is relatively limited data on the differences in haematological indices of malaria-infected people in rural, peri-urban and urban settlements, especially in the forested zones. The aim of this study was to investigate the haematological indices of malaria-infected individuals across these different settlements.

Methods

We carried out a cross-sectional study targeting hospital attendees. In order to avoid bias and avoid including only 'sick'

or symptomatic participants, we extended our sampling to include 'healthy' participants who accompanied their relatives or friends to the hospital.

Ethical statement

The protocol for data collection was reviewed and approved by the Committee on Human Research Publication and Ethics of the Kwame Nkrumah University of Science and Technology (CHRPE/KNUST) and the Komfo Anokye Teaching Hospital (CHRPE/KATH), (Ref: CHRPE AP/018/18). All study participants provided written informed consent prior to study enrolment, with parental or guardian consent obtained for children.

Study sites

The study was conducted concurrently at the Kumasi South Hospital (KSH), the Kuntanase Government Hospital (KGH) and the Agona Government Hospital (AGH) in the Ashanti region of Ghana. KSH is located in Atonsu, a suburb of Kumasi, the regional capital and second largest city in Ghana, and served as the urban site. KGH is situated in Kuntanase, the capital of the Bosomtwe district. The Bosomtwe district is one of the 27 districts in the Ashanti region and is located approximately 28 kilometres from Kumasi. AGH is in the Sekyere East district and is located approximately 37 kilometres away from Kumasi. KGH and AGH served as the peri-urban and rural study sites, respectively. The urban, peri-urban and rural sites served as proxies for high, medium and low transmission transects. People in the Agona area receive over 11,000 mosquito bites annually, with 800 of them being infectious bites, while people in the Kumasi area receive approximately 400 bites and around 200 infectious bites per year. The transmission intensity in the Kuntanase area is between these two^{10,11}.

Study participants

The sample size was determined using the binomial model. Confidence intervals of 95% and a precision level of 5% was used. In the equation below, n is the sample size, z is the critical value of the standard normal distribution at 5% level (1.96), p is the estimated malaria prevalence, $q = 1 - p$ and d is the precision level. The prevalence of malaria had previously been determined by Paintsil *et al.* (2019) to be about 26%¹².

$$n = z_a^2 pq/d^2$$

The minimum sample size required was calculated to be 295; however, we sampled 601 participants to make up for the different transmission seasons (January to December 2018) across which samples were collected. The study targeted people accessing healthcare at the various hospitals. This included patients referred to the laboratory for malaria tests and accompanying caregivers who were not sick. The purpose of the study was explained to potential participants using a participant information leaflet to seek their informed consent, a copy of which is provided as *Extended data*¹³. An interpreter was employed to translate the written document into the local dialect (Akan) for those who could not read. In addition, thumbprints were obtained for those who could not sign/write.

Demographic data such as age, gender and insecticide-treated net (ITN) use were obtained from participants using a semi-structured questionnaire, a copy of which is provided as *Extended data*¹³.

Exclusion criteria

People who were critically ill (requiring hospitalisation) and those who declined to consent were excluded from the study. Infants under six months (<6 months) were also excluded.

Haematological analysis

From each participant, 2 ml of venous blood was drawn by a trained phlebotomist in the hospital laboratory. This was transferred into an EDTA tube with a unique participant identifier. The blood sample was used to prepare thin and thick films for microscopic examination and automated complete blood counts (CBCs). Blood parameters were estimated using the Sysmex XP-300 Automated Haematology Analyzer. The cell counter provided data on red blood cell (RBC) count, haemoglobin (Hb) level, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) level, WBC, lymphocyte, neutrophil, platelet counts, mean platelet volume (MPV), plateletcrit and the red blood cell distribution width (RDW).

Parasitological analysis

From the EDTA-treated blood, 6 µL and 3 µL were used to prepare thick and thin blood films, respectively. After air-drying, the thin blood smears were fixed with methanol and both smears subsequently stained with 10% Giemsa for 15 minutes. Two experienced microscopists independently examined the slides under ×100 oil immersion to determine the presence or absence of malaria parasites. Parasites were quantified after counting 200 or 500 WBCs. Parasite densities were calculated as parasite per microliter of blood (parasite counted / WBCs counted × total WBC in 1µL of blood). A slide was only declared negative when no malaria parasite was seen after scanning 100 high power fields (HPFs).

Data collection technique

The data were gathered by the authors using standardized questionnaire. At the end of each day, all researchers came together to check the completeness of each individual questionnaire and match them to the lab specimens taken. Prior to the survey we had pre-tested the questionnaire in a community setting to ensure that people understood the questions. Feedback were used to improve the questionnaire before the actual survey was carried out.

Statistical analysis

The data collected were coded and entered into Microsoft Excel 2016. The data were checked for completeness. Samples with missing data were excluded from the analysis. Thus, three samples with missing microscopy data were excluded completely from all analyses. Data analysis was performed using GraphPad Prism v6 (GraphPad Software, Inc., San Diego, CA, USA). Data normality was checked using the Shapiro-Wilk normality test. For normally distributed data, comparisons were carried out using one-way ANOVA, whilst data not conforming to the normal distribution were compared using the Kruskal-Wallis or Mann-Whitney Tests. Pairwise multiple

comparison across communities was done using Dunn’s multiple comparison test. For the logistic regression analyses, 19 samples were excluded due to incomplete data, bringing the sample count to 579. The regression analyses were carried out using the *glm* function in R-3.6.2¹⁴. Results were considered statistically significant if $p \leq 0.05$.

Results

In total, 598 participant samples were examined for parasite prevalence and density. A further 19 samples were excluded from the haematological analysis because the haematological indices could not be determined. **Table 1** gives a summary of the demographic profile of the study population.

Out of the 598 samples analysed in the study, 75.4% ($n=451$) were female¹³. The overall median age was 27 years ($IQR=19-40$) and there was a statistically significant difference in the age profiles of participants from the three study sites ($H= 20.8$; $p<0.0001$). Study participants in the rural area were comparatively younger, with a median age of 24.5 years ($IQR=14-35$), followed by those in the peri-urban area, with a median age of 27 years ($IQR=19.5-41.5$) and finally those in the urban area, with a median age of 29 years ($IQR=22.5-43.0$).

Table 1. Baseline characteristics of participants and study areas.

Baseline characteristics	n (%)	P value
Age(yrs)		
0-5		
Urban	11(19.6)	0.006
Peri-urban	16(28.6)	
Rural	29(51.8)	
6-14		
Urban	8(21.1)	0.004
Peri-urban	8(21.1)	
Rural	22(57.8)	
15+		
Urban	185(36.7)	<0.0001
Peri-urban	171(33.9)	
Rural	149(29.5)	
Gender(Female)		
Urban	161(78.9)	0.070
Peri-urban	150(76.9)	
Rural	139(65.5)	
ITN usage		
Urban	66(32.3)	0.7932
Peri-urban	57(29.2)	
Rural	60(30.3)	
Settlement		
Urban	203 (33.9)	0.884
Peri-urban	195 (32.6)	
Rural	200 (33.4)	
Malaria prevalence		
Urban	24 (11.8)	<0.0001
Peri-urban	26 (13.3)	
Rural	43 (21.3)	

The results were considered significant if $p \leq 0.05$

This observation is consistent with expectations as, typically, the youth tend to leave their rural communities in search of jobs as they get older.

16% ($n=93$) of the study participants had *Plasmodium falciparum* infection, confirmed by microscopy. Children between the ages of six and 14 (inclusive) recorded both the highest rates of malaria prevalence (34.8%) and the lowest rates of ITN usage (28.9%) while children less than five years constituted the majority of those who slept under insecticide-treated mosquito nets (58.9%). There was a significant difference in the median parasite densities of infected study participants across the different age groups ($H=8.64$; $p=0.0133$). Children under five years harboured the highest number of parasites, with a median parasite density of 665 ($IQR=327-1038$), followed by children 6–14 years, with a median density of 504 ($IQR=160-4139$), and study participants ≥ 15 years, with a median density of 24.5 ($IQR=13-40$). Study participants from the urban area reported the highest ITN usage (32.3%; $n=66/204$), with people from the peri-urban (29.2%; $n=57/195$) and rural areas (30.3%; $n=61/202$) reporting slightly lower percentages of usage ($p=0.7932$). In this case, however, the highest malaria prevalence was recorded in rural inhabitants (21.3%; $n=43/202$), followed by the peri-urban area (13.3%; $n=26/195$), with the urban centre recording the lowest prevalence (11.8%, $n=24/204$). There was also a significant difference in the parasite densities of infected participants across the three communities ($H=8.41$; $p=0.0149$). Study participants in the peri-urban area recorded the highest median parasite density (568; $IQR=190-1312$), followed by the rural area (224; $IQR=126-1198$), with the urban area recording the lowest median parasite density (167; $IQR=20.5-311.5$) (Figure 1).

Age is a significant demographic predictor of malaria infection status

To test the relationship between the various demographic parameters and infection status, we carried out univariate logistic regression analyses of each predictor against the response

variable. In order to avoid discarding potentially significant variables, we used a relaxed significance threshold of $p \leq 0.25$. All the demographic predictors passed this threshold with the exception of ITN usage ($p=0.838$), which was subsequently discarded. The remaining variables were used to create a multivariate logistic model, with age emerging as the only significant predictor of infection status (Table 2).

Malaria causes significant changes in several haematological parameters

The median haematological parameters of the infected and non-malaria groups were compared using the Mann-Whitney test due to the non-parametric distribution of the data. There were no significant differences in the median counts of neutrophils, lymphocytes or WBCs between the infected and non-infected groups; however, individuals with malaria had significantly lower median values of RBCs ($p=0.017$), Hb ($p=0.0165$), haematocrit ($p=0.0015$), MCV ($p=0.00139$), platelets ($p<0.0001$) and plateletcrit ($p<0.0001$) compared to malaria-negative individuals (Table 3).

Youth, anaemia and thrombocytopaenia are associated with increased odds of malaria positivity

To determine key predictors of infection status, we created multivariate models using the six significant haematological parameters from Table 2, as well as the only significant demographic variable: age (Table 3). Model selection was done using the forward stepwise regression method. As some of the haematological parameters were highly correlated with each other (RBC, HB and HCT; PLT and PCT), we decided to only select the correlated variables with the largest effect sizes as inclusion of correlated predictors reduces the precision of the estimated coefficients and inflates estimates of the standard error. The analysis identified age ($p=2.06 \times 10^{-5}$), haemoglobin level ($p=0.046$) and plateletcrit ($p=0.0001$) as being significantly inversely associated with malaria positivity (Table 4). As all three predictors are continuous variables, care must be taken when interpreting the estimated coefficients. Applying the

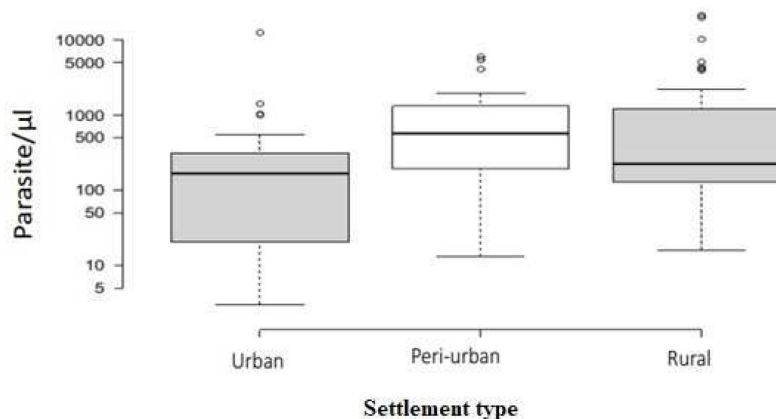


Figure 1. Parasite density across the three types of settlements. Centre lines show the median counts; box limits indicate the 25th and 75th percentiles as determined by R; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Widths of boxes are proportional to square-roots of the number of observations, Urban=24, Peri-urban=26 and Rural=43 sample points. The Y-axis shows the estimated number of parasites per microlitre in blood in logarithmic scale. The results were considered significant if $p \leq 0.05$.

Table 2. The association between malaria infection status and age is independent of gender and settlement type.

Predictor	Coeff. est.	Std. error	Z-value	OR (95% CI)	p-value
Intercept	-0.70	0.27	-2.56	0.50 (0.29 — 0.85)	0.010494
Sex: M	0.11	0.27	0.42	1.12 (0.66 — 1.88)	0.675678
Age	-0.03	0.01	-3.48	0.97 (0.96 — 0.99)	0.000496
Community: Peri-urban	-0.47	0.28	-1.67	0.63 (0.36 — 1.08)	0.094920
Community: Urban	-0.52	0.29	-1.82	0.59 (0.34 — 1.04)	0.068121

Results were considered significant if $p \leq 0.05$.

Table 3. Levels of haematological parameters in infected vs uninfected study participants.

Variable	Malaria-infected	Uninfected	P-value
	Median (IQR)	Median (IQR)	
Red blood cells (RBC) ($\times 10^6 / \mu\text{L}$) ^a	4.16 (3.78 – 4.67)	4.42 (3.93 – 4.85)	0.0170
Haemoglobin (g/dL) ^a	11.10 (9.5 – 12.4)	11.60 (10.40 – 12.80)	0.0165
Haematocrit (%) ^a	39.30 (34.40 – 44.60)	42.80 (38.20 – 46.20)	0.0015
Mean corpuscular volume (MCV) (fL) ^a	94.20 (89.3 – 100.5)	97.80 (91.70 – 102.70)	0.00139
Mean corpuscular haemoglobin (MCH) (pg) ^a	26.60 (25.2 – 28.0)	26.80 (24.80 – 28.50)	0.5413
Red cell distribution width (RDW-SD) (fL) ^a	50.90 (47.40 – 57.10)	51.00 (47.70 – 57.40)	0.7782
Red cell distribution width (RDW-CV) (%) ^a	14.30 (14.30 – 15.90)	13.80 (12.60 – 15.80)	0.1482
Platelet ($\times 10^3 / \mu\text{L}$) ^a	128.00 (72.0 – 186.0)	172 (119.0 – 229.0)	<0.0001
Plateletcrit (PCT) (%) ^a	0.15 (0.08 – 0.22)	0.20 (0.13 – 0.26)	<0.0001
Platelet distribution width (PDW) (fL) ^a	16.30 (14.30 – 19.60)	16.20 (13.60 – 19.05)	0.1544
Mean platelet volume (MPV) (fL) ^a	11.20 (10.20 – 12.30)	11.20 (10.10 – 12.20)	0.9878
Platelet large cell ratio (P-LCR) (%) ^a	35.70 (29.40 – 43.0)	35.50 (28.30 – 43.90)	0.7465
White blood cells (WBC) ($\times 10^3 / \mu\text{L}$) ^b	3.50 (2.20 – 5.35)	3.50 (2.40 – 4.80)	0.5078
Neutrophil ($\times 10^3 / \mu\text{L}$) ^a	1.00 (0.50 – 2.10)	1.00 (0.70 – 1.70)	0.9702
Lymphocyte ($\times 10^3 / \mu\text{L}$) ^a	1.80 (1.20 – 3.0)	2.20 (1.50 – 3.10)	0.8222

^aT-test; ^bMan-Whitney U; IQR=Interquartile range

Table 4. Demographic and haematological predictors of malaria infection status.

Predictor	Coeff. est.	Std. error	Z-value	OR (95% CI)	p-value
Intercept	1.45	0.66	2.19	4.264 (1.162 — 15.656)	0.028841
HGB	-0.11	0.06	-2.00	0.894 (0.801— 0.997)	0.045799
Age	-0.03	0.01	-4.26	0.966 (0.951— 0.981)	2.06*10⁻⁵
PCT	-5.03	1.32	-3.81	0.006 (0.0004 — 0.0869)	0.000139

The results were considered significant if $p \leq 0.05$.

exponential function to the estimated coefficient of a continuous variable (e^β) produces the odds ratio associated with unitary increments of the variable.

Anaemia and thrombocytopaenia comprise two of the most common complications associated with malaria. Anaemia and acute anaemia are defined as having Hb levels <11g/dl or <5g/dl,

respectively¹⁵, while thrombocytopenia is defined as platelet count $<150 \times 10^3/\mu\text{L}$ ¹⁶. 48.4% ($n=45/93$) of all malaria-infected individuals in this study were anaemic compared to 33.3% ($n=164/492$) of the control group ($p=0.0054$), with two malaria-infected participants matching the criteria for acute anaemia. In addition, 58.1% ($n=54/93$) of participants from the infected group were thrombocytopenic.

While all the variables included in the final model are statistically significant, that does not necessarily mean that the model is a good fit for the data. As logistic regression deals with odds and probabilities, we can use the model to predict the probability of being malaria-positive. In order to map our logistic regression values to one of the two response categories (infected or uninfected), we first defined a decision threshold ($p>0.21$) that balanced model sensitivity with specificity, and made our predictions based on this threshold. The model correctly identified 78.4% of malaria-negative individuals but only 50% those who were positive (Table 5).

Discussion

We investigated the effect of malaria on haematological parameters of people in three different demographic settlements: urban, peri-urban and rural communities. By 2050, it is predicted that 58% of people in sub-Saharan Africa will be living in urban areas, compared with approximately 40% currently¹⁷. This is expected to have a significant impact on the prevalence and clinical outcomes of infectious diseases like malaria as the increasing urban population further widens urban-rural economic and resource divides. In this study, people from the rural area recorded the highest prevalence of malaria compared to people from the urban and peri-urban areas, a finding that is consistent with results from other studies conducted in Ghana^{18,19}. Rural areas are often described as intense and perennial transmission areas as there are often several suitable *Anopheles* breeding sites available, coupled with poor access and/or adherence to vector control measures by rural inhabitants^{20,21}. The relatively younger age observed in the rural area may also be a contributing factor to the high prevalence observed in this study area, as children represent a high risk group for malaria infection²². In line with this, the majority of infected individuals in the rural area were children less than five years. Children under five years may not have a fully developed immune system and are, therefore, more susceptible to infections. Adults, on the other hand, have relatively stronger immune systems and often have partial

immunity to malaria from previous exposure²³. This might explain why the older generation recorded the lowest parasite densities.

Consistent with findings from other studies^{2,6,18}, this study also found significant differences in several red blood cell parameters between malaria-infected and uninfected study participants. *Plasmodium falciparum*, the parasite that causes the most severe form of malaria in humans, invades and multiplies inside red blood cells in a destructive cycle that is responsible for much of the severity and mortality associated with the disease^{24,25}. Haemolytic mechanisms are usually employed by the host immune system to eliminate parasitized red blood cells in a process that may lead to anaemia^{26,27}. Anaemia is considered to be one of the most common complications of malaria, especially in children and pregnant women²⁸. The present study revealed significantly lower Hb levels in the infected population compared to the uninfected group (Table 3); however, a substantial proportion of the uninfected study participants also met the criteria for anaemia. The suspected anaemia cases observed in the control population may be due in part to poor nutritional status, undetectable malaria infection or, to a lesser extent, helminth infections². Peripheral leukocyte or WBC counts have also been noted as being in the low to normal range during malaria, a phenomenon which is counterintuitive as one would expect increased production of WBCs during infection. Malaria-infected individuals have been reported to have significantly lower lymphocyte levels likely as a result of their withdrawal from the peripheral circulation and sequestration in lymph tissue, rather than actual depletion of the cell population²⁹. However, there are reports of both increased (leukocytosis)⁴ and decreased (leucopenia)^{2,7} WBC levels in malaria-infected individuals. The present study did not observe any significant changes in the WBCs levels of participants with malaria compared to the uninfected group. One of the most striking results from the study was the low levels of platelet and plateletcrit (a measure of total platelet mass) observed in malaria-infected study participants compared to uninfected participants, a finding that is consistent with results from other studies^{6,7,16}. This observation is particularly common in *Plasmodium falciparum*^{4,30} and *Plasmodium vivax* infections^{31,32}. In this present study, 58.1% of malaria-infected individuals were thrombocytopenic. There are several hypotheses about the reduction of platelets during malaria infection. This abnormality may be the result of blood clots developing in the bloodstream, thereby blocking small blood vessels. The intermittent clotting subsequently depletes the number of circulating platelets in the bloodstream³³. The reduction in platelet levels may also be attributable to an immune-mediated mechanism, whereby specific immunoglobulin G (IgG) produced as a result of the parasite invasion, forms a complex with parasite antigens. The resulting complex then binds to and damages platelets, with damaged platelets subsequently removed from circulation^{33,34}. Ultimately, plateletcrit was the strongest predictor of infection status, with 99.4% increased odds of being malaria-positive for each unit increase in plateletcrit levels. In spite of this, the

Table 5. Confusion matrix comparing the actual outcomes to the predicted outcomes.

	Negative	Positive
Pred. Neg.	382	46
Pred. Pos.	105	46

The predictions were classified using a decision threshold of $p > 0.21$

full predictive model performed rather poorly as only 50% of infected individuals were correctly classified as such. Further studies are needed to more precisely elucidate the relationship between malaria infection status and various host demographic and haematological parameters. Limitations of this study included a skewed gender-balance and a lack of information on recent medical histories as there are many other diseases and conditions that may affect haematological values and could potentially affect the interpretation of the results.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Impact of malaria on haematological parameters of urban, peri-urban and rural patients in the Ashanti region of Ghana. <https://doi.org/10.7910/DVN/NGBMZM>¹³

This project contains the following underlying data:

- Combined data.xlsx (raw demographic, haematological and parasitological data for all participants)
- Combined Data_Age.tab (raw demographic, haematological and parasitological data for all participants, including ages)
- Malaria Infected population.xlsx (raw demographic, haematological and parasitological data for malaria infected participants)
- Malaria Uninfected.xlsx (raw demographic, haematological and parasitological data for uninfected participants)

- Peri-Urban population.xlsx (raw demographic, haematological and parasitological data for participants in the peri-urban area)
- Rural population.xlsx (raw demographic, haematological and parasitological data for participants in the rural area)
- Urban population.xlsx (raw demographic, haematological and parasitological data for participants in the urban area)
- Data Dictionary.xlsx

Extended data

Harvard Dataverse: Replication Data for: Impact of malaria on haematological parameters of urban, peri-urban and rural patients in the Ashanti region of Ghana. <https://doi.org/10.7910/DVN/NGBMZM>¹³

This project contains the following extended data:

- CHRPE Participant Information Leaflet_Malaria.pdf
- Questionnaire- Malaria.pdf

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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We are grateful to the Kumasi South, Kuntanase and the Agona district hospitals for their support during data collection. We are also grateful to the KNUST Graduate Assistantship programme which supported AHM and SKA. KB, the corresponding author, is an AAS affiliate (2017–2022).

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Version 3

Reviewer Report 10 July 2020

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Kwadwo Asamoah Kusi 

Department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana

The authors have made significant improvements based on the last review, but remnants of the requested portions needing review remain. For example, the concluding statement of the abstract is expected to directly respond to the study object of comparing haematological indices across the three sites, but it still reports comparison of infected and uninfected persons.

Wherever this infected/uninfected comparison is made, it is not clarified whether it is for the entire data set from the three sites, or a per site analysis. These are minor issues that will greatly enhance the paper as it is.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology of infectious diseases, vaccine discovery and development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 16 June 2020

<https://doi.org/10.21956/aasopenres.14189.r27489>

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Deus S. Ishengoma 

Tanga Research Centre, National Institute for Medical Research (NIMR), Tanga, Tanzania

Abstract:

- The following statement; “Age was significantly associated with the odds of malaria positivity (OR: 0.97; CI:0.96 — 0.99)” should have p-value. Despite a narrow confidence interval, the OR is almost 1.0 which suggests that the association cannot be significantly different. Based on table 2, I still recommend to report adjusted OR rather than crude OR which can potentially suffer from bias and confounding.

Introduction:

- In the second statement, “Despite the decrease in incidence of mortality due to malaria in the last decade, the most recent World Malaria Report revealed that **two hundred and twenty-eight million** clinical cases of malaria were reported, resulting in no less than 405,000 deaths, the majority of which were in sub-Saharan Africa”, the number of cases should be written in figures (i.e 228 million).

Methods:

- The authors stated that, “Babies younger than six months, patients who were critically ill and those dissenting to give their consent were excluded from the study”. However, babies (<6 months old) are not mentioned in the revised manuscript. This should be corrected.
- I would recommend to add a separate sub-section on collection of demographic data. The details provided in the responses to my previous comments should be summarized and incorporated in the manuscript. “*The data, that is to say, the questionnaire were administered by the authors of this article and not by research assistants. Authors are very experienced in conducting similar surveys from different parts of the country and in other research projects. At the end of everyday all researchers came together to check the completeness of each individual questionnaire and match them to the lab specimen taken. Prior to the survey we had pre-tested the questionnaire in a community setting to ensure people understood and that questions were effective. Feedback were used to improve the questionnaire before the actual survey was carried out.*”

Results:

- For the following statement, “Study participants from the urban area reported the highest ITN usage (32.3%; $n=66/204$), with people from the peri-urban (29.2%; $n=57/195$) and rural areas (30.3%; $n=61/202$) reporting significantly lower percentages of usage”; please provide a p-value.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antimalarial drug resistance and Genomic epidemiology of malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 08 June 2020

<https://doi.org/10.21956/aasopenres.14189.r27488>

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Kwadwo Asamoah Kusi

Department of Immunology, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Legon, Ghana

- The authors have not provided the ethics approval number and also not indicated exclusion criteria that are likely to affect haematological parameters. E.g. being immunocompromised through HIV infection can affect haematological indices. Acknowledging the fact however, that authors have identified this as a study limitation.
- Table 1 should be re-organized to present information for each of the three hospitals used, and include a column with statistical analysis outcomes (p values) comparing parameters amongst the three sites. Analysis of the entire data set should follow this format, since comparing the measured parameters across the three transmission zones is what the study set out to do. I see from the tracked changes version that such results comparing the three sites were initially presented, but have now been removed. Knowing which parameters are different in Table 1 will be a useful guide for identifying potential confounders for regression analysis.
- The analysis should compare the different haematological, demographic and clinical parameters between the three sites. The regression analysis should predict malaria risk using the haematological parameters as independent variables, and correcting for potential confounders such as age and possibly the study site, in order for the analysis to directly answer the research question.
- Direct comparison of infected with uninfected persons for all data assumes that the different transmission settings will have no impact on outcomes. This analysis should be done per site before combining all data.
- Also, the table legend describes the study results. The legend should only describe how the information in the table was generated, not the results/data. Kindly remove all results from the legend. Figure 1 legend is a perfect example, with no data in legend, just a description of how the figure was created.
- Figure 1 needs the horizontal axis title. The vertical axis has parasites/ul

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology of infectious diseases, vaccine discovery and development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 09 Jul 2020

Kingsley Badu, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

We have responded to all the concerns raised by the reviewer. The major changes made in the manuscript are;

1. We provide information on the ethical committee and approval number. The Committee on Human Research Publication and Ethics of the Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital (CHRPE/KNUST), reviewed and approved the study protocol (Ref: CHRPE AP/018/18).
2. We have re-organized Table 1, which now shows the statistical comparison amongst the three sites for each parameter.
3. We have included Figure 1 horizontal axis title.

Thank you

Competing Interests: We declare no competing interest

Version 1

Reviewer Report 27 January 2020

<https://doi.org/10.21956/aasopenres.14060.r27312>

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Deus S. Ishengoma

Tanga Research Centre, National Institute for Medical Research (NIMR), Tanga, Tanzania

General comments:

The study aimed at assessing haematological changes among malaria patients residing in three different types of settlements. However, the study design and data collection methods could not be tailored to this overall aim. Thus, the manuscript needs major revisions before it can be accepted for publication.

Specific comments:

Abstract:

1. The design of this study cannot allow to investigate the changes in haematological parameters but rather to assess the status of the parameters at the time of the study. The first statement should be changed to take into account this fact.
2. I would recommend to change the way the results are presented in the abstract:
 1. The prevalence of malaria infection in the three settlements should be mentioned.
 2. The results of the analyses which compared malaria infected and non-infected patients should state which group had higher or lower haematological values rather than stating that there was a significant difference. This does not provide useful and adequate information to the readers.
 3. The results need to be reorganized to provide a more local flow of the findings. It may be useful to first compare all haematological parameters (overall) among infected vs non-infected patients; and then presenting the same values in infected (with or without symptoms) and non-infected asymptomatic patients from the three settlements.
3. The last statement in the abstract which states that, "Atypical results from routine haematological findings of anaemia and thrombocytopaenia, may be indicative of malaria, ..." is not supported by the results presented in the abstract. Furthermore, it is generally known that malaria infection (particularly severe malaria) is associated with anaemia and thrombocytopaenia. This statement needs to be revised, otherwise it can be dropped.

Introduction:

1. The authors should use the data presented in the most recent WHO report of 2019, and should also note that the report normally presents data of the past year. For example, WHO report of 2019 contains malaria data of 2018 and not the same year (2019).
2. In the second paragraph, the authors mentioned that infection with malaria parasites trigger haematological changes. Apart from providing some citations, they could not give a brief account of those changes. It is important to provide a reader with the type of changes and the mechanisms associated with such changes.

Methods:

1. This was a hospital-based study which targeted patients with symptoms of malaria but later the investigators decided to include asymptomatic relatives. Was this the original plan or it was adopted just to avoid bias?
2. What were the inclusion and exclusion criteria?
3. How were patients sampled and recruited? Did the investigators target all patients attended at the study hospitals?
4. When was the study conducted?
5. Study sites: how and why were sites selected for this study? What criteria were used to select the hospitals?

6. What were the justifications/rationale for using the formula presented on page 3 and malaria prevalence in determining the sample size? I think that the expected differences in haematological parameters among the three settlements should have been taken into account in determining the sample size.
7. How were demographic and clinical data collected? Who collected the data? And what tools were used and how was quality control of the data performed?
8. Since some haematological data are significantly influenced by environment factors (e.g. altitude) and nutrition, were these data collected? If not, why?
9. I would recommend to re-analyse the data to determine the impact of malaria on haematological parameters by linear/logistic regression or similar analysis to determine the potential contribution of malaria infection on different parameters with adjustment for different co-variables such as age, sex, residence etc.

Results:

1. I would recommend presenting a baseline table showing key demographics (sex, age, ITNs use), prevalence and parasite density in the three study settlements.
2. The results show that 75.5% of the participants were female. Was this deliberately planned to collect more samples from female participants? Due to the impact of sex on some parameters, this should have been avoided from the study design and sampling.
3. The age differences of participants were also different among participants from the three settlements. This should have also been avoided in the study design in order to get comparable groups.
4. I would recommend analysing the variations and impact of ITNs, malaria prevalence, age and settlement using logistic regression rather than χ^2 -test.
5. The results comparing different parameters in symptomatic and asymptomatic participants should also be presented.
6. On page 4, the authors stated that they tested for the relationship between anaemia and thrombocytopaenia in malaria-infected patients. However, it is not mentioned in the method how this was done. It is important to have details in the data analysis section to show how the different tests were done.

Discussion and conclusion:

1. The authors mentioned that they investigated haematological changes in malaria patients in three settlements. However, the nature and design of this study cannot allow to assess the trends and changes in such parameters, which can better be assessed in a well-designed longitudinal study; with a design such as cohort or case-control study.
2. The differences in haematological parameters reported in both infected and non-infected participants in the three settlements should be thoroughly discussed. The possible explanations for such differences must be provided.

3. The study had many limitations in terms of design (targeted symptomatic patients but later switched to collecting asymptomatic care-givers as well), selection criteria (biased age and sex of patients), lack of data on altitude of residence, socio-economic and nutritional status, and other factors with significant impacts on haematological parameters. These and other limitations need to be well reported and taken into account when drawing the conclusion of the study.

Other comments.

1. Under figure 1, it is mentioned that analysis was done using R software but this was not mentioned in the data analysis section.
2. The authors should revise the document to take care of typos.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antimalarial drug resistance and Genomic epidemiology of malaria

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Jun 2020

Kingsley Badu, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Response to reviewers' comments

We are very grateful for the comments provided by the reviewers of this manuscript. Please see below for our responses.

- **Abstract:** The design of this study cannot allow to investigate the changes in haematological parameters but rather to assess the status of the parameters at the time of the study. The first statement should be changed to take into account this fact.

Response: *We appreciate the reviewer's comment and agree that investigating changes in haematological parameters was outside the scope of this study. We have changed the wording to better reflect the actual aims of the study*

- I would recommend to change the way the results are presented in the abstract:
 - The prevalence of malaria infection in the three settlements should be mentioned.

Response: *We have now included this information in the abstract.*

- The results of the analyses which compared malaria infected and non-infected patients should state which group had higher or lower haematological values rather than stating that there was a significant difference. This does not provide useful and adequate information to the readers.

Response: *We have revised the text to include information on which group had higher or lower haematological values.*

- The results need to be reorganized to provide a more logical flow of the findings. It may be useful to first compare all haematological parameters (overall) among infected vs non-infected patients; and then presenting the same values in infected (with or without symptoms) and non-infected asymptomatic patients from the three settlements.

Response: *We have re-organised the results presented in the abstract to provide a more logical flow of the main findings of the study.*

- The last statement in the abstract which states that, "Atypical results from routine haematological findings of anaemia and thrombocytopaenia, may be indicative of malaria, ..." is not supported by the results presented in the abstract. Furthermore, it is generally known that malaria infection (particularly severe malaria) is associated with anaemia and thrombocytopaenia. This statement needs to be revised, otherwise it can be dropped.

Response: *We have 'dropped' the statement in line with the reviewer's suggestion.*

Introduction:

- The authors should use the data presented in the most recent WHO report of 2019, and should also note that the report normally presents data of the past year. For example, WHO report of 2019 contains malaria data of 2018 and not the same year (2019).

Response: *We have revised the manuscript to reflect the most recent malaria data available from the WHO.*

- In the second paragraph, the authors mentioned that infection with malaria parasites trigger haematological changes. Apart from providing some citations, they could not give a brief account of those changes. It is important to provide a reader with the type of changes and the mechanisms associated with such changes.

Response: *We have now provided a brief account of the most common haematological changes associated with malaria.*

Methods:

- This was a hospital-based study which targeted patients with symptoms of malaria but later the investigators decided to include asymptomatic relatives. Was this the original plan or it was adopted just to avoid bias?

Response: *The study recruited all study participants at the beginning of the study.*

- What were the inclusion and exclusion criteria?

Response: *Since the study was carried out in the hospital laboratories, prospective participants were all who had been referred to the hospital laboratory for tests to be conducted, as well as any family members or friends who accompanied them. Babies younger than six months, patients who were critically ill and those dissenting to give their consent were excluded from the study. We have revised the text to make this information clearer.*

- How were patients sampled and recruited? Did the investigators target all patients attended at the study hospitals?

Response: *We targeted all patients who had been referred to the hospital's laboratory, together with any accompanying persons. And of-course only those who gave consent to participate*

- When was the study conducted?

Response: *The study was conducted from January to December 2018.*

- Study sites: how and why were sites selected for this study? What criteria were used to select the hospitals?

Response: *We selected the hospitals based on our knowledge of the malaria transmission cline. This ranges from, AGH – moderately high; KGH – moderate; KSH – relatively low*

- What were the justifications/rationale for using the formula presented on page 3 and malaria prevalence in determining the sample size? I think that the expected differences in haematological parameters among the three settlements should have been taken into account in determining the sample size.

Response: *Unfortunately, there isn't any prior information on differences in haematological parameters of residents of the three settlements. However, the malaria prevalence used was from a study that sought to predict malaria infection using the haematological parameters of patients from a hospital in a nearby town that can be classified as a peri-urban community.*

- How were demographic and clinical data collected? Who collected the data? And what tools were used and how was quality control of the data performed?

Response: *The data, that is to say, the questionnaire were administered by the authors of this article and not by research assistants. Authors are very experienced in conducting similar surveys from different parts of the country and in other research projects. At the end of everyday all researchers came together to check the completeness of each individual questionnaire and match them to the lab specimen taken. Prior to the survey we had pre-tested the questionnaire in a community setting to ensure people understood and that questions were effective. Feedback were used to improve the questionnaire before the actual survey was carried out.*

- Since some haematological data are significantly influenced by environment factors (e.g. altitude) and nutrition, were these data collected? If not, why?

Response: *We appreciate the reviewer's comments on altitude and nutrition. However altitude will not be of any effect in our study sites, all of which are between 250m to 300m asl. Unlike the mountainous areas many parts of Kenya and Tanzania, Ghana is relatively flat. We did not collect information on other variable such as nutrition or intestinal helminths and others. We have been studying these communities for a while, we have other studies that focus on nutrition and other infections. However, this study focused on malaria. We do not expect the influence of 'other environmental' parameters to significantly differ in the population.*

- I would recommend to re-analyse the data to determine the impact of malaria on haematological parameters by linear/logistic regression or similar analysis to determine the potential contribution of malaria infection on different parameters with adjustment for different co-variables such as age, sex, residence etc.

Response: *We have re-analysed the data using logistic regression and believe the results are now easier to interpret (Tables 2 & 4).*

Results:

- I would recommend presenting a baseline table showing key demographics (sex, age, ITNs use), prevalence and parasite density in the three study settlements.

Response: *We appreciate the opportunity to include any additional information that makes our data easier to follow. We have now included a baseline table of demographic information (Table 1).*

- The results show that 75.5% of the participants were female. Was this deliberately planned to collect more samples from female participants? Due to the impact of sex on same parameters, this should have been avoided from the study design and sampling.

Response: *We appreciate the concern raised by the reviewer. The main aim of the study was to assess the status of the haematological parameters. At the time of recruitment, these were the respondents who agreed to take part in the study after they were taken through the consenting process hence the high numbers of female participants were not deliberately planned. This notwithstanding, we entirely agree that gender category should ideally be as balanced it is practicable.*

- The age differences of participants were also different among participants from the three settlements. This should have also been avoided in the study design in order to get comparable groups.

Response: *This is a cross sectional study, where individual sent to the lab were approached for consent, we could not discriminate among willing participants. The age groups presented are study participants there were presented at the hospital and consented to participate. That said, future studies will take this into account.*

- I would recommend analysing the variations and impact of ITNs, malaria prevalence, age and settlement using logistic regression rather than χ -test.

Response: *We are very grateful to the reviewer for this suggestion. We have now re-analysed the data using logistic regression (Tables 2 & 4).*

- The results comparing different parameters in symptomatic and asymptomatic participants should also be presented.

Response: *The only available infection categories were the malaria-positive and malaria negative groups. We intended to further categorise the study participants into symptomatic and asymptomatic based on temperature readings. Unfortunately, our temperature readings could not reliably distinguish between the two categories. This is a limitation of this study.*

- On page 4, the authors stated that they tested for the relationship between anaemia and thrombocytopaenia in malaria-infected patients. However, it is not mentioned in the method how this was done. It is important to have details in the data analysis section to show how the different tests were done.

Response: *We have revised this section and completely removed the analysis on the relationship between anaemia and thrombocytopaenia in malaria-infected patients.*

Discussion and conclusion:

- The authors mentioned that they investigated haematological changes in malaria patients in three settlements. However, the nature and design of this study cannot allow to assess the trends and changes in such parameters, which can better be assessed in a well-designed longitudinal study; with a design such as cohort or case-control study.

Response: *We have re-worded the statements to reflect reporting on the status of the hematological parameters, not necessarily the "changes".*

- The differences in haematological parameters reported in both infected and non-infected participants in the three settlements should be thoroughly discussed. The possible explanations for such differences must be provided.

Response: *We discuss possible reasons for differences in haematological parameters between all infected and non-infected participants. However, it is now apparent that "settlement" is not significantly associated with malaria positivity. It is, correlated with "age" and this is likely the reason for the positive result using the χ^2 -test.*

- The study had many limitations in terms of design (targeted symptomatic patients but later switched to collecting asymptomatic care-givers as well), selection criteria (biased age and sex of patients), lack of data on altitude of residence, socio-economic and nutritional status, and other factors with significant impacts on haematological parameters. These and other limitations need to be well reported and taken into account when drawing the conclusion of the study.

Response: *We thank the reviewer for bringing up this very important point. We have added a brief section on the limitations of this study towards the end of the manuscript. However, we maintain that 1. Altitude is not an issue in our study settings as it is in Tanzania and other East African altitudinal transects. The socio-economic and nutritional status are NOT severe confounding factors as in other studies we know they are similar in the populations.*

Other comments.

- Under figure 1, it is mentioned that analysis was done using R software but this was not mentioned in the data analysis section.

Response: *This has been stated appropriately.*

- The authors should revise the document to take care of typos.

Response: *Typos have been taken care of.*

Competing Interests: No

Reviewer Report 18 September 2019

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The manuscript by Mutala *et al.* assesses malaria-related haematological changes in three different transmission zones and reports statistically significant differences in some of the measured indices between malaria infected and uninfected persons, and higher levels of some indices in infected persons in peri-urban areas compared to levels in persons in the rural and urban areas.

The paper will require some significant revision to better communicate the study findings.

Major comments:

1. Authors describe the three study locations as rural, peri-urban and urban. It is unclear from the write up why it is necessary to compare these three demographic settings for haematological differences. If this is being done because of possible differences in transmission intensity however, then it may be more appropriate for the authors to present some current malaria transmission data from these sites and on that basis rather, proceed to refer to these as different transmission intensity settings.
2. Authors should clearly state whether this is a cohort or case control study. Authors should also describe any exclusion criteria that were applied during sampling as the conclusions of the study and potential application of the data may only be valid if certain exclusion criteria were applied. There are some common conditions/infections that could also impact these haematological indices, and without accounting for these, it may not be appropriate to conclude that the observed differences in the indices are due to malaria infection.
3. The manner of results presentation makes comprehension difficult. Authors refer to all 598 participants whose samples were analyzed as patients at some times (second paragraph of the results section), and even though on the basis of the study design, participants can be categorized into those who reported clinically sick, those with asymptomatic infections and those who may be uninfected (by microscopy), no such breakdown in terms of age within these three groups is given in the first subsection of the results. For example, how many of the persons accompanying sick people to the hospital were parasite positive?
4. Most of the subsequent comparisons refer to an infected group and a control group, and it is unclear whether the infected group is in reference to the clinically ill group alone or a combined group of clinically ill and asymptomatic persons. If the latter, what was the reason for combining these two distinct groups as they are likely to have very different haematological profiles?
5. The age range of study participants is not provided. The youngest age groups is given as 0 – 5 years (Table 2).
6. Table 1 seems to be a comparison between infected and uninfected participants from all 3 study sites, but if the goal of the study was to assess differences across different settlements, then why is

it relevant to combine data from the three study sites and compare between infected and uninfected persons? A combined presentation of information in Table 1 and Table 3 will be more consistent with the aim of this study, to compare infected and uninfected (maybe sick and not sick rather) for each community.

7. Overall, the data presentation needs to be overhauled. That aside, the presented data does not justify the conclusion that changes in haematological indices can be used as indicative factors for malaria, especially when they depended on the community in which they were measured. I would agree with authors if the indices were consistently different between sick and not-sick persons in all three study sites. Moreover, the unavailability of exclusion criteria in this write up makes it difficult to agree that these observations are due to malaria infections.

Minor comments:

1. Abstract, penultimate line before the conclusions section – "...levels amongst patients from urban, ...", not "...between patients...".
2. "Thrombopaenia", not "thrombopenia", to be consistent with all other British spelling of words (parasitaemia, haematological).
3. Authors should provide the protocol approval number.
4. The statistical analysis section should be updated to reflect the fact that the R software was also used for some graphics (Figure 1).
5. Second sentence of the results section should read: "In total, 598 participant samples...", not "...participants samples...".
6. "Kruskal-Wallis", not "Kruskall-Wallis" (statistical analysis section, Legend to Table 3).
7. Discussion section, lines 5/6 – which group do the authors refer to as non-malaria patients?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology of infectious diseases, vaccine discovery and development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 05 Jun 2020

Kingsley Badu, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

We are very grateful to the reviewer, his comments has enabled us clarify and improve the quality of the manuscript. Here we provide point by point response to the comments raised by the reviewer.

Major Comments

- Authors describe the three study locations as rural, peri-urban and urban. It is unclear from the write up why it is necessary to compare these three demographic settings for haematological differences. If this is being done because of possible differences in transmission intensity however, then it may be more appropriate for the authors to present some current malaria transmission data from these sites and on that basis rather, proceed to refer to these as different transmission intensity settings.

Response: *We thank the reviewer for this comment. It makes more biological sense to compare on the basis of transmission. We have provided background information on the malaria transmission intensity and associated references in the manuscript. Whereas people in the Agona area (we describe as rural and high transmission) receive over 11,000 mosquito bites with over 800 infectious bites, the Kumasi area receives about 400 bites and about 200 infectious bite per year. The Kuntanase area is moderate in between the two [Abonuusum et al 2011, and Basing et al 2014] The citations are below*

- Authors should clearly state whether this is a cohort or case control study. Authors should also describe any exclusion criteria that were applied during sampling as the conclusions of the study and potential application of the data may only be valid if certain exclusion criteria were applied. There are some common conditions/infections that could also impact these haematological indices, and without accounting for these, it may not be appropriate to conclude that the observed differences in the indices are due to malaria infection.

Response: *We thank the reviewer for bringing up this very important point. This was an observational study with cross-sectional sampling design in which participants were recruited from three hospitals located in different transmission intensity settings. Since participants were recruited from hospitals, we referred to them as "patients"; however, it is now apparent that this term is misleading so we have removed most references to "patients" from the manuscript. We are also aware that many viral and bacterial infections present with malaria-like symptoms, and may also affect haematological indices similarly. This is one of the limitations of our study.*

- The manner of results presentation makes comprehension difficult. Authors refer to all 598 participants whose samples were analysed as patients at some times (second paragraph of the results section), and even though on the basis of the study design, participants can be categorized into those who reported clinically sick, those with asymptomatic infections and

those who may be uninfected (by microscopy), no such breakdown in terms of age within these three groups is given in the first subsection of the results. For example, how many of the persons accompanying sick people to the hospital were parasite positive?

Response: *We have re analysed the data based on reviewer's comments also echoed by Reviewer 1.*

We believe the data is now much more clearer to understand. We refer to two categories as malaria infected and not infected due to challenges in distinguishing between symptomatic and asymptomatics earlier described

- Most of the subsequent comparisons refer to an infected group and a control group, and it is unclear whether the infected group is in reference to the clinically ill group alone or a combined group of clinically ill and asymptomatic persons. If the latter, what was the reason for combining these two distinct groups as they are likely to have very different haematological profiles?

Response: *The infected group refers to those who tested positive for malaria, regardless of symptoms. We pooled symptomatic and asymptomatic participants together as there was no other way to distinguish between the two groups due to problems with our temperature readings.*

- The age range of study participants is not provided. The youngest age groups is given as 0 – 5 years (Table 2).

Response: *The age range of study participants has been presented in Table 1. Participants were grouped into three categories: 0–5 years, 6–14 years and 15+ years.*

- Table 1 seems to be a comparison between infected and uninfected participants from all 3 study sites, but if the goal of the study was to assess differences across different settlements, then why is it relevant to combine data from the three study sites and compare between infected and uninfected persons? A combined presentation of information in Table 1 and Table 3 will be more consistent with the aim of this study, to compare infected and uninfected (maybe sick and not sick rather) for each community.

Response: *We are grateful for this comments from the reviewer to improve our manuscript. After re-analysing the data using logistic regression, it is clear that “settlement” is not significantly associated with malaria positivity. It is, however, correlated with “age” and this is likely the reason for the positive result using the χ^2 -test. As settlement is no longer a predictor of malaria positivity, we decided to completely remove Table 3.*

- Overall, the data presentation needs to be overhauled. That aside, the presented data does not justify the conclusion that changes in haematological indices can be used as indicative factors for malaria, especially when they depended on the community in which they were measured. I would agree with authors if the indices were consistently different between sick and not-sick persons in all three study sites. Moreover, the unavailability of exclusion criteria in this write up makes it difficult to agree that these observations are due to malaria infections.

Response: *Our data presentation has been ‘overhauled’ as recommended by reviewers, data has been re analyzed. Thus the conclusion has been subsequently re-worded and we have included the exclusion criteria in the methods section as requested.*

Minor comments:

- Abstract, penultimate line before the conclusions section – "...levels amongst patients from urban, ...", not "...between patients...".

Response: *We thank the reviewer for this comment. Sentence corrected as suggested.*

- "Thrombopaenia", not "thrombopenia", to be consistent with all other British spelling of words (parasitaemia, haematological).

Response: *We thank the reviewer for this observation. The manuscript has been revised to effect these changes.*

- Authors should provide the protocol approval number.

Response: *The approval number has been included in the manuscript.*

- The statistical analysis section should be updated to reflect the fact that the R software was also used for some graphics (Figure 1).

Response: *This has been included in the revised manuscript.*

- Second sentence of the results section should read: "In total, 598 participant samples...", not "...participants samples...".

Response: *The proposed correction has been effected in the manuscript.*

- "Kruskal-Wallis", not "Kruskall-Wallis" (statistical analysis section, Legend to Table 3).

Response: *This correction has been effected in the manuscript. Thank you.*

- Discussion section, lines 5/6 – which group do the authors refer to as non-malaria patients?

Response: *The sentence has been modified in the manuscript.*

Abonuusum A, Owusu-Daako K, Tannich E, May J, Garms R, Kruppa T. Malaria transmission in two rural communities in the forest zone of Ghana. *Parasitol Res.* 2011;108(6):1465-1471. doi:10.1007/s00436-010-2195-1

A.W. Basing*, S. Tay. Malaria transmission dynamics of the Anopheles mosquito in Kumasi, Ghana. *16th ICID Abstracts / International Journal of Infectious Diseases* 21S (2014) 1–460

Competing Interests: No