



Haematological Parameters Associated with Malaria and Its Controls

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Authors' contributions

This work was carried out in collaboration between both authors. Authors UAF and AD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UAF and AD managed the analyses of the study. Author UAF managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This research aimed to evaluate the haematological parameters associated with malaria and its controls.

Materials and Methods: A convenient cross-sectional technique was used for the study for which the sample size was determined by using the formula; $n = Z^2 (P) (1-P) / (A)^2$. The haematological profile was performed using the Sysmex 2000i automated blood cell counter machine.

Results and Discussion: The erythrocyte profiles (RBC, HB, HCT, RDW-SD and RDW-CV) are highly affected by malaria, whereas MCH, MCHC, and MCV did not show significant variations

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between the positive malaria cases and negative malaria cases. Means of haemoglobin concentrations, RBC count and HCT values for cases with positive malaria were significantly lower than negative malaria cases and controls for all the age groups and sexes.

Conclusion: The study showed that there were haematological profiles between the positive and negative malaria cases and this can be used in conjunction with clinical and microscopic parameters to heighten the suspicion of malaria as well as prompt initiation of therapy for diagnosing malaria.

Keywords: *Haematological parameters; leukocytosis; parasite density; plasmodium; haemoglobinopathy*

1. INTRODUCTION

Malaria is an infectious disease caused by a protozoan called *Plasmodium* (phylum Apicomplexa) that is transmitted through the bite of an infected Anopheles mosquito. The species that causes this infection in human include *P. falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi* and *Plasmodium vivax* [1]. The report indicated that the deaths of 1.5 to 2.7 million per annum are attributed to 300-500 million acute cases of malaria that occurs worldwide each year [2]. However, *Plasmodium falciparum* (*P. falciparum*) is the major cause of the disease and is responsible for about 90% of malaria infections and 80% of malaria deaths in sub-Saharan Africa for which Ghana is not an exception [3,1]. In Ghana, the estimated cases of malaria reported in children below 5 years were nearly 4 million with approximately 21 thousand deaths and fatality rate of 0.53% [4]. The increase in malaria infections is an impediment to the world's population and is as a result of deteriorating health systems, growing drug and insecticide resistance, climate change, natural disasters and armed conflict [5,6]. In general, malaria accounts for 10% of Africa's disease burden and cost the continent \$12 billion annually [7,8,9,10]. The report indicates that in Ghana, funding provided by the government from the Global Fund, the World Bank and bilateral donors to control malaria was close to US\$ 60 million and US\$ 40 million in 2006 and 2007 respectively [4].

The haematological profile is also known as haemogram which comprises full blood count (FBC), full blood exam (FBE) or blood panel is a test that gives information about the cells in a patient's blood [11]. It is used for clinical purposes, monitoring, screening and case finding for example of patients with symptoms such as fatigue or weakness, infection,

inflammation, bruising, or bleeding [11]. The abnormal high or low blood counts may be due to the presence of disease for which blood count tests are performed in medicine to provide an overview of a patient's general health status [11]. These tests comprise haemoglobin, haematocrit, red cell indices, red cell distribution width (RDW), total and differential leukocyte counts, and platelet counts which are used as a routine test for patients to complement diagnosis of diseases [12]. Report indicated that haematologic aberrations are the most common complications encountered in malaria and play a major role in the fatality [13,14,15]. These changes associated with malaria infection are well recognized but specific changes may vary with the level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity [16]. This study aimed to evaluate hematological parameters associated with malaria and its controls. The haematological changes would enable the differentiation of malaria from other diseases that are present with similar symptoms such as anaemia and thrombocytopenia which are common among patients with *Plasmodium falciparum* [17].

2. MATERIALS and METHODS

2.1 Study Area

The study was carried out in the following Polyclinics using random sampling technique including Mamprobi, Ussher town, Dansoman, Princess Marie and La in Accra Metropolis in the Greater Accra region of Ghana. The region has an estimated population of 1.6 million and is located in the coastal savannah zone with an average annual rainfall of 730 mm. Malarial transmission in the region

is between May to October with perennial and hyper-endemic seasonal peak rainy season.

2.2 Ethical Issues

Ethical approval was obtained from the Research and Ethical Review Committee of the University of Ghana Medical School, College of Health Science Korle-Bu, Ghana.

2.3 Study Population and Sample Size Determination

The samples were drawn from the population of patients who attended the Polyclinics/Hospitals laboratory from January to August 2009 with fever or clinical signs and symptoms suggestive of malaria based on World Health Organization (WHO) criteria. A convenient cross-sectional study from each of the five (5) study sites was used to obtain a total of 414 and 214 cases. The sample size was determined by using the formula; $n = Z^2 (P) (1-P) / (A)^2$; Where n= Minimum sample size, Z= Confidence level (1.96), P= Prevalence of malaria in Accra (14.8%) and A= Allowable error = 0.05. Based on the above formula, the calculated minimum sample size of 300 subjects was enrolled for the study. All subjects who presented to the Polyclinic/Hospital Laboratory with request cards from specified clinicians indicating suspected malaria were included in the study. The clinicians in each of the study sites were briefed and given an abstract of the study. The selection of the cases for the study depended on their expertise and was required to indicate by writing the diagnosis on the laboratory request card.

2.4 Administration of Questionnaire

A structured questionnaire was also administered to each consenting volunteer to document information on demographics, current symptoms and previous malaria episodes and treatments. Two hundred and Fourteen (214) apparently healthy Blood donors and Children from first cycle Schools who were located in the areas where the cases were obtained and whose peripheral blood film screen was negative for the malaria parasite served as controls.

2.5 Laboratory Analysis

Tubes were transported in an ice chest within 4 h to the Central Laboratory, where cell counts were performed using Sysmex XT-2000i automated haematology analyzer. All samples taken for the day were processed starting with the very first subject's sample. Whenever samples had to be delayed beyond the 4 h, they were kept in a refrigerator at 2°C - 8°C after which they were brought to room temperature before processing by allowing it to warm at a minimum of 15 mins, then mixed, by rotation, for at least 5 mins.

2.6 Automated Counting

2.6.1 Complete blood count and differential test using sysmex XT-2000i Automated haematology analyzer

The Sysmex XT-2000i automated haematology analyzer installed at Central Laboratory of the Korle-Bu Teaching Hospital was used for the test analysis. Standardization, calibration of instrument and processing of samples were done according to the manufacturer's instructions. Quality control of the Sysmex XT-2000i was determined on a daily basis by analysis of three different manufacturer-provided samples (low, normal and high) with known cell counts. The rapid diagnostic tests, Paracheck® Malaria *P.falciparum* (Orchid Biomedical Systems, India), was used to screen control subjects for malaria according to the manufacturer's instruction.

2.7 Statistical Analysis

Data collected were entered into a database and analyzed using a statistical software package, SPSS version 8.1, Excel and Epi-info.

3. RESULTS

3.1 Haematological Profiles Predictive of Malaria

Haematological profiles predictive of malaria were carried out for the most significant predictors of malaria using the likelihood ratios for children less than 5 and 6-16 years and adult males and females in (Table 1) and (Table 2) respectively.

Table 1. Likelihood ratios for various haematological parameters in the diagnosis of malaria in children

Children <5 Years			Children 6-16 Years		
Variables	Likelihood ratios	P values	Variables	Likelihood ratios	P values
HB (g/dl) <11.0	1.64	<0.001	HB (g/dl) <11.5	2.72	<0.001
RBC(x10 ¹² /L)<4.00	6.71*	<0.001	RBC(x10 ¹² /L) <4.00	9.06*	<0.001
HCT (%) <34.0	4.05*	<0.001	HCT (%) <35.0	2.77*	<0.001
MCH (pg) <24.0	0.24	<0.001	MCH (pg) <25.0	1.32	<0.001
MCV (fl) <75.0	0.39	<0.001	MCV (fl) <77.0	1.00	1.00
MCHC (g/dl) <31.0	0.96	0.655	MCHC (g/dl) <31.0	0.63	<0.001
RDW-SD (fl) >47.0	1.82	<0.001	RDW-SD (fl) >47.0	0.71	0.002
RDW-CV(%)>17.0	0.82	0.479	RDW-CV (%) >17.0	0.55	<0.001
PLT (x10 ⁹ /L) <200	10.17*	<0.001	PLT (x10 ⁹ /L) <170	3.39*	<0.001
PDW (fl) >16.0	3.92*	<0.001	PDW (fl) >16.0	3.86*	<0.001
MPV (fl) <9.4	0.87	0.258	MPV (fl) <9.4	4.01*	<0.001
P-LCR (%) < 21.0	1.90	<0.001	P-LCR (%) < 21.0	6.20*	<0.001
PCT (%) <0.15	7.61*	<0.001	PCT (%) <0.15	2.22	<0.001
TWBC (x10 ⁹ /L) >15.0	1.00	1.000	TWBC (x10 ⁹ /L)>13.0	8.92*	<0.001
NEUT# (x10 ⁹ /L) >8.0	1.64	<0.001	NEUT# (x10 ⁹ /L)>8.0	4.23*	<0.001
LYMP# (x10 ⁹ /L) <6.0	1.23	0.106	LYMP# (x10 ⁹ /L)<1.0	0.74	0.003
MONO# (x10 ⁹ /L) >1.0	1.08	0.411	MONO# (x10 ⁹ /L) >1.0	13.2*	<0.001
EO# (x10 ⁹ /L) <0.1	1.02	0.820	EO# (x10 ⁹ /L) <0.1	2.09	<0.001
BASO# (x10 ⁹ /L) >0.1	4.85*	<0.001	BASO# (x10 ⁹ /L) >0.1	1.00	1.00

*= Haematological profiles with the most significant predictors for the presence of malaria for children less than 5 and 6-16 years
Reference range used was obtained from Dacie and Lewis [18]

Table 2. Likelihood ratios for various hematological parameters in diagnosis of malaria in adults

Adult males above 16 years			Adult females above 16 years		
Variables	Likelihood ratios	P values	Variables	Likelihood ratios	P values
HB (g/dl) <13.0	0.65	<0.001	HB (g/dl) <12.0	3.89*	<0.001
RBC(x10 ¹² /L) <4.40	1.56	<0.001	RBC (x 10 ¹² /L) <4.00	7.68*	<0.001
HCT (%) <38.0	6.16*	<0.001	HCT (%) <35.0	3.81*	<0.001
MCH (pg) <23.0	1.89	<0.001	MCH (pg) <24.0	0.87	0.243
MCV (fl) <72.0	1.48	0.006	MCV (fl) <71.0	2.48	<0.001
MCHC(g/dl) <30.0	1.12	0.260	MCHC (g/dl) <30.0	0.87	0.243
RDW-SD (fl) >49.2	1.20	0.173	RDW-SD (fl) >47.0	1.79	<0.001
RDW-CV (%) >17.6	0.69	0.027	RDW-CV (%) >16.0	0.80	0.061
PLT (x 10 ⁹ /L) <145	6.17*	<0.001	PLT (x 10 ⁹ /L) <140	10.20*	<0.001
PDW (fl) <9.8	0.34	<0.001	PDW (fl) <9.4	0.41	<0.001
MPV (fl) <9.2	9.82*	<0.001	MPV (fl) >12.4	2.47	<0.001
P-LCR(%) >44.6	0.91	0.170	P-LCR (%) >42.0	0.20	<0.001
PCT (%) <0.16	3.11*	<0.001	PCT (%) <0.15	8.52*	<0.001
TWBC(x 10 ⁹ /L) <3.2	1.64	<0.001	TWBC (x 10 ⁹ /L) <3.2	4.58*	<0.001
NEUT#(x10 ⁹ /L) <1.20	2.33	<0.001	NEUT# (10 ⁹ /L) <1.40	1.61	<0.001
>4.60	1.37	0.007			
NEUT% >70.0	2.39	<0.001	NEUT% >65.0	2.03	<0.001
LYMP# (x 10 ⁹ /L) <1.13	4.80*	<0.001	LYMP# (x 10 ⁹ /L) <1.20	6.63*	<0.001
LYMP% <24.0	2.17	<0.001	LYMP% <28.0	2.54	<0.001
MONO# (x 10 ⁹ /L) >0.74	2.48*	<0.001	MONO# (x 10 ⁹ /L) >0.70	1.33	0.030
MONO% >13.6	1.37	0.007	MONO% >12.0	6.83*	<0.001
EO# (x 10 ⁹ /L) <0.02	2.33	<0.001	EO# (x 10 ⁹ /L) <0.02	3.61*	<0.001
EO% <0.31	3.84*	<0.001	EO% <0.36	1.97	<0.001
BASO# (x 10 ⁹ /L) <0.01	1.72	<0.001	BASO# (x 10 ⁹ /L) <0.01	2.70	<0.001
BASO% <0.10	2.81	<0.001	BASO% <0.10	0.59	<0.001

*= Haematological parameters with the most significant predictors for the presence of malaria for adult males and females; Reference ranges used was obtained from Akuetteh [19]

3.2 Correlation between Each Haematological Profile and Parasite Density

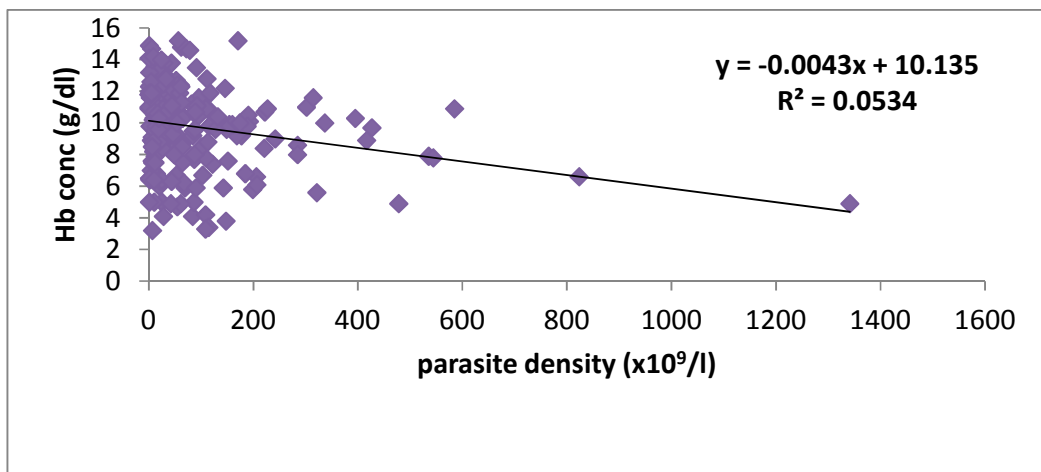


Fig. 1. Association between haemoglobin and parasite density

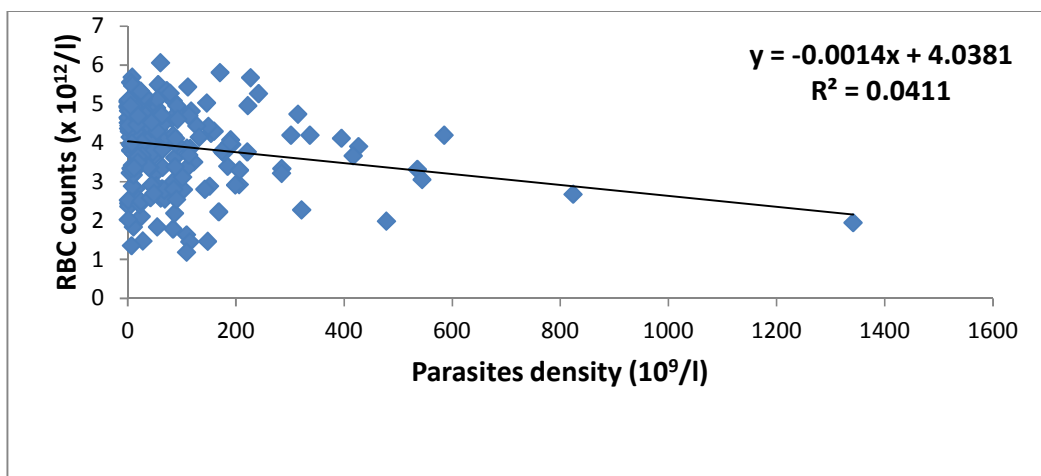


Fig. 2. Association between RBC count and parasite density

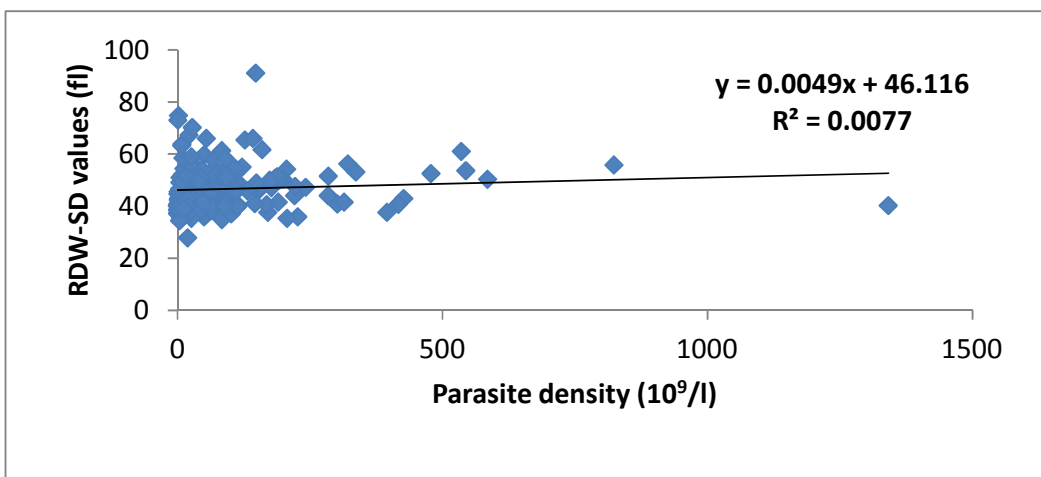


Fig. 3. Association between RDW-SD and parasite density

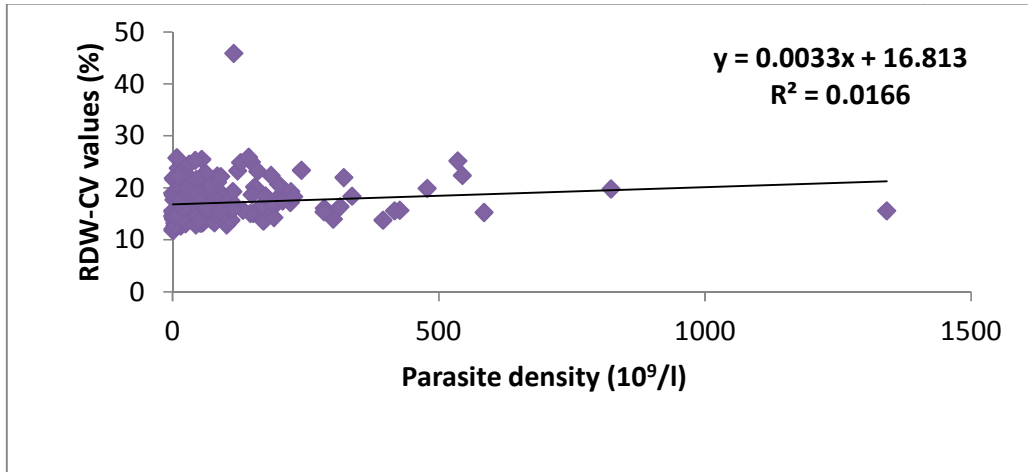


Fig. 4. Association between RDW-CV and parasite density

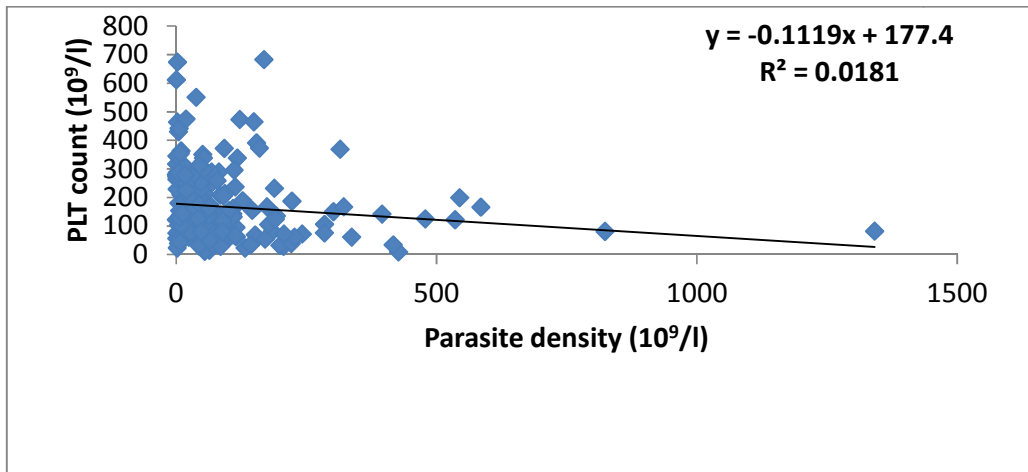


Fig. 5. Association between platelet count and parasite density

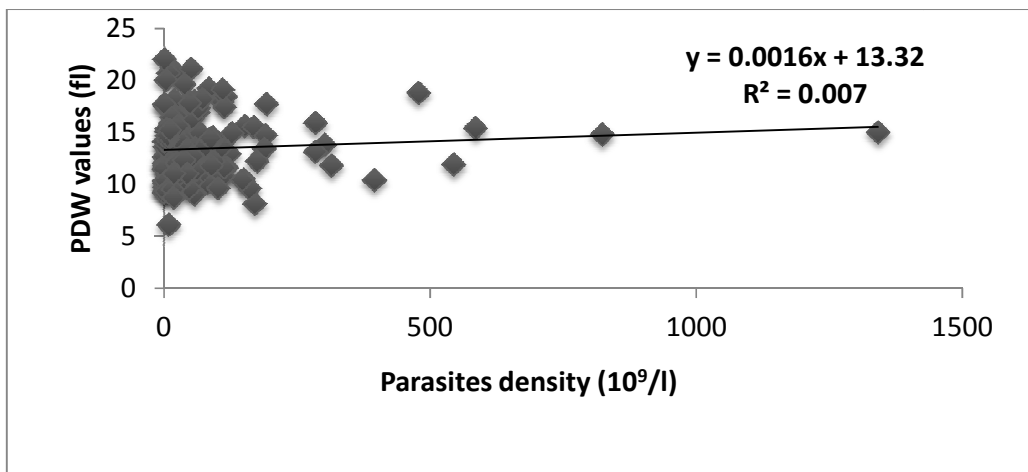


Fig. 6. Association between PDW values and parasite density

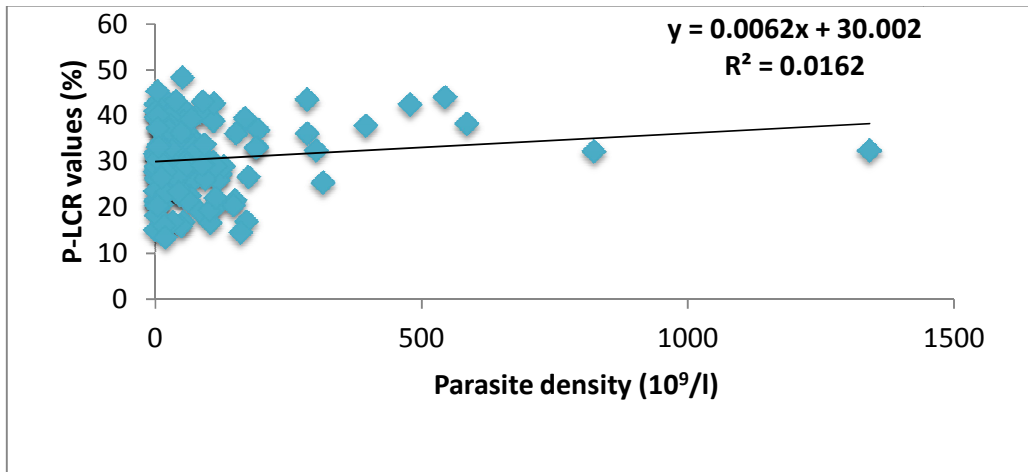


Fig. 7. Association between P-LCR and parasite density

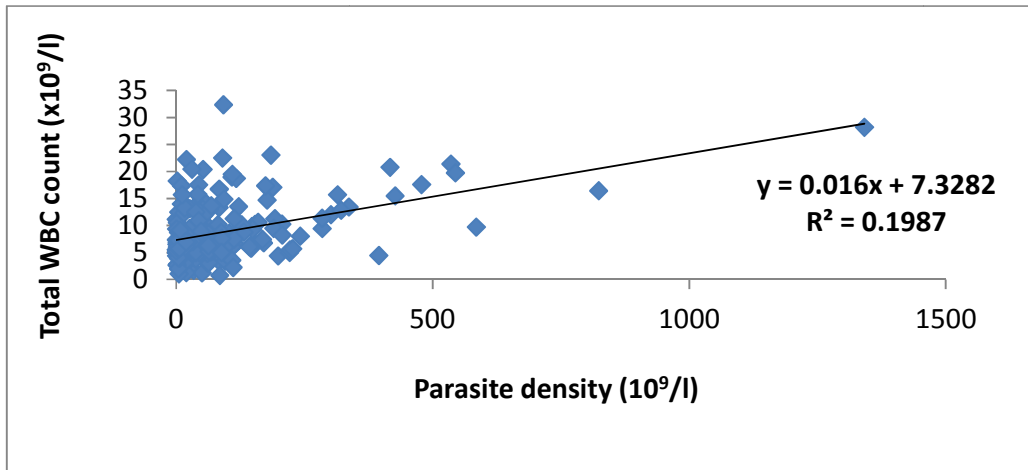


Fig. 8. Association between total WBC count and parasite density

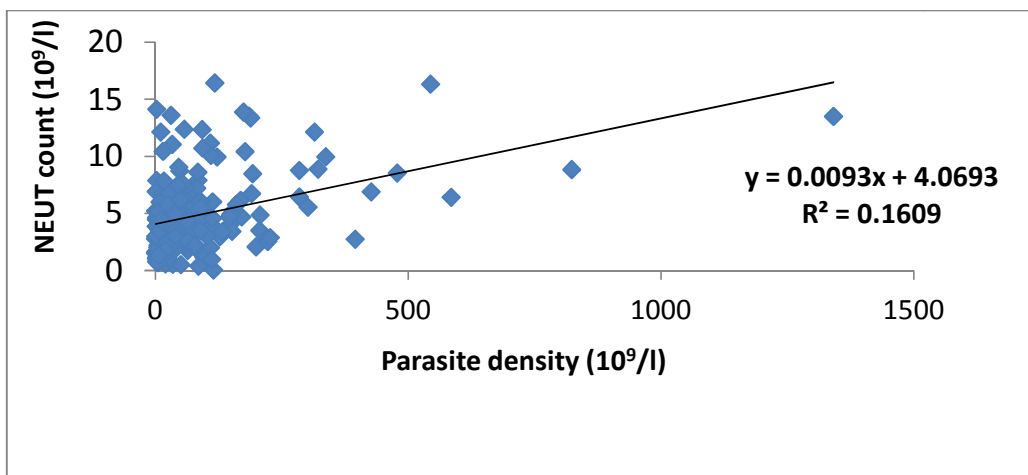


Fig. 9. Association between absolute neutrophil count and parasite density

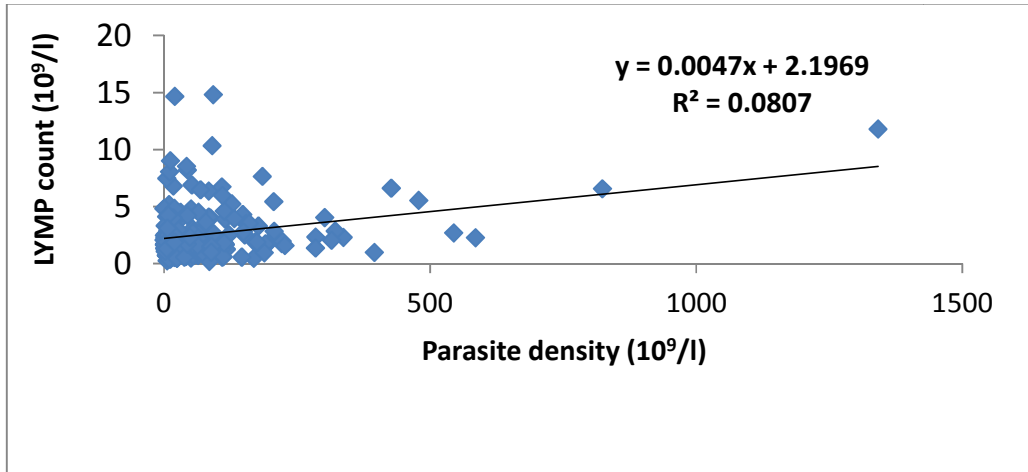


Fig. 10. Association between absolute lymphocyte count and parasite density

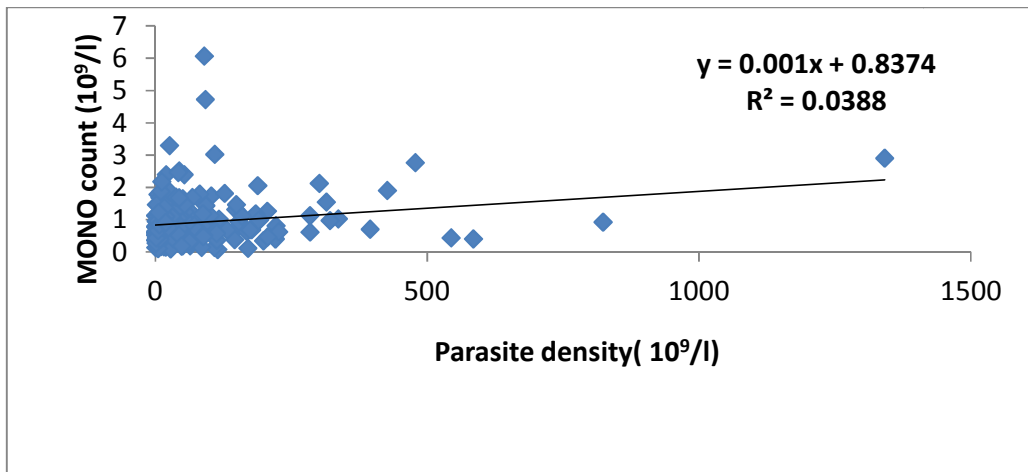


Fig. 11. Association between absolute monocyte count and parasite density

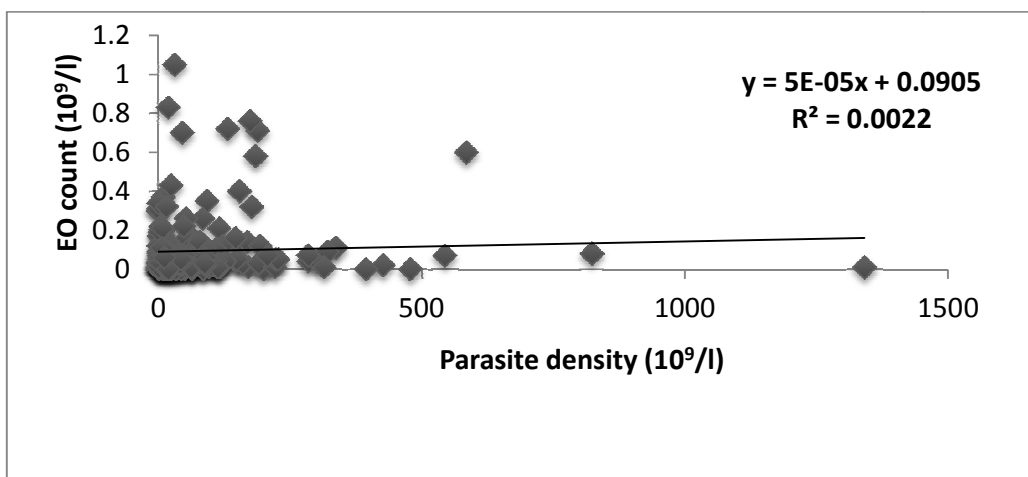


Fig. 12. Association between absolute eosinophil count and parasite density

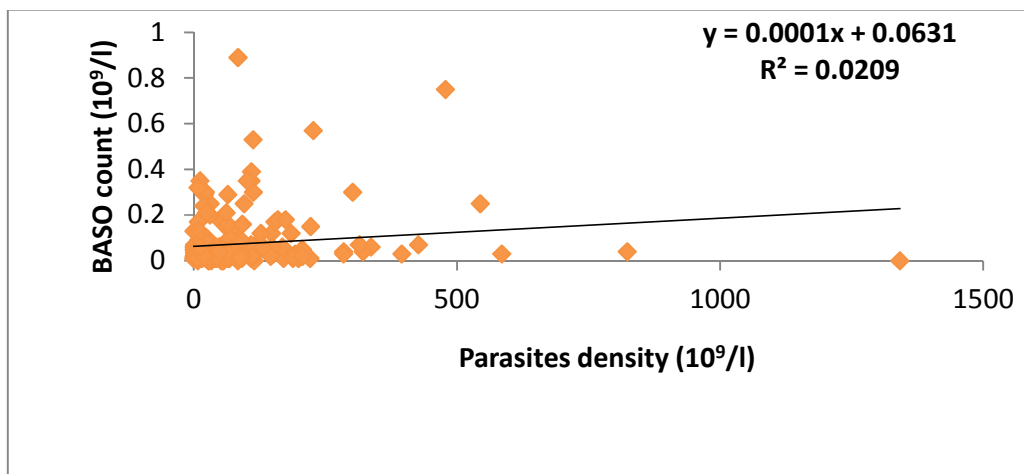


Fig. 13. Association between absolute basophil count and parasite density

4. DISCUSSION

In the study (Tables 1 and 2) it was identified that various hematological profiles give likelihood indication of diagnosing malaria but there was a variation on age and sex. Anemias was not a good predictor of malaria for children less than 5 years, 6-16 years and adult females and have been confirmed by a previous study in India where they observed likelihood of 1.95 of Hb at < 10g/dl. This could be attributed to low hemoglobin concentrations associated with these categories probably due to poor nutrition and physiological variations [20]. However, from (Table 2) anemia was 3.89 times more likely to be associated with malaria in adult males RBC's and HCT were better predictors of malaria in the various age categories than Hb.

A platelet count was better predictors of malaria in all the age and sex categories, a previous observation which this study also confirmed [21]. In a study on over two thousand patients with fever, Erhart et al. [22] reported platelet count of less than $150 \times 10^3/\mu\text{l}$ increases the likelihood of malaria by 12-15 times whiles Lathia et al. [23] reported likelihood of malaria by 5.04 at $150 \times 10^3/\mu\text{l}$ and Laura et al. [24] reported 14.7 for *P. falciparum* infection at $150 \times 10^3/\mu\text{l}$. The likelihood of 10.17, 3.39, 6.17 and 10.2 was reported at platelets counts less than $200 \times 10^3/\mu\text{l}$, $170 \times 10^3/\mu\text{l}$, $140 \times 10^3/\mu\text{l}$ and $145 \times 10^3/\mu\text{l}$ for children under 5, 6-16 years, adult females and males respectively.

PCT presented in (Tables 1 and 2) with a significant likelihood of 7.61 and 8.02 for children

less than 5 years and adult males respectively. The reason for this observation is attributed to the fact that PCT is proportional to platelets counts just as HCT is proportional to HB and RBC count.

Another striking observation in this study is the increase in the likelihood of MPV (4.01 and 9.82) for children, 6-16 years and adult females respectively. There is no literature to support this observation but may be due to the presence of increase younger platelets in positive malaria cases the same way increase in MCV is associated with reticulocytosis in malaria.

In this study, leukocytosis, absolute neutrophilia, monocytosis and eosinopenia were observed to be good predictors of malaria in children between 6-16 years of age with the likelihood of 8.92, 4.23, 13.2 and 2.09 respectively. For children less than 5 years, absolute basophilia was the only leukocyte predictor associated with the presence of malaria. However, leukopenia, absolute lymphopenia, monocytosis and eosinopenia were profiles that gave high likelihood ratio for adult males whiles absolute lymphopenia and eosinopenia were the only strong predictors of malaria for adult females.

There was a strong negative association between HB and parasite density ($r = -0.23$). This means that higher parasites density is associated with lower HB concentrations. The coefficient of determination ($r^2 = 5.3\%$), (Fig. 1).

There was a strong negative association between RBC count and parasite density ($r = -$

0.203). This suggests that higher parasites density is associated with lower RBC count. The coefficient of determination ($r^2 = 4.1\%$), (Fig. 2).

There was no association between MCV, MCH and MCHC values and parasites density ($r = -0.05, -0.08$ and -0.02 respectively). This means that higher parasites density is not associated with lower MCV, MCH and MCHC values respectively. The coefficient of determination ($r^2 = 0.41\%, 0.68\%$ and 0.05% respectively).

There was a weak positive association between RDW-SD values and parasites density ($r = 0.09$). This indicates that higher parasites density is associated with higher RDW-SD values. The coefficient of determination ($r^2 = 0.8\%$), (Fig. 3). There was a weak positive association between RDW-CV values and parasites density ($r = 0.13$). This means that higher parasites density is associated with higher RDW-CV values. The coefficient of determination ($r^2 = 1.7\%$), (Fig. 4).

There was a weak negative association between platelets count and parasites density ($r = -0.13$). This suggests that higher parasites density is associated with lower platelets count. The coefficient of determination ($r^2 = 1.8\%$), (Fig. 5).

There was a very weak positive association between PDW values and parasites density ($r = 0.08$). This means that higher parasites density is associated with higher PDW values. The coefficient of determination ($r^2 = 0.7\%$), (Fig. 6).

There was no association between MPV and PCT values and parasites density ($r = -0.009, -0.0015$ respectively). This means that higher parasites density is not associated with lower MPV and PCT values respectively.

There was a weak positive association between P-LCR values and parasites density ($r = 0.13$). This indicates that higher P-LCR values are associated with higher parasites density. The coefficient of determination ($r^2 = 1.7\%$), (Fig. 7).

There is a strong positive association between total WBC count and parasite density ($r = +0.45$). This suggests that higher parasites density is associated with high total WBC counts. The coefficient of determination ($r^2 = 20\%$) (Fig. 8).

There is a strong positive association between absolute neutrophil count and parasite density ($r = +0.40$). This means that higher parasites

density is associated with higher absolute neutrophil count. The coefficient of determination ($r^2 = 16\%$), (Fig. 9). There is a weak positive association between absolute lymphocyte count and parasite density ($r = +0.28$). This means that higher parasites density is associated with higher absolute lymphocytes counts. The coefficient of determination ($r^2 = 8.0\%$) (Fig. 10).

There is a strong positive association between absolute monocyte count and parasite density ($r = +0.20$). This suggests that higher parasites density is associated with higher absolute monocyte count. The coefficient of determination ($r^2 = 4.0\%$), (Fig. 11).

There is a very weak positive association between absolute eosinophil count and parasite density ($r = +0.05$). This means that higher parasites density is associated with higher absolute eosinophil count. The coefficient of determination ($r^2 = 0.23\%$), (Fig. 12). There is a weak positive association between absolute basophil count and parasite density ($r = +0.14$). This means that higher parasites density is associated with higher absolute basophil count. The coefficient of determination ($r^2 = 2.1\%$), (Fig. 13).

6. CONCLUSION

The haematological profiles give likelihood indication of diagnosing malaria but there was variation on age and sex. Anaemia, low RBC count, HCT, PLT, PCT, leukopenia, absolute lymphopenia, monocytosis and eosinopenia can heighten the suspicion of malaria in adult males. The degree of anaemia, low HCT, low RBC, low platelets, leukocytosis, absolute neutrophilia, monocytosis and lymphopenia is associated with the parasites density level. Haematological profiles can be used in addition to the clinical and microscopic parameters to heighten the suspicion of malaria and prompt initiation of the therapy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Research and Ethical Review Committee of the University of Ghana Medical School, College of Health Science Korle-Bu, Ghana.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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