

1 Hematological parameters associated with malaria and its 2 controls

3 ABSTRACT

4 **Aims** This research aimed to evaluate the hematological parameters associated with malaria
5 and its controls.

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

10 **Results and Discussion:** The erythrocyte profiles (RBC, HB, HCT, RDW-SD and RDW-
11 CV) are highly affected by malaria, whereas MCH, MCHC, and MCV did not show
12 significant variations between the positive malaria cases and negative malaria cases. Means
13 of haemoglobin concentrations, RBC count and HCT values for cases with positive malaria
14 were significantly lower than negative malaria cases and controls for all the age groups and
15 sexes.

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

20 **Keywords:** Haematological parameters, leukocytosis, parasite density, *Plasmodium*,
21 haemoglobinopathy

22 23 Introduction

24 Malaria is an infectious disease caused by a protozoan called *Plasmodium* (phylum
25 Apicomplexa) that is transmitted through the bite of an infected *Anopheles* mosquito. The
26 species that causes this infection in human include *P. falciparum*, *Plasmodium malariae*,
27 *Plasmodium ovale*, *Plasmodium knowlesi* and *Plasmodium vivax*¹. The report indicated that
28 the deaths of 1.5 to 2.7 million per annum are attributed to 300-500 million acute cases of

29 malaria that occurs worldwide each year². However, *Plasmodium falciparum* (*P. falciparum*)
30 is the major cause of the disease and is responsible for about 90% of malaria infections and
31 80% of malaria deaths in sub-Saharan Africa for which Ghana is not an exception^{3,1}. In
32 Ghana, the estimated cases of malaria reported in children below 5 years were nearly 4
33 million with approximately 21 thousand deaths and fatality rate of 0.53%⁴. The increase in
34 malaria infections is an impediment to the world's population and is as a result of
35 deteriorating health systems, growing drug and insecticide resistance, climate change, natural
36 disasters and armed conflict^{5,6}. In general, malaria accounts for 10% of Africa's disease
37 burden and cost the continent \$12 billion annually^{7,8,9,10}. The report indicates that in Ghana,
38 funding provided by the government from the Global Fund, the World Bank and bilateral
39 donors to control malaria was close to US\$ 60 million and US\$ 40 million in 2006 and 2007
40 respectively⁴.

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42 The haematological profile is also known as haemogram which comprises full blood count
43 (FBC), full blood exam (FBE) or blood panel is a test that gives information about the cells
44 in a patient's blood¹¹. It is used for clinical purposes, monitoring, screening and case finding
45 for example of patients with symptoms such as fatigue or weakness, infection, inflammation,
46 bruising, or bleeding¹¹. The abnormal high or low blood counts may be due to the presence
47 of disease for which blood count tests are performed in medicine to provide an overview of
48 a patient's general health status¹¹. These tests comprise haemoglobin, haematocrit, red cell
49 indices, red cell distribution width (RDW), total and differential leukocyte counts, and
50 platelet counts which are used as a routine test for patients to complement diagnosis of
51 diseases¹². Report indicated that haematologic aberrations are the most common
52 complications encountered in malaria and play a major role in the fatality¹³. These changes
53 associated with malaria infection are well recognized but specific changes may vary with the
54 level of malaria endemicity, background haemoglobinopathy, nutritional status,
55 demographic factors, and malaria immunity¹⁴. This study aimed to evaluate hematological
56 parameters associated with malaria and its controls. The haematological changes would
57 enable the differentiation of malaria from other diseases that are present with similar
58 symptoms such as anaemia and thrombocytopenia which are common among patients with
59 *Plasmodium falciparum*¹⁵.

60

61 **Materials and Methods**

62 **Study area**

63 The study was carried out in the following Polyclinics using random sampling technique
64 including Mamprobi, Ussher town, Dansoman, Princess Marie and La in Accra Metropolis in
65 the Greater Accra region of Ghana. The region has an estimated population of 1.6 million and
66 is located in the coastal savannah zone with an average annual rainfall of 730 mm. Malarial
67 transmission in the region is between May to October with perennial and hyper-endemic
68 seasonal peak rainy season.

69

70 **Ethical Issues**

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

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74 **Study Population and Sample size Determination**

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76 The samples were drawn from the population of patients who attended the
77 Polyclinics/Hospitals laboratory from January to August 2009 with fever or clinical signs and
78 symptoms suggestive of malaria based on World Health Organization (WHO) criteria. A
79 convenient cross-sectional study from each of the five (5) study sites was used to obtain a
80 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
81 malaria in Accra (14.8%) and A = Allowable error = 0.05. Based on the above formula, the
82 calculated minimum sample size of 300 subjects was enrolled for the study. All subjects who
83 presented to the Polyclinic/Hospital Laboratory with request cards from specified clinicians
84 indicating suspected malaria were included in the study. The clinicians in each of the study
85 sites were briefed and given an abstract of the study. The selection of the cases for the study
86 depended on their expertise and was required to indicate by writing the diagnosis on the
87 laboratory request card.

88

89 **Administration of Questionnaire**

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91 A structured questionnaire was also administered to each consenting volunteer to document
92 information on demographics, current symptoms and previous malaria episodes and
93 treatments. Two hundred and Fourteen (214) apparently healthy Blood donors and Children

94 from first cycle Schools who were located in the areas where the cases were obtained and
95 whose peripheral blood film screen was negative for the malaria parasite served as controls.

96 **Laboratory Analysis**

97

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

104 **Automated Counting**

105 **Complete blood count and differential test using Sysmex XT-2000i Automated** 106 **haematology Analyzer**

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

115 **Statistical analysis**

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

119

120 **Results**

121 **Haematological profiles predictive of malaria**

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

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

131

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($\times 10^{12}/L$)<4.00	6.71*	<0.001	RBC($\times 10^{12}/L$) <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 ($\times 10^9/L$) <200	10.17*	<0.001	PLT ($\times 10^9/L$) <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 ($\times 10^9/L$)	1.00	1.000	TWBC ($\times 10^9/L$)>13.0	8.92*	<0.001

>15.0						
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	

132 *= Haematological profiles with the most significant predictors for the presence of malaria
 133 for children less than 5 and 6-16 years

134 **Reference range used was obtained from Dacie and Lewis¹⁶.**

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

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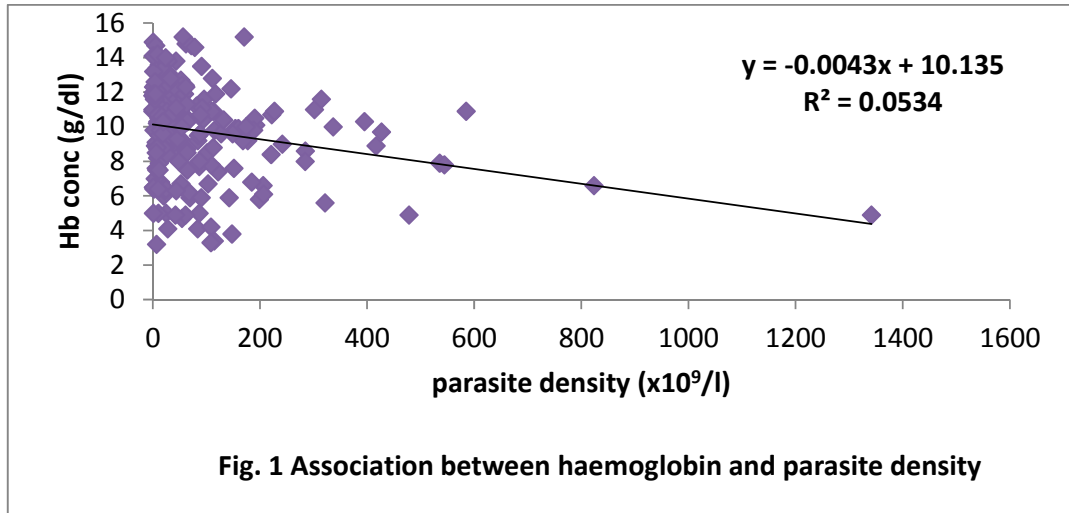
ADULT MALES ABOVE 16 YEARS			ADULT FEMALES ABOVE 16 YEARS		
Variables	Likelihood ratios	P values	Variables	Likelihood ratios	P values
HB (g/dl) <13.0	0.65	<0.001	HB (g/dl) <12.0	3.89*	<0.001
RBC(x10 ¹² /L) <4.40	1.56	<0.001	RBC (x 10 ¹² /L) <4.00	7.68*	<0.001
HCT (%) <38.0	6.16*	<0.001	HCT (%) <35.0	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 >4.60	2.33 1.37	<0.001 0.007	NEUT# (10 ⁹ /L) <1.40	1.61	<0.001
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

141 *= Haematological parameters with the most significant predictors for the presence of
142 malaria for adult males and females.

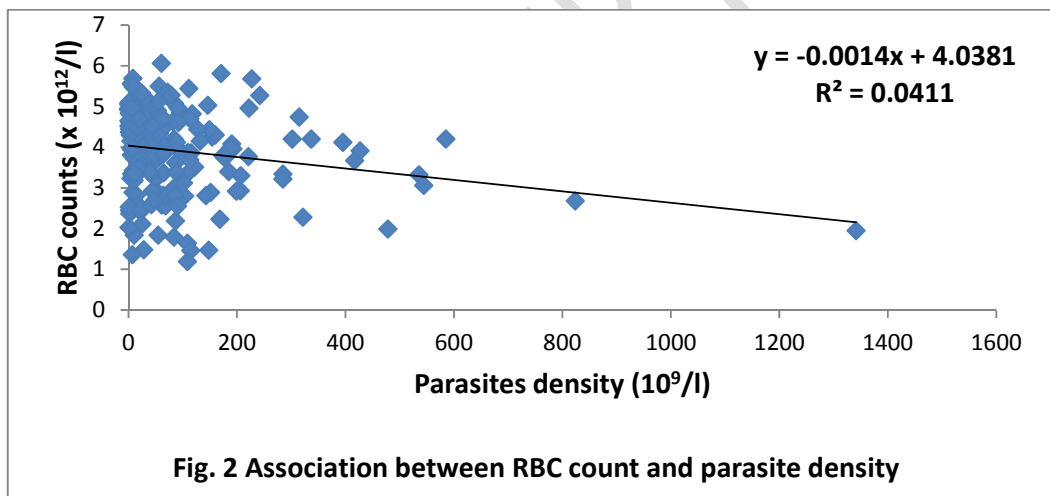
143 Reference ranges used was obtained from Akuetteh¹⁷.

144 **Correlation between each haematological profile and parasite density**



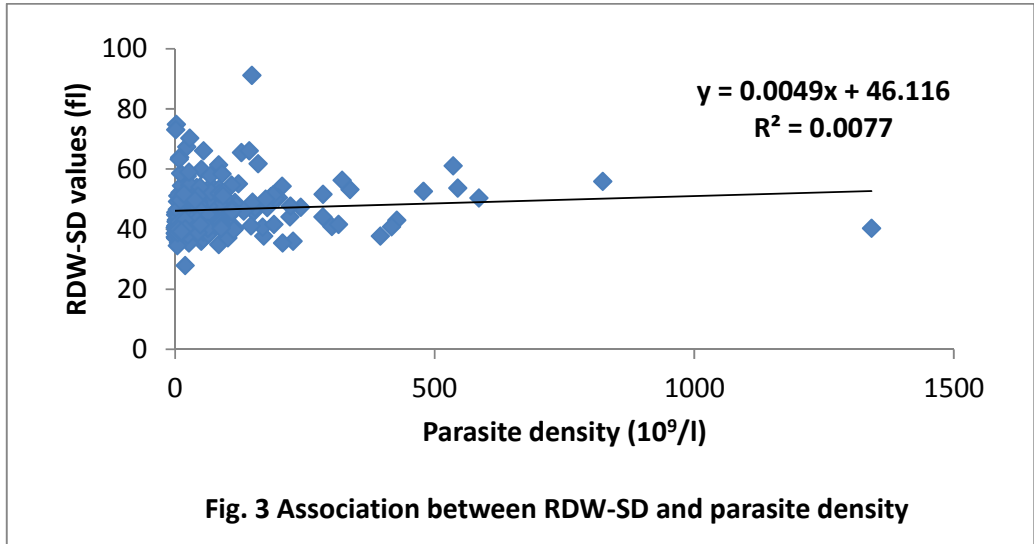
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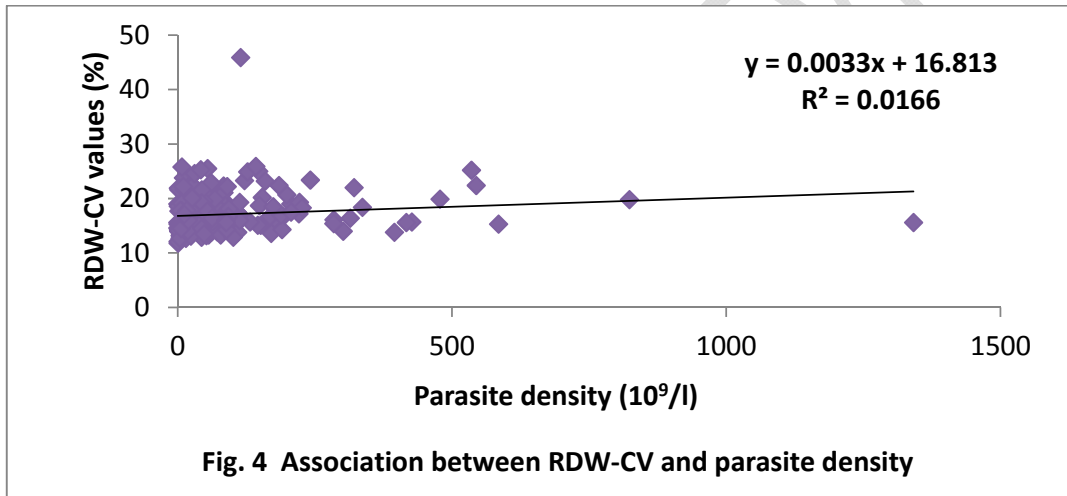


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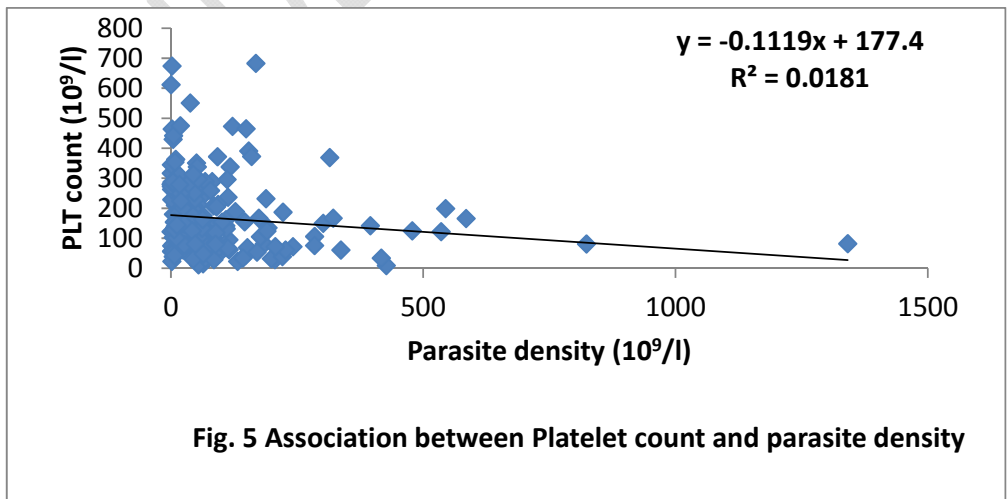
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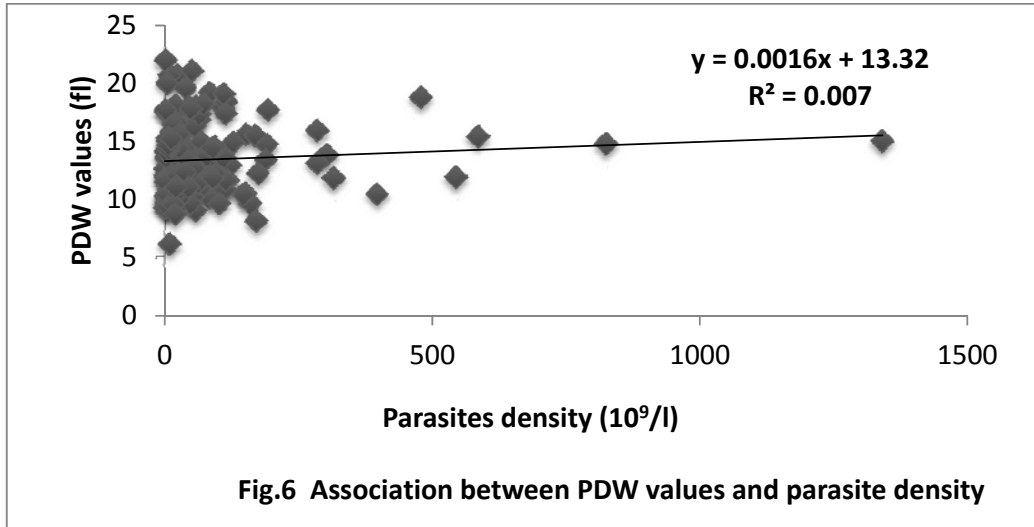
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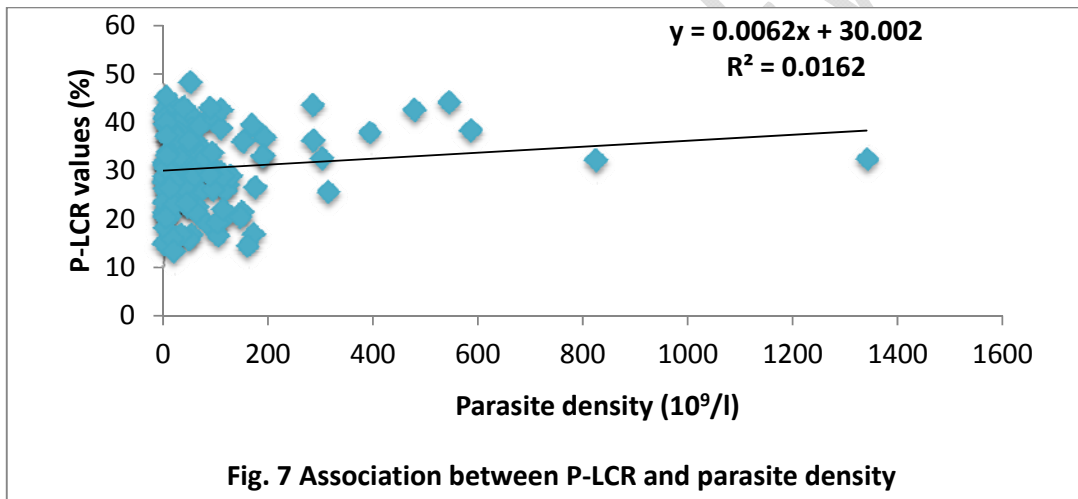
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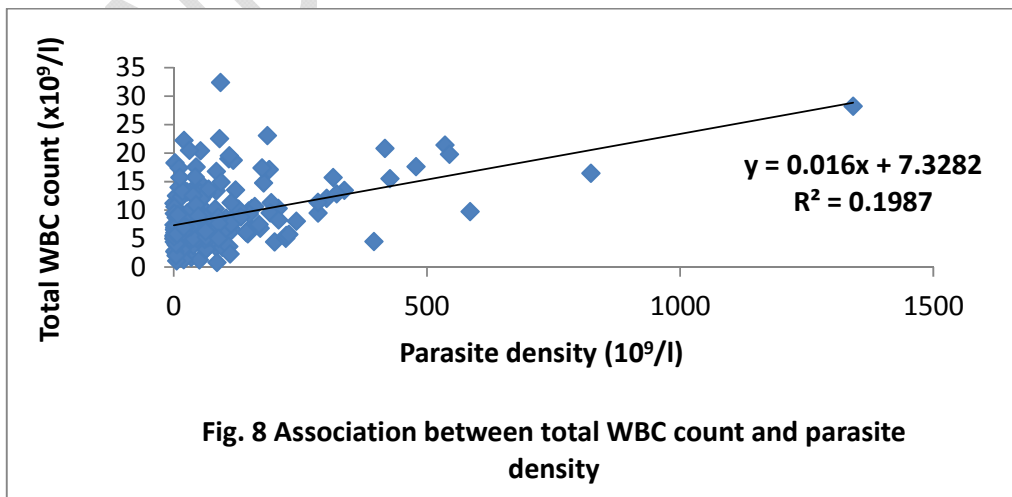
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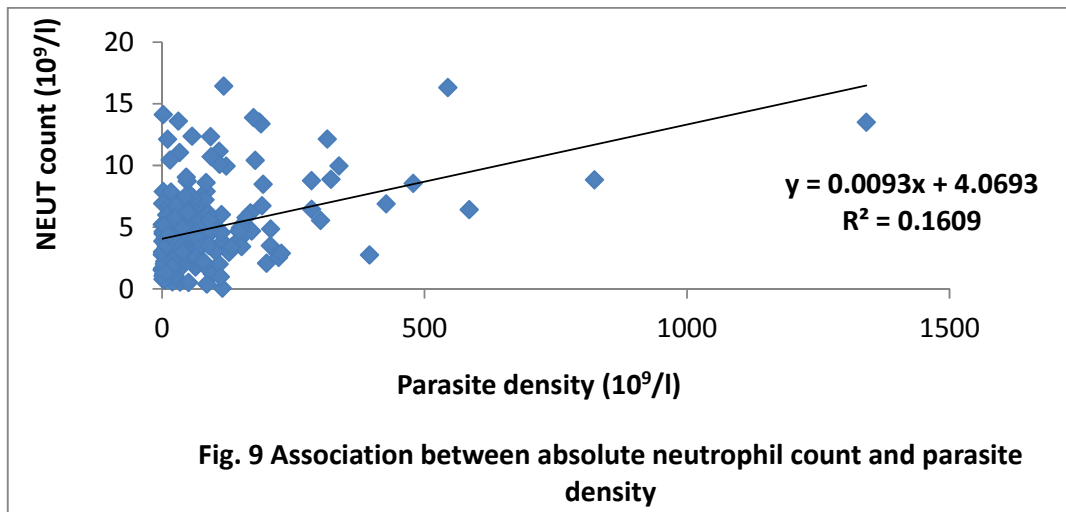
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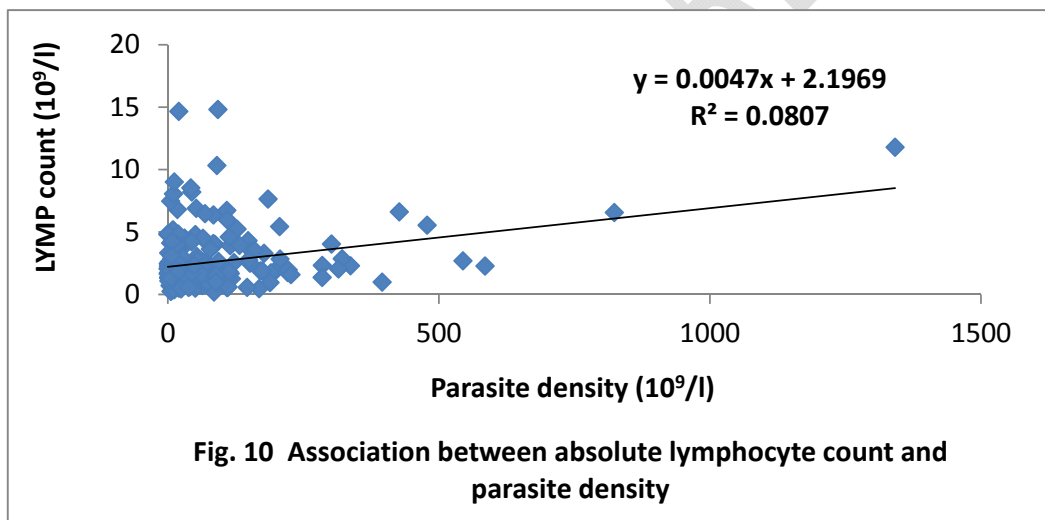
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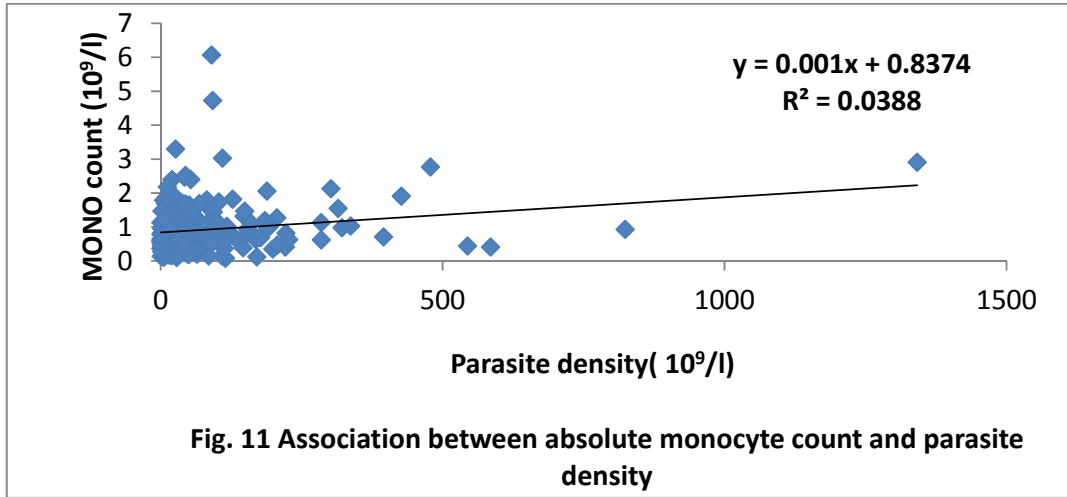
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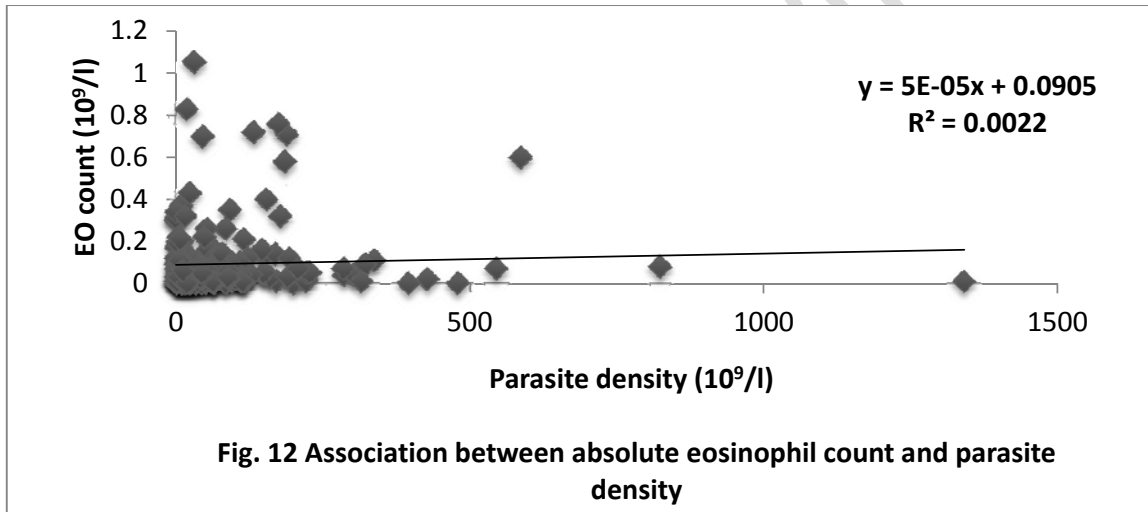


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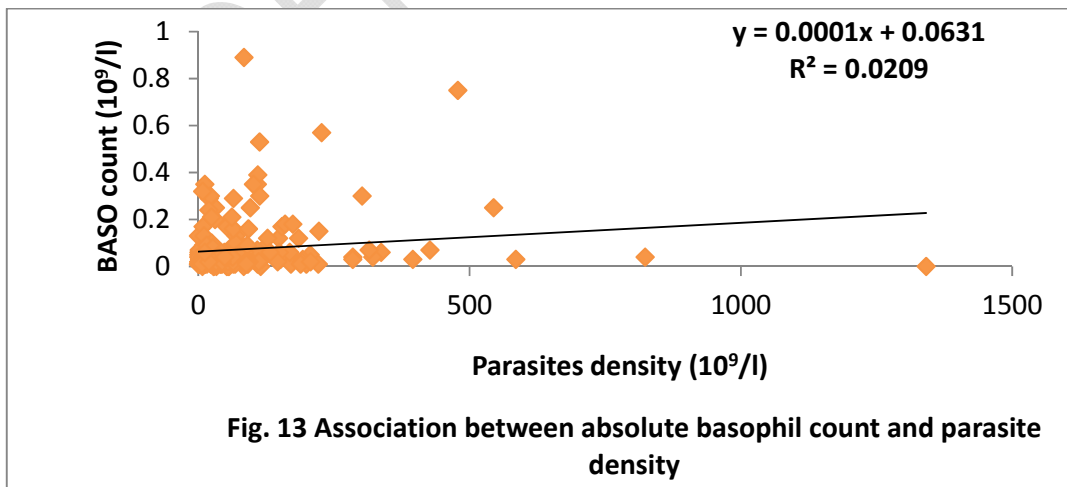


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170 **Discussion**

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

180 A platelet count was better predictors of malaria in all the age and sex categories, a previous
181 observation which this study also confirmed. In a study on over two thousand patients with
182 fever, Erhart *et al.*¹⁸ reported platelet count of less than $150 \times 10^3/\mu\text{l}$ increases the likelihood
183 of malaria by 12-15 times whiles Lathia *et al.*¹⁹ reported likelihood of malaria by 5.04 at $150 \times$
184 $10^3/\mu\text{l}$ and Laura *et al.*²⁰ reported 14.7 for *P. falciparum* infection at $150 \times 10^3/\mu\text{l}$. The
185 likelihood of 10.17, 3.39, 6.17 and 10.2 was reported at platelets counts less than $200 \times$
186 $10^3/\mu\text{l}$, $170 \times 10^3/\mu\text{l}$, $140 \times 10^3/\mu\text{l}$ and $145 \times 10^3/\mu\text{l}$ for children under 5, 6-16 years, adult
187 females and males respectively.

188 PCT presented in (Table 1 and 2) with a significant likelihood of 7.61 and 8.02 for children
189 less than 5 years and adult males respectively. The reason for this observation is attributed to
190 the fact that PCT is proportional to platelets counts just as HCT is proportional to HB and
191 RBC count.

192 Another striking observation in this study is the increase in the likelihood of MPV (4.01 and
193 9.82) for children, 6-16 years and adult females respectively. There is no literature to support
194 this observation but may be due to the presence of increase younger platelets in positive
195 malaria cases the same way increase in MCV is associated with reticulocytosis in malaria.

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

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

206 There was a strong negative association between RBC count and parasite density ($r = -0.203$).
207 This suggests that higher parasites density is associated with lower RBC count. The
208 coefficient of determination ($r^2 = 4.1\%$), (figure 2).

209 There was no association between MCV, MCH and MCHC values and parasites density ($r = -$
210 $0.05, -0.08$ and -0.02 respectively). This means that higher parasites density is not associated
211 with lower MCV, MCH and MCHC values respectively. The coefficient of determination (r^2
212 $= 0.41\%, 0.68\%$ and 0.05% respectively).

213 There was a weak positive association between RDW-SD values and parasites density ($r =$
214 0.09). This indicates that higher parasites density is associated with higher RDW-SD values.
215 The coefficient of determination ($r^2 = 0.8\%$), (figure 3). There was a weak positive association
216 between RDW-CV values and parasites density ($r = 0.13$). This means that higher parasites
217 density is associated with higher RDW-CV values. The coefficient of determination ($r^2 =$
218 1.7%), (figure 4).

219 There was a weak negative association between platelets count and parasites density ($r = -$
220 0.13). This suggests that higher parasites density is associated with lower platelets count.
221 The coefficient of determination ($r^2 = 1.8\%$), (figure 5).

222 There was a very weak positive association between PDW values and parasites density ($r =$
223 0.08). This means that higher parasites density is associated with higher PDW values. The
224 coefficient of determination ($r^2 = 0.7\%$), (figure 6).

225 There was no association between MPV and PCT values and parasites density ($r = -0.009, -$
226 0.0015 respectively). This means that higher parasites density is not associated with lower
227 MPV and PCT values respectively

228 There was a weak positive association between P-LCR values and parasites density ($r =$
229 0.13). This indicates that higher P-LCR values are associated with higher parasites density.
230 The coefficient of determination ($r^2 = 1.7\%$), (figure 7).

231 There is a strong positive association between total WBC count and parasite density ($r =$
232 $+0.45$). This suggests that higher parasites density is associated with high total WBC counts.
233 The coefficient of determination ($r^2 = 20\%$) (Figure 8)

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

236 neutrophil count. The coefficient of determination ($r^2 = 16\%$), (figure 9) There is a weak
237 positive association between absolute lymphocyte count and parasite density ($r = +0.28$). This
238 means that higher parasites density is associated with higher absolute lymphocytes counts.
239 The coefficient of determination ($r^2 = 8.0\%$) (Figure 10).
240 There is a strong positive association between absolute monocyte count and parasite density
241 ($r = +0.20$). This suggests that higher parasites density is associated with higher absolute
242 monocyte count. The coefficient of determination ($r^2 = 4.0\%$), (figure 11).
243 There is a very weak positive association between absolute eosinophil count and parasite
244 density ($r = +0.05$). This means that higher parasites density is associated with higher
245 absolute eosinophil count. The coefficient of determination ($r^2 = 0.23\%$), (figure 12). There
246 is a weak positive association between absolute basophil count and parasite density ($r =$
247 $+0.14$). This means that higher parasites density is associated with higher absolute basophil
248 count. The coefficient of determination ($r^2 = 2.1\%$), (figure 13).

249

250 **Conclusions**

251

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

259

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