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ORIGINAL ARTICLE

Immunological, haematological, and clinical attributes of rural and urban malaria: a case–control study in Ghana

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Abstract To compare clinical presentations, haematological and immunological parameters in urban and rural malaria patients. Clinically suspected malaria patients, resident in either rural or urban communities, were selected from seven health facilities in the Greater Accra region of Ghana. For each suspected malaria patient, parasites were detected microscopically and quantified subsequently. In each study site, an equal number of cases and age-matched controls were selected. In both cases and controls, clinical presentations, nutritional status, haematological, and immunological parameters were profiled. A total of 149 malaria patients and 149 nonmalaria controls were selected. Compared to rural dwellers with malaria, parasitaemia was significantly higher in both males and females and in the various age groups in urban dwellers with malaria. Additionally, mean lymphocytes, haemoglobin, haematocrit, mean cell haemoglobin, platelets, and mean platelet volume levels were significantly lower in urban dwellers with malaria. However, TNF-α, IL-6, and IL-12 levels in urban dwellers with malaria were significantly higher, while IL-10, $CD4^+$, $CD3^+$, $CD8^+$ T-cells levels and $CD4^+$/ $CD3^+$ ratio were significantly lower in urban dwellers with malaria. Furthermore, chills, diarrhoea, fever, and pallor were significantly associated with urban dwellers with malaria. This study concluded that urban dwellers are more prone to severe malaria while rural dwellers tend to have more measured immune response against malaria infection, and therefore experienced better controlled inflammatory processes associated with mild form of the disease.

Keywords T-cell profiling in malaria · Malaria immunology · Comparative haematological profiling in malaria · Rural and urban malaria · Ghana

Abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
CD	Cluster of differentiation
fL	Femtoliter (equivalent to 10^{-15} L)
GM	Geometric mean
IL	Interleukin
MCH	Mean cell haemoglobin
MCV	Mean cell volume
RBC	Red blood cells
TNFα	Tumor necrosis factor alpha

Introduction

Malaria is intra-erythrocytic parasitic infection caused by *Plasmodium falciparum*, *P. ovale, P. malaria*, *P. vivax,* and *P. knowlesi* (White 2008). These parasites are responsible for an average of over 400,000 mortalities yearly (World

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2019). In Ghana, over 95% of malaria cases are attributable to *P. falciparum* (Asante et al. 2011).

Haematological alterations that occur during the asexual stage of the life cycle of the malaria parasite reported to characterize malaria may lead to adverse biochemical changes (Costa et al. 2006). Erythrocytes infected with *P. falciparum* induce increase in secretion of cytokines such as tumor necrosis factor alpha (TNFα), interleukin (IL)-1, IL-10 and interferon gamma (IFNγ). These cytokines and intracellular *P. falciparum* induce red cells lysis which leads to low red cell count and attendant abnormalities in haematological profile (Ghosh and Shetty 2008).

Malaria transmission and incidence tend to be higher in rural areas compared to urban communities (Tatem et al. 2013). This is due to lack of improved housing, poor drainage facilities, lack of better health care, lack of knowledge of prevention of malaria (Omumbo et al. 2005), and abundance of mosquito vectors in suitable temperatures and humid climate (Grillet 2000). Although the reverse is true of urbanized communities, increasingly high cases of malaria have been reported in urbanized communities (Maghendji-Nzondo et al. 2016; Parnell and Walawege 2011; Yadouléton and Allagbé 2010; Klinkenberg et al. 2010).

In classical *P. falciparum* malaria, fever, chills, anorexia, epigastric discomfort, nausea, vomiting, and diarrhoea are common (Uzzan et al. 2006). Routine laboratory examinations may show various degrees of anaemia. In most cases, the white blood cell count is usually normal, but leucopenia may be observed. Thrombocytopenia is common as well as elevation of serum transaminases and lactic dehydrogenase. Moderately or markedly elevated bilirubin concentrations may also be observed (Ahiboh et al. 2008; Chiwakata et al. 2001). However, it is not clearly established if malaria impacts differently on urban and rural patients. Therefore, this study aimed at comparing the haematological, immunological (TNF-α, IL-12, IL-10, IL-6, CD4, CD3, and CD8) and clinical profiles (abdominal discomfort, chills, fever, vomiting, diarrhea, nausea and palor) of malaria patients dwelling in urban and rural communities in the Greater Accra region of Ghana. TNF-α, IL-6 and IL-12, and IL-10 were chosen based on their known proinflammatory and anti-inflammatory and immunoregulatory properties, respectively (Jason et al. 2001; Torre et al. 2002).

Materials and methods

Study sites and study design

The study samples were collected from health facilities located in both rural and urban communities in the Greater Accra region of Ghana. In all, five districts were involved in this study. The districts were Ga West, Ga South, Ashaiman, Ada East and Accra Metropolis. The rural sites were Ada (Ada East), Mayera and Oduman (Ga West), and Obom (Ga South) while the urban sites were Amasaman (Ga West), Maamobi (Accra metropolis) and Ashaiman (Ashaiman district). Health facilities used for this study were Ga West Municipal Hospital in Amasaman, Obom Health Centre, Mayera Health Centre, Oduman Health Centre, Ada East Government Hospital, Maamobi General Hospital and Ashaiman Polyclinic. The study participants were selected strictly based on residence in rural and urban communities.

Details of study area

Figure 1 is the map of the Greater Accra region detailing the study region and sites. In Ada East and Ga South districts, rural communities predominate while Ga West is made up of almost equal numbers of rural and urban communities. Ashaiman and Accra metro are entirely urban communities. In Ga West municipality, the municipal hospital, Ga West Municipal Hospital is located in the municipal capital, Amasaman. Mayera health centre is located in Mayera, south-east of Amasaman, a distant of about 15 km from Amasaman. Oduman health centre is also located in Oduman, north-east of Amasaman, a distant of about 20 km from Amasaman. The geographical locations of the study centers were Ga West Municipal Hospital, Amasaman (5.7020708, −0.2992889), Ashaiman Polyclinic (5.6856, −0.0398) and Ada East District Hospital (5.8956754, 0.5340865) and the health centres (latitude, longitude) were Mayera Health Centre (5.720578, −0.2703561), Oduman Health Centre (5.64171, −0.3302) and Obom Health Centre (5.7361, −0.4395).

Sample size

This cross-sectional study sampled at least 198 malaria cases from rural and urban communities based on 15.1% prevalence of malaria in the region (Diallo et al. 2017). Sample size was calculated using the Cochrane's formula, $n = z^2p(1\text{-}p)/d^2$, where n = sample size, p = prevalence of malaria in Greater Accra Region, z = confidence level at 95% (standard value of 1.96), d = margin of error at 5% (standard value of 0.05). Adjusting for 10% nonresponse rate, a sample size of 218 was obtained. A minimum of 109 suspected malaria cases were recruited from both rural and urban dwellers. For each case recruited age-matched controls were also collected. Cases and controls were collected from March to July 2018.

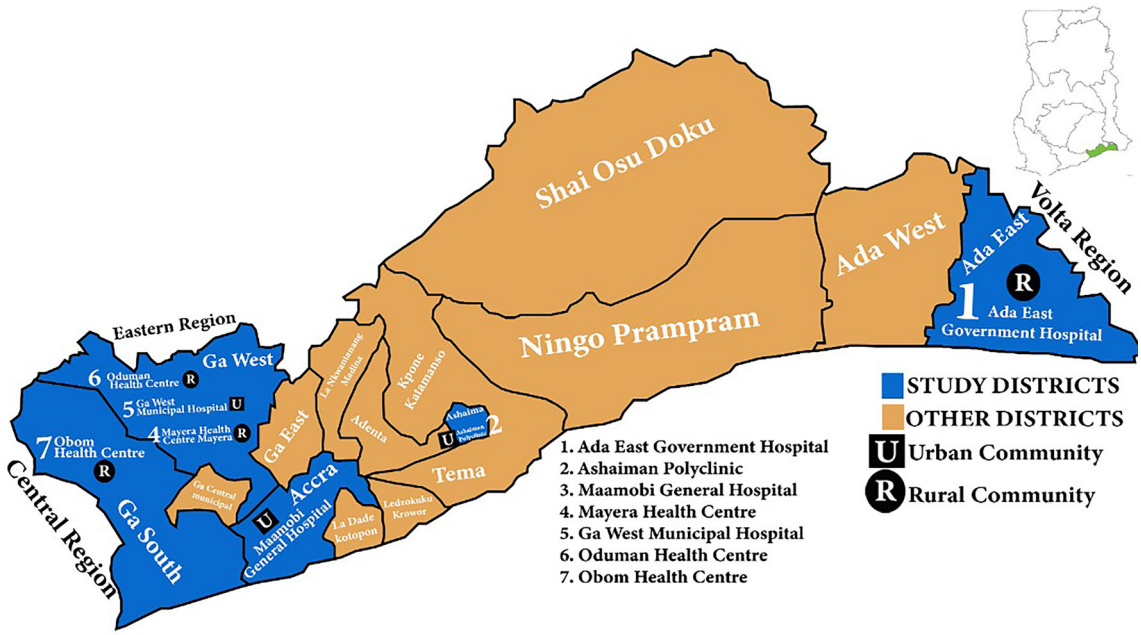

Fig. 1 Map of study districts and the sites (map was authors' own production)

Sampling and sampling strategy

The suspected malaria cases were sampled randomly by selecting the first four malaria cases each day which consented to be part of the study. On each day, an equal numbers of malaria suspected cases that were negative for malaria were also collected as control cases. To ensure uniformity in the clinical assessment, digital infra-red noncontact thermometers (Kinlee, Guangdong), digital blood pressure meters (Omron, Kyoto-Japan), and digital weighing scales (Omron, Kyoto-Japan) were calibrated and distributed to the study sites.

Blood sample collection

Five mL of whole blood was collected from suspected malaria patients with prior written consent from accompanying parents and guardians. Patients above 18 years consented for themselves. The blood samples were kept on ice packs until they arrive at Ga West Municipal Hospital Laboratory where all analyses were done.

Laboratory procedures

Blood smears for malaria parasite quantification

Thick blood smears were prepared on a new microscope slide for each blood sample. Smears were stained with locally prepared Giemsa stain (pH = 6.8). Parasite densities were estimated by dividing the number of asexual parasites per at least 200 leukocytes and multiplied by the estimated WBC of the patients/μL of blood (Alves-Junior et al. 2014).

Hematological profiling procedure

Peripheral blood's haematological parameters were determined using Urit 5160 haematological analyzer (Guangzhou, China). The 5-part differential analyzer works on the principle of laser beam multidimensional cell classification, flow cytometry for white cell differentiation, white and red cell estimation. Platelets were counted by optical and electrical impedance principles and haemoglobin concentration measured by cyanide-free colorimetric method. All other parameters were calculated automatically.

Nutritional assessment and determination of body mass index

Body mass index (BMI), protein, and albumin concentrations were used to assess nutritional statuses as previously used (Norgan 1994; Prenner et al. 2014). BMI was calculated as the ratio of weight in kilogram and square of height in meters. The height and body weight were taken by Seca 213 portable stadiometer (New Zealand) and Omron digital weighing scale (Omron, Kyoto-Japan), respectively. The total protein and albumin concentrations (from plasma samples) were measured by PKL-125 Italia fully automated chemistry analyser using Medsource endpoint reagents (Ozone Biomedicals Pvt Ltd, Haryana, India).

Cytokines profiling using enzyme immuno-assay

Plasma levels of interleukin (IL)-12, IL-10, IL-6, and tumor necrosis factor alpha (TNF-α) were determined by using Cusabio Biotech sandwich ELISA kit (Wuhan, China). Strict manufacturer's protocols were followed. Cytokines in stored plasma (below −20 °C) were analyzed on the same day using Mindray MR-96A ELISA plate reader (Shenzhen, China).

Enumeration of CD4 and CD3 T cells

CD3 and CD4 T cells were enumerated using BD FACS count flow cytometer (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, the CD4/CD3 reagent tube was brought to room temperature, vortexed for approximately 5 s each, and then opened with the coring station. Subsequently, 50 µL of whole blood was added to a reagent tube, vortexed upright, and incubated in the workstation for one hour. After incubation, 50 µL of formalin was added, vortexed, and incubated until the samples were analyzed. The samples were then vortexed and immediately run on BD FACS count flow cytometer. The absolute values of CD4, CD3 T cells, and the CD4/CD3 ratio were then automatically printed out by an inbuilt thermal printer.

Interpretation of results

The Ghanaian normal reference range for $CD4^+$ T lymphocytes is 660–1493 cell/µL, $CD3^+$ T lymphocytes is 1140–2685 cell/µL and CD4/CD3 ratio is 0.51–0.65. A person is said to be immuno-suppressed if the $CD4^+$ T lymphocytes, $CD3^+$ T lymphocytes, and CD4/CD3 ratio are less than 660 cells/µL 1140 cells/µL and 0.50 respectively. $CD8^+$ cells (normal ranges 187–1139 cells/µL) were calculated by subtracting CD4 cells from CD3 cells (Ampofo et al. 2006).

Statistical analyses

The statistical analyses were done by Graphpad Prism version 8.4.3.686. One-way analysis of variance (ANOVA) was used to test the level of differences among urban, rural, and control patients' samples. Tukey post hoc analysis was used to perform multiple comparisons among the parameters. Additionally, student t-test was used to test the differences in geometric mean of parasite densities in the malaria cases. Furthermore, Chi-square tool was used to test the association of clinical variables to residential status. In all inferential statistics, *p*-value less than 0.05 was considered statistically significant.

Results

Cases and controls selected for the study

A total of 298 suspected malaria patients participated in this study. Of this total, 166 patients were rural dwellers ($n = 83$ were infected with malaria parasites and $n = 83$ were not). From urban-dwelling patients, 132 patients were selected ($n = 66$ were infected with malaria parasites and $n = 66$ were not). The distributions of the malaria cases and controls in each health facility, situated in either rural or urban communities, are presented in Table 1.

Comparison of cases and controls

Table 2 represents demographic and clinical parameters of the malaria cases and control patients. In both cases and controls, the number in each age range reduces with increasing age. The number of individuals belonging to each age range in both cases and controls were comparable. Similarly, the gender distributions between cases and controls was also comparable. However, mean temperature readings were significantly higher in cases than controls (38.6 °C vs. 37.8 °C, $p = 0.041$). It was also found that all clinical findings assessed were associated with malaria.

Parasitological characteristics of the study patients

Parasitological analysis revealed that the geometric means of parasitaemia among males were higher in urban dwellers than rural dwellers (15,169 vs. 11,031 parasites/µL of blood). Additionally, parasitaemia was significantly higher in each age group in urban dwellers than rural dwellers. Comparing parasitaemia among each age group, level in 0–5 year age group, in both urban and rural dwellers, were higher than the level seen in the other age groups. Similarly, higher mean parasite densities were seen in 6–10 year group compared to 11–15 and 16–20 year groups. However, while mean parasitaemia in 11–15 year group in rural dwellers did not differ from density seen in the 16–20 year group (10,478 vs. 8901 parasites/µL, $p > 0.05$), in urban dwellers, mean parasitaemia density was significantly higher in 11–15 year age group compared to 16–20 year group (15,489 vs. 11,254 parasites/µL, $p < 0.05$). On the other hand, a similar trend was seen in the females. Parasitaemia was higher in female urban dwellers (18,365 vs. 14,008 parasites/µL, $p = 0.001$). Among the female age groups, significant higher densities were seen in each age group. In female rural dwellers, parasitaemia was higher in the 0–5 year age group compared to the other age groups. Likewise, parasitaemia in the 6–10 year age group was higher than densities seen in

Table 1 Samples collection sites and number of samples

Parameters	Study samples	
Sample collection sites		
Rural patients	Cases (n = 83)	Controls (n = 83)
Ada East hospital	17 (20.5%)	17 (20.5%)
Mayera health center	13 (15.7%)	13 (15.7%)
Oduman health center	23 (27.7%)	23 (27.7%)
Obom health center	30 (36.1%)	30 (36.1%)
Urban patients	Cases (n = 66)	Controls (n = 66)
Ga West hospital	29 (43.9%)	29 (43.9%)
Maamobi Gen. hospital	13 (20.0%)	13 (20.0%)
Ashaiman polyclinic	24 (36.4%)	24 (36.4%)

11–15 year and 16–20 year groups. However, in urban dwellers, parasite density was only significantly higher in the 0–5 year group. Densities in the other age groups did not differ from each other (Table 3).

Haematological parameters in malaria cases

The levels of peripheral blood neutrophils, eosinophils, and monocytes in infected cases did not significantly differ between controls and cases (Table 4). However, whereas leukocytes count, lymphocyte count, basophil count and mean cell haemoglobin (MCH) were significantly lower in controls compared to cases, red blood cells (RBC) count, haemoglobin, mean cell volume (MCV), haematocrit and platelet count were significantly higher in controls compared to cases. Additionally, Tukey post hoc analysis identified total white blood cells, lymphocytes, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, platelets and mean platelet volume levels in urban dwellers to be significantly different from rural dwellers. Lymphocytes (2.08 ± 0.16 vs. 2.15 ± 0.05), haemoglobin (10.53 ± 1.75 vs. 12.04 ± 1.45), haematocrit (27.88 ± 3.93 vs. 31.71 ± 3.93), mean cell haemoglobin (28.29 ± 3.39 vs. 30.58 ± 1.97), platelets (126.3 ± 62.52 vs. 166.80 ± 75.20) and mean platelet volume (8.60 ± 0.87 vs. 10.02 ± 1.55) levels in urban dwellers were found to be significantly lower than rural dwellers.

Table 2 The demographic and clinical findings of the study participants

Parameters	Rural		Urban		Total		p-value
	Controls (n = 83)	Cases (n = 83)	Controls (n = 66)	Cases (n = 66)	Controls (n = 149)	Cases (n = 149)	
Age range (years)							
0–5	37 (44.6%)	45 (54.2%)	18 (27.3%)	18 (27.3%)	55 (36.9%)	63 (42.3%)	0.39 (0.532)
6–10	28 (33.7%)	21 (25.3%)	25 (37.9%)	27 (40.9%)	53 (35.6%)	48 (32.2%)	0.13 (0.667)
11–15	7 (8.4%)	8 (9.6%)	17 (25.8%)	14 (21.2%)	24 (16.1%)	22 (14.7%)	0.07 (0.784)
16–20	11 (13.3%)	9 (10.8%)	6 (9.1%)	7 (10.6%)	17 (11.4%)	16 (10.7%)	0.03 (0.869)
Gender							
Males	39 (47.0%)	30 (36.1%)	19 (28.8%)	20 (30.3%)	58 (38.9%)	50 (33.6%)	0.43 (0.509)
Females	44 (53.0%)	53 (63.9%)	47 (71.2%)	46 (69.7%)	91 (61.1%)	99 (66.4%)	0.21 (0.650)
Clinical findings							
Mean temperature (°C)	37.7 ± 0.3	38.1 ± 0.3	37.8 ± 0.4	39.0 ± 0.7	37.8 ± 0.4^{g}	38.6 ± 0.6^{g}	2.9 (0.041)*
Abdominal discomfort	33 (39.8%)	52 (62.7%)	36 (54.5%)	51 (77.3%)	69 (46.3%)	103 (69.1%)	4.2 (0.038)
Chills	29 (24.9%)	61 (73.4%)	36 (54.5%)	62 (94.0%)	65 (43.6%)	123 (82.6%)	11.1 (0.008)
Diarrhoea	11 (13.2%)	37 (44.6%)	17 (25.8%)	48 (72.7%)	28 (18.8%)	85 (57.0%)	21.3 (< 0.001)
Fever	42 (50.6%)	63 (75.9%)	39 (59.1%)	62 (94.0%)	81 (54.4%)	125 (83.9%)	5.6 (0.017)
Nausea	23 (27.7%)	67 (80.7%)	30 (45.5%)	55 (83.3%)	53 (35.6%)	122 (81.9%)	17.5 (< 0.001)
Pallor	17 (20.5%)	39 (47.0%)	38 (57.6%)	45 (68.2%)	55 (36.9%)	84 (56.4%)	4.1 (0.041)
Vomiting	9 (10.8%)	48 (57.8%)	16 (19.3%)	41 (62.1%)	25 (16.8%)	89 (59.7%)	26.6 (< 0.001)

*Test statistic and p-value determined by T-test. The other statistics are presented at Chi statistic x^2 (p-value)

Table 3 Age-gender segregation of malaria parasitaemia

Gender	Number of patients (n = 149)	GM of parasite densities (parasites/μL)		p-value
		Rural dwellers n (parasite count)	Urban dwellers n (parasite count)	
Males	30 (11,031)*	20 (15,169)*	0.032	
0–5 years	19 (38.0%)	14 (19,578)[a]	5 (28,015)[a]	0.011
6–10 years	17 (34.0%)	10 (13,201)[a, b]	7 (19,090)[a, b]	0.008
11–15 years	9 (18.0%)	3 (10,478)[a, b]	6 (15,489)[a, b, c]	0.021
16–20 years	5 (10.0%)	3 (8901)[a, b]	2 (11,254)[a, b, c]	0.013
Females	53 (14,008)*	46 (18,365)*	0.001	
0–5 years	44 (44.4%)	31 (17,336)[a]	13 (21,505)[a]	0.038
6–10 years	31 (31.3%)	11 (13,407)[a, b]	20 (16,888)[a]	0.027
11–15 years	13 (13.1%)	5 (12,114)[a, b]	8 (15,509)[a]	0.009
16–20 years	11 (11.1%)	6 (11,456)[a, b]	5 (16,358)[a]	0.016

GM – geometric mean, n – number of patients in category, *Significant difference in parasitaemia between males and females, [a, b, c] parasitaemia differed significantly in respective groups

Table 4 Haematological parameters among confirmed cases and controls

Parameters	Mean ± standard deviation			p-value
	Controls (n = 149[a])	Rural (n = 83)	Urban cases (n = 66)	
White blood cells ($\times 10^9$/L)	4.99 ± 1.12	6.43 ± 1.14	7.95 ± 1.93	0.003
Neutrophils ($\times 10^9$/L)	3.0 ± 0.14	4.0 ± 0.05	5.25 ± 0.15	0.113
Lymphocytes ($\times 10^9$/L)	1.87 ± 0.14	2.15 ± 0.05*	2.08 ± 0.16*	0.012
Eosinophil ($\times 10^9$/L)	0.16 ± 0.03	0.19 ± 0.012	0.23 ± 0.02	0.503
Monocytes ($\times 10^9$/L)	0.12 ± 0.02	0.23 ± 0.02	0.38 ± 0.03	0.068
Basophils ($\times 10^9$/L)	0.01 ± 0.002	0.03 ± 0.002	0.03 ± 0.003	0.015
Red blood cells ($\times 10^{12}$/L)	5.08 ± 0.46	4.02 ± 0.39	3.62 ± 0.57	0.022
Haemoglobin (g/dL)	12.26 ± 1.39	12.04 ± 1.45*	10.53 ± 1.75*	0.039
Haematocrit (%)	36.79 ± 3.89	31.71 ± 3.93*	27.88 ± 3.93*	0.009
Mean cell volume (fL)	86.51 ± 8.12	77.47 ± 6.31*	79.62 ± 10.80*	0.011
Mean cell haemaoglobin (pg)	24.23 ± 2.95	30.58 ± 1.97*	28.29 ± 3.39*	0.002
Platelet ($\times 10^9$/L)	242.90 ± 65.53	166.80 ± 75.20*	126.3 ± 62.52*	0.001
Mean platelet volume (fL)	8.61 ± 0.99	10.02 ± 1.55*	8.60 ± 0.87*	0.012
Plateletcrit (%)	0.21 ± 0.06	0.16 ± 0.07	0.16 ± 0.11	0.006
Platelet large cell ratio	16.76 ± 4.62	23.32 ± 5.02	22.78 ± 4.01	0.031

*Significant differences between rural and urban parameters, [a]The control values presented in the table were mean values of all the control cases selected from both rural and urban communities

However, mean cell volume levels were significantly higher in urban dwellers (79.62 ± 10.80) compared to rural dwellers (77.47 ± 6.31).

Nutritional status of cases and controls

The body mass index, total protein, and albumin levels of urban dwellers with malaria were statistically not different from control values. However, levels in rural dwellers with malaria were significantly lower than control values and levels observed in urban dwellers with malaria (Table 5).

Levels of immunological markers in malaria patients

Presented in Table 6 are the immunological parameters of the malaria cases, tested by one-way analysis of variance (ANOVA). Whereas serum IL-10 and CD4 + cells were

significantly higher in controls than cases, TNF- α, IL-12, IL-6, CD3 + , and CD8 + cell count were significantly lower in controls compared to cases. Tukey post hoc analysis found that TNF-α (392.6 ± 149.6 vs. 284.9 ± 93, $p = 0.02$), IL-6 (239.6 ± 80.3 vs. 186.3 ± 35.3, $p = 0.0015$) and IL-12 (130.2 ± 40.6 vs. 102.8 ± 52.8, $p = 0.008$) levels in urban residents were significantly higher than their rural counterparts. However, the reverse was observed in IL-10 (110.6 ± 41.3 vs. 158.8 ± 44.2, $p < 0.0001$). In addition, the levels of $CD4^+$ T-cells, $CD3^+$ T-cells, and $CD8^+$ T-cells in urban-infected residents were significantly lower compared to rural residents.

Association of clinical findings to dwelling places

Of the seven clinical findings reported by patients, four of them were strongly associated with urban residents (Table 7). The associated clinical findings were chills (94.0%, $x^2 = 10.7$, $p = 0.001$), diarrhoea (72.7%, $x^2 = 11.9$, $p = 0.0006$), fever characterized by temperature above 38 oC (94.0%, $x^2 = 8.9$, $p = 0.003$) and pallor (68.2%, $x^2 = 6.7$, $p = 0.001$).

Discussion

In Ghana, malaria is prevalent in both urban (Diallo et al. 2017) and rural communities (Babayara and Addo 2018). In this case–control study, we report that Ghanaian urban dwellers were hard hit with malaria compared to rural dwellers, even though the nutritional status assessment was significantly poor in rural dwellers. In both age and gender categories, parasitaemia was significantly higher in urban dwellers than rural dwellers. Although the actual causality of this observation was not explored in the present study, a review of previous studies in this subject area offers some insights as to the plausible causes of the significantly higher parasitaemia in urban dwellers compared to rural dwellers. Low transmission intensity of malaria in urban areas (Padilla et al. 2015) is due partly to factors such as improved housing and limited mosquito breeding sites and abundance (Kokwaro 2009). In Ghana, the predominant malaria causing *Anopheles* species in urban communities were identified as *An. gambiae* (*s.l.*) of which more than 99% are *An. coluzzii* and *An. gambiae* (*s.s.*) (Mattah et al. 2017) while in rural Ghana, *An. gambiae s.l.* and *An. funestus* constitute over 94% of malaria causing mosquito population (of which almost 98% of *An. gambiae.s.l.* were identified as *An. gambiae s.s.*). Relatively fewer numbers of *An. pharoensis* and *An. rufipes* has also been identified in rural communities (Appawu et al. 2004). Entomological inoculating rates (EIR) of female Anopheles mosquitoes have also been reported to be significantly reduced in urbanized communities (Robert et al. 2003). For these reasons, transmission of malaria in urban areas is expected to be low with its attendant lower levels of multiclonality. Multiclonality of malaria parasites is directly related to malaria immune status. In communities with stable malaria transmission, multiclonality tends to increase as host immunity to malaria sharpens (Smith et al. 1999). Additionally, several studies have also shown an inverse relationship between multiclonality and severity of malaria (al-Yaman 1997; Farnert et al. 1999; Muller et al. 2001). Hence, the hyperparasitaemia observed in urban residents in the current study could be as a result of incompetent malaria immunity that characterizes the urban dwellers observed in this study. In such patients, malaria parasites will undergo unchecked multiplication from few parasites to tens of millions of parasites within a short time (Dietz et al. 2006; Simpson et al. 2002). As malaria parasites multiply in such individuals, peripheral blood haematological indices are radically deranged. Not surprisingly, the urban dwellers studied in this study recorded significantly low levels of lymphocytes, haemoglobin, haematocrit, mean cell haemoglobin, and platelets. This trend may be sequalae to the hyper-parasitaemia reported herein, further corroborating the earlier findings. In both rural and urban dwellers with malaria, thrombocytopenia was observed with levels further reduced in urban dwellers. Tumour necrosis factor alpha (TNF-α) which promotes cellular

Table 5 Anthropometric and nutritional status of the participants

Parameters	Mean ± standard deviation			*p*-value
	Controls ($n = 149$[a])	Rural cases ($n = 83$)	Urban cases ($n = 66$)	
Body mass index (kg/m^2)	23.1 ± 3.7	19.1 ± 2.9*	24.5 ± 3.4	0.011
Total protein (g/L)	75.7 ± 4.2	69 ± 3.6*	78.3 ± 5.1	0.008
Albumin (g/L)	42.8 ± 4.6	34.1 ± 2.8*	44.1 ± 5.0	0.037

Underweight (< 18.5), Normal (18.5 to −24.9), Overweight (25.0 to −29.9), Obese (> 30.0) (World Health Organization 2020), *Significantly different from control values, [a]The control values presented in the table were mean values of all the control cases selected from both rural and urban communities

Table 6 Levels of immunological parameters among confirmed cases and controls

Parameters	Mean ± standard deviation			*p*-value
	Controls (n = 149[a])	Rural (n = 83)	Urban cases (n = 66)	
TNF-α (pg/mL)	83.9 ± 46.51	284.9 ± 93*	392.6 ± 149.6*	< 0.001
IL-12 (pg/mL)	75.92 ± 17.42	102.8 ± 52.8*	130.2 ± 40.6*	< 0.001
IL-10 (pg/mL)	190.5 ± 63.2	158.8 ± 44.2*	110.6 ± 41.3*	< 0.001
IL-6 (pg/mL)	71.7 ± 17.19	186.3 ± 35.3*	239.6 ± 80.3*	< 0.001
$CD4^+$ T-cells (cells/μL)	944 ± 244	703 ± 110*	511 ± 175*	< 0.001
$CD3^+$ T-cells (cells/μL)	1533 ± 254	1840 ± 431*	1630 ± 330*	< 0.001
$CD8^+$ T-cells (cells/μL)	588 ± 273	1337 ± 359	1149 ± 345	< 0.001
$CD4^+$/ $CD3^+$ ratio	0.62 ± 0.69	0.38 ± 0.25*	0.31 ± 0.19*	< 0.001

*Significant differences between rural and urban parameters, [a] The control values presented in the table were mean values of all the control cases selected from both rural and urban communities

Table 7 Clinical findings of the study participants

Clinical finding	Rural dwellers (n = 83)	Urban dwellers (n = 66)	x^2 (*p*-value)
Abdominal discomfort			3.7 (0.055)*
Yes	52 (62.7%)	51 (77.3%)	
No	31 (37.3%)	15 (22.7%)	
Chills			10.7 (0.001)**
Yes	61 (73.4%)	62 (94.0%)	
No	22 (26.5%)	4 (6.0%)	
Diarrhoea			11.9 (0.0006)**
Yes	37 (44.6%)	48 (72.7%)	
No	46 (55.4%)	18 (27.3)	
Fever (temp > 38.0 °C)			8.9 (0.003)**
Yes	63 (75.9%)	62 (94.0%)	
No	20 (24.1%)	4 (6.0%)	
Nausea			0.7 (0.404)
Yes	67 (80.7%)	55 (83.3%)	
No	16 (19.3%)	9 (16.7%)	
Pallor			
Yes	39 (47.0%)	45 (68.2%)	6.7 (0.01)**
No	44 (53.05)	21 (31.8%)	
Vomiting			0.3 (0.595)
Yes	48 (57.8%)	41 (62.1%)	
No	35 (42.2%)	25 (37.9%)	

*Confirmed by accompanying adults for children under 10 years

**Significant association

apoptosis, is reported to be inversely related to platelet count (Raza et al. 2013). Urban dwellers with malaria recorded higher levels of TNF-α than rural dwellers, hence the reduction in their platelet count compared to rural dwellers conformed to earlier publications.

Low levels of lymphocytes observed in urban dwellers reflected in significantly reduced T-lymphocyte subpopulations specifically $CD4^+$ T-cells, $CD3^+$ T-cells, and $CD8^+$ T-cells. In urban dwellers with malaria, mean $CD4^+$ T cells and mean $CD4^+/CD3^+$ ratios were not only significantly reduced but also fell below the lower reference ranges established for Ghanaians by Ampofo et al. (2006). There is compelling evidence to confirm that $CD4^+$ T-cells play an important role in clearance of pathogens in

humans and in animal models (Soghoian and Streeck 2010). Specifically, in humans, $CD4^+$ T-cell subtype, T-helper 2 (Th2) is responsible for controlling parasitic infections such as *Plasmodium falciparum* mediated by interleukin (IL)-4, IL-5, IL-13 and IL-10 (Zhu and Paul 2008). In this study, mean $CD4^+$ T-cells and mean $CD4^+/CD3^+$ ratios were significantly lower; suggesting their immunocompromised states. Therefore, these categories of individuals are highly susceptible to infections as well as complications associated with the infections, hence higher parasitaemia in urban dwellers with malaria. High parasitaemia correlated with high body temperatures (> 38.0 °C) in the study participants of urban origin. Additionally, in urban dwellers with malaria, hyperthermia directly correlated with TNF-α, IL-6 and IL-12. However, high level of TNF-α have been associated with associated with fever and headache in malaria patients (Kwiatkowski and Nowak 1991) and disease severity and complications (Shaffer et al. 1991). In effect, urban dwellers are more likely to develop severe malaria compared to rural dwellers and by inference, urban dwellers may be more prone to malaria complications than rural dwellers. Based on the foregoing, urban dwellers with malaria must be treated as an emergency case, with all the attention to reduce malaria parasitaemia within the shorted possible time with the ultimate aim to subside inflammatory response and tissue damage.

The protective effect of IL-10 was observed in this study which corroborates earlier reports that high level of IL-10 is beneficial to the host by modulating the *P. falciparum* parasite-induced inflammatory response (Perera et al. 2013; Day et al. 1999). IL-10 has been shown in a previous study to be inversely related to TNF-α and other proinflammatory cytokines (Ho et al. 1998), a phenomenon that was observed in this study. More importantly, IL-10 has been shown to be directly produced by $CD4^+$ T cells. It exerts its activity by suppressing inflammation via the inhibition of T cell functions and the upstream activities of antigen-presenting cells (Kumar et al. 2019). The findings from this study indicated a corresponding reduction in both $CD4^+$ T cells and IL-10 in urban residents. This observation confirms a reduced control of inflammatory responses and the attendant heightening of host tissue damage in urban dwellers (Ado and Langhorne 2012). It has been demonstrated experimentally in mice that reduced IL-10 levels exacerbates disease pathology and enhanced proinflammatory cytokine (IFN-γ and TNF-α) activity (Li et al. 1999).

Taken together, elevation of TNF-α, IL-6, IL-12, and reduction in IL-10 could be responsible for the simultaneous presentations of chills, diarrhoea, fever, and pallor as observed in urban dwellers with malaria. These presentations are usually associated with severe and prolonged inflammation as a result of sustained higher levels of proinflammatory cytokines.

Conclusion

In summary, urban dwellers exhibited severer forms of malaria compared to rural dwellers with malaria. This could be as a result of reduced or no malaria immunity which occurs due to irregular exposure of urban dwellers to infected mosquito bites. This situation predisposes infected individuals to severe outcomes of malaria Additionally, combinations of symptoms such as abdominal discomfort, chills, diarrhoea, fever, nausea, pallor, and vomiting observed in the urban dwellers could be due to cytokine storm as a result of prolong inflammation.

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Data availability The study data have been deposited at Harvard dataverse (https://doi.org/10.7910/DVN/DMUDQ3).

Declarations

Conflicts of interest The authors have none to declare.

Consent to participate Written consent to participate was sought from each participant or relatives or guardians of children below 18 years.

Ethics approval Ethical approval for the study was obtained from Ghana Health Service Ethical Review Committee (study number: GHS-REC002/03/18).

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