



RESEARCH ARTICLE

Impact of malaria on haematological parameters of urban, peri-urban and rural patients in the Ashanti region of Ghana: a cross-sectional study [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: This study aimed at investigating haematological changes in malaria patients across different demographic settlements. 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 rarely been studied.

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. Giemsa-stained blood smears were examined to detect and quantify malaria parasitaemia, while haematological parameters were measured using a haematology analyser.

Results: The rural settlement had the highest malaria prevalence compared to the other study communities ($p=0.009$). The difference in parasite densities across the three communities was also significant ($p=0.0149$). When the malaria-infected population was compared to the uninfected, there were differences in red blood cell count ($p=0.0170$), haemoglobin levels ($p=0.0165$), mean corpuscular volume ($p=0.0139$) and platelet counts ($p<0.0001$). The difference in median white blood cell (p -value <0.0001), neutrophil (p -value <0.0001) and lymphocyte (p -value <0.0269) count were significantly higher in infected patients from the peri-urban area compared to malaria patients from the rural and urban areas. There were also significant differences in platelet ($p=0.0002$), plateletcrit ($p=0.0041$), mean platelet volume ($p=0.0009$) and platelet large cell ratio ($p=0.0046$) levels between patients from the urban, peri-urban and rural areas.

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Conclusions: Patients infected with malaria generally had low red blood cell, haemoglobin and platelets in comparison to uninfected patients. There were also significant differences in several haematological parameters between malaria-infected patients from the three demographic settlements. Atypical results from routine haematological assays, especially findings of anaemia and thrombocytopenia, may be indicative of malaria and, in cases where the infection is asymptomatic, may improve diagnosis by prompting a more thorough search for the parasite in the peripheral circulation.

Keywords

malaria, anaemia, parasitaemia, WBC count, thrombocytopenia,

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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 malaria cases increased by about two million in 2018 compared to 2017, resulting in approximately 435,000 deaths, the majority of which were reported in sub-Saharan Africa¹.

The introduction of malaria parasites into the host peripheral blood by an infected female *Anopheles* mosquito triggers changes in many host haematological parameters. These changes may subsequently affect the general physiology of the host, resulting in a number of clinical manifestations^{2,3}. In fact, the most common complications arising from malaria are associated with changes in host haematological parameters that play a role in malaria pathogenesis, with thrombocytopenia being the most common⁴. Haematological parameters that are most often affected include the relative numbers of circulating cell types such as platelets, granulocytes, lymphocytes and erythrocytes, as well as parameters like haemoglobin concentration. A recent study showed a significant reduction in platelet, erythrocyte and leukocyte levels in malaria-infected study participants compared to the control group⁵. Moreover, Kotepui *et al.* (2014) reported that low platelet, white blood cell (WBC) and lymphocyte counts are important predictors of malaria infection and, when used with other clinical methods, could improve malaria diagnosis and treatment⁶. Yet another study has reported elevated leukocyte levels in the malaria-infected group compared to uninfected study participants⁷.

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

Methods

This is a hospital-based study. In order to avoid bias and prevent including only 'sick' or symptomatic participants, we extended our sampling to include 'healthy' participants who accompanied their relatives or friends to the hospital. This increased the chance of having both symptomatic and asymptomatic participants.

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 (KNUST) and the Komfo Anokye Teaching Hospital (CHRPE/KATH). All study participants provided written informed consent prior to study enrolment, with parental or guardian consent obtained for children.

Study sites

We carried out a hospital-based, cross-sectional study that 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 kilometers 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.

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%¹⁰.

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

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 patients accessing healthcare at the various hospitals. They included patients referred to the laboratory for malaria test 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*¹¹. An interpreter was employed to translate the written document into their local dialect (Akan) for those who could not read. In addition, thumbprints were obtained for those who could not sign/write. Patients who were critically ill and those refusing consent were excluded from the study. All age groups were considered for the study except infants under six months. Demographic data such as malaria drug use and insecticide-treated net (ITN) use were obtained from participants using a semi-structured questionnaire, a copy of which is provided as *Extended data*¹¹.

Haematological analysis

From each participant, 2 ml of venous blood was drawn by a trained phlebotomist in the hospital laboratory as part of their routine medical care. This was transferred into an EDTA tube with a unique patient 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 $\times 100$ 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 \times total WBC in 1 μL of blood). A slide was only declared negative when no malaria parasite was seen after scanning 100 high power fields (HPFs).

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 analysis. 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. Categorical data on community and age groups were compared using Pearson's chi-squared test. Results were considered statistically significant if $p \leq 0.05$.

Results

Three samples had demographic data only and thus were excluded completely. In total, 598 participants samples were examined for parasite prevalence and density of infections. A further 16 were excluded from the haematological analysis because the sample obtained was either too small or the haematological indices were not determined.

Out of the 598 analysed in the study, 75.4% ($n=451$) were female¹¹. While the overall median age was 27 years ($IQR=19-40$), there was a statistically significant difference in the age profiles of patients from the three study sites ($p<0.0001$). Patients 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 ($IQR=19.5-41.5$). Patients in the urban area were the oldest, with a median age of 29 years ($IQR=22.5-43.0$). This observation is conceivable because, typically, the youth tend to leave their rural communities in search of jobs as they get older. 16% ($n=93$) of study participants had *Plasmodium falciparum* infection, confirmed by microscopy, while the remaining uninfected patients were used as controls.

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 was no significant difference in the median counts of neutrophils, lymphocytes or WBCs between the infected and non-infected groups; however, malaria-infected patients in our study population ($n=93$) 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 control patients (Table 1).

ITN usage and malaria prevalence vary significantly with age and settlement

There was a significant relationship between ITN usage and malaria prevalence ($\chi^2=48.41$; $p<0.0001$). When we tested the relationship between these two variables and age or settlement, we found a statistically-significant association between age and ITN usage ($p<0.0001$), with children less than five years constituting the majority of those who slept under insecticide-treated mosquito nets, while children between the ages of six and 14 (inclusive) recorded the lowest ITN usage. Malaria prevalence was also significantly related to age ($p<0.0001$), with the highest prevalence seen in children from six to 14 years of age (Table 2). Furthermore, there was a significant difference in the parasite densities of infected patients 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 patients 6–14 years, with a median density of 504 ($IQR=160-4139$), and patients ≥ 15 years, with a median density of 24.5 ($IQR=13-40$).

As shown in Table 2, ITN usage was also significantly related to settlement type ($p=0.0017$). Patients from the urban area reported the highest ITN usage (32.3%; $n=66/204$), with patients from the peri-urban (29.2%; $n=57/195$) and rural areas (30.3%; $n=61/202$) reporting significantly lower percentages of usage. Malaria prevalence was similarly significantly related to settlement type ($p=0.0144$). In this case, however, the highest malaria prevalence was recorded in rural patients (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 a significant difference in the parasite densities of infected patients across the three communities ($H=8.41$; $p=0.0149$). Patients 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).

Haematological indices differ significantly in patients from different areas

The median haematological parameter values of malaria-infected patients from the three study areas were compared

Table 1. 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 2. Relationship between age & settlement type and insecticide-treated net (ITN) usage & malaria prevalence.

	ITN usage (%)	χ^2	P-value	Malaria prevalence	χ^2	P-value
Age						
0–5	58.9			26.8		
6–14	28.9			34.8		
15+	30.2	34.34	<0.0001	12.7	21.29	<0.0001
Settlement						
Urban	32.3			11.8		
Peri-urban	29.2			13.3		
Rural	30.3	10.73	0.0017	21.3	8.05	0.0179

χ^2 is the chi-squared value. The results were considered significant if $p \leq 0.05$.

using the Kruskal-Wallis test. The difference in median WBC ($p < 0.0001$), neutrophil ($p < 0.0001$) and lymphocyte ($p < 0.0269$) counts were significantly different across the three communities and higher in infected patients from the peri-urban area compared to malaria patients from the rural and urban areas. Red blood cell indices such as MCV ($p = 0.0006$), RDW-SD ($p = 0.0005$) and RDW-CV ($p = 0.0218$) were significantly different across the three communities, with the lowest values recorded from malaria patients from the peri-urban area compared to those from the rural and urban areas. There were also significant differences in platelet ($p = 0.0002$), plateletcrit ($p = 0.0041$), mean platelet volume ($p = 0.0009$) and platelet large

cell ratio ($p = 0.0046$) levels between patients from the urban, peri-urban and rural areas (Table 3).

Anaemia and thrombocytopenia comprise two of the most common complications associated with malaria. Anaemia and acute anaemia are defined as having Hb levels $< 11\text{g/dl}$ or $< 5\text{g/dl}$, respectively¹², while thrombocytopenia is defined as platelet count $< 150 \times 10^3 / \mu\text{L}$ ¹³. It was found that 48.4% ($n = 45/93$) of all malaria-infected patients in this study were anaemic compared to 33.3% ($n = 164/492$) of the control group ($p = 0.0054$), with two malaria-infected patients matching the criteria for acute anaemia. In addition, 58.1% ($n = 54/93$) of patients from the infected

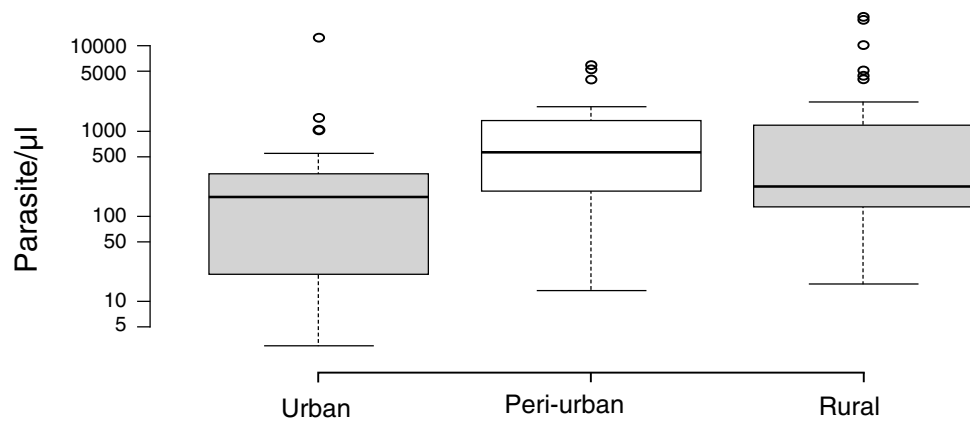


Figure 1. Parasite density across the three types of settlements. Centre lines show the median counts; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Widths of boxes are proportional 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 3. Haematological parameter levels in infected patients from the three communities.

Variable	Urban Median (IQR)	Peri-urban Median (IQR)	Rural Median (IQR)	P-value
Red blood cells (RBC) ($\times 10^6 /\mu\text{L}$) ^a	4.16 (3.50 – 4.39)	4.12 (3.81– 4.89)	4.23 (3.72 – 4.67)	0.3758
Haemoglobin (g/dL) ^a	10.60 (8.85–11.45)	11.70 (10.30–12.90)	11.10 (9.30–12.45)	0.1455
Hematocrit (%) ^a	40.80 (33.20– 44.30)	38.05 (34.40 – 41.60)	41.40 (36.10–44.85)	0.2797
Mean corpuscular volume (MCV) (fL) ^a	97.45 (90.20–103.5)	90.35 (86.4–92.0)	97.2 (90.25–103.0)	0.0006
Mean corpuscular haemoglobin (MCH) (pg) ^b	97.45 (90.20–103.15)	90.35(86.40–92.20)	97.20 (92.25–103.0)	0.3488
Red cell distribution width (RDW-SD) (fL) ^b	55.95(50.20– 66.00)	47.40 (44.80–51.30)	50.90 (48.70–57.30)	0.0005
Red cell distribution width (RDW-CV) (%) ^b	15.00(13.70–16.50)	14.45(13.40–16.30)	13.60(12.30–15.05)	0.0218
Platelet ($\times 10^3/\mu\text{L}$) ^b	121.50 (87.0–87.0)	195.50 (138.0–233.0)	103 (55.0–170)	0.0002
Plateletcrit (PCT) (%) ^a	0.12 (0.08– 0.17)	0.24 (0.16– 0.28)	0.12 (0.06– 0.18)	0.0041
Platelet distribution width (PDW) (fL) ^a	16.00(13.70–19.80)	15.95(14.60–19.40)	17.60 (15.05–19.65)	0.3497
Mean platelet volume (MPV) (fL) ^a	10.30 (9.45–11.15)	12.00 (10.90–12.50)	11.30 (9.90–12.15)	0.0009
Platelet large cell ratio (P-LCR) (%) ^a	29.90 (24.15–38.75)	41.55 (34.60–46.50)	34.70 (29.90–41.95)	0.0046
White blood cells (WBC) ($10^3/\mu\text{L}$) ^b	1.85 (1.35–3.60)	4.70 (3.40–6.70)	3.50 (2.35–5.20)	<0.0001
Neutrophil ($10^3/\mu\text{L}$) ^b	0.50 (0.30–1.30)	2.20 (1.60–3.30)	0.90 (0.50–1.55)	<0.0001
Lymphocyte ($10^3/\mu\text{L}$) ^a	0.90 (0.30–2.40)	2.10 (1.40–2.80)	2.00 (1.40–3.50)	0.0269

^aANOVA; ^bKruskal-Wallis test; IQR=Interquartile range

group were thrombocytopenic. We therefore tested the relationship between these two conditions and settlement type to determine if the occurrence of either anaemia or thrombocytopenia in malaria-infected patients was influenced by the type of settlement. The prevalence of thrombocytopenia was significantly influenced by the type of settlement ($p=0.0006$),

with malaria-infected patients in the urban area recording the highest prevalence. There was a strong relationship between anaemia and settlement type, although it was not statistically significant ($p=0.072$). Similar to thrombocytopenia, the highest prevalence of anaemia was observed in patients from the urban area (Table 4).

Table 4. Relationship between anaemia & thrombocytopenia and settlement type.

Settlement	Anaemia (%)	χ^2	P-value	Thrombocytopenia (%)	χ^2	P-value
Urban	66.7			75.0		
Peri-urban	52.9			26.9		
Rural	46.5	5.25	0.072	67.4	14.74	0.0006

χ^2 is the chi-squared test statistic. The results were considered significant if $p \leq 0.05$.

Discussion

We investigated haematological changes in malaria patients across three different demographic settlements: urban, peri-urban and rural communities. Consistent with findings from other studies^{2,6,14}, this study found significant differences in several red blood cell parameters between malaria-infected and non-malaria patients. *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^{15,16}. Haemolytic mechanisms are usually employed by the host immune system to eliminate parasitized red blood cells in a process that may lead to anaemia^{17,18}. Anaemia is considered to be one of the most common complications of malaria, especially in children and pregnant women¹⁹. The present study revealed significantly lower Hb levels in the infected population compared to the uninfected group (Table 1); 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 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. The lack of increased numbers of circulating WBCs is believed to be due to the redistribution and sequestration of leukocytes in organs like the spleen, rather than actual depletion of the cell population²⁰. 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 patients 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 patients compared to uninfected patients, a finding that is consistent with results from other studies^{6,7,13}. This trend has been well recognized and consistently found in *Plasmodium falciparum*^{4,21} and *Plasmodium vivax* infections^{22,23}. In this present study, 58.1% of malaria-infected patients 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 bloodstream²⁴. 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^{24,25}.

By 2050, it is predicted that 58% of people in sub-Saharan Africa will be living in urban areas, compared with approximately 40% currently²⁶. 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, patients from the rural area recorded the highest prevalence of malaria compared to patients from the urban and peri-urban areas, a finding that is consistent with results from other studies conducted in Ghana^{14,27}. 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^{28,29}. 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 infection³⁰. 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 exposure³¹. This might explain why the older generation recorded the lowest parasite densities. A comparison of haematological parameters of malaria-infected patients across the three areas revealed significant differences in the levels of key platelet and WBC indices, including plateletcrit, platelet, WBC, lymphocyte and neutrophil counts. Interestingly, higher levels of all the above blood indices were recorded in patients from the peri-urban area compared to those from the rural and urban areas, though the significance of this observation is unclear at present.

Observation of abnormal values of haematological parameters that are associated with malaria infection may prompt a more vigilant search for the parasite in routine microscopic diagnosis and could help avoid missing cases of malaria. Furthermore,

differences in the haematological parameters of malaria patients can also serve as an auxiliary criterion for malaria diagnosis, especially in cases of low parasite density. With the increasing rates of urbanisation in Africa, it is more important than ever to better understand the impact of such socio-geographic and demographic changes on infectious diseases.

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>¹¹

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>¹¹

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).

Grant information

AHM and SKA received graduate assistantship from the KNUST College of Science. KB received funds for laboratory supplies from the Africa Research Excellence Fund (AREF) Research Development Fellowship [MRF-157-0007-F-BADU]. Kingsley Badu is an Affiliate of the African Academy of Sciences.

Acknowledgements

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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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 thrombocytopenia 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-variates 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 some 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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Kwadwo Asamoah Kusi 

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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):14651471. 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