



***Plasmodium falciparum* Induces Significant Changes in Haematological Profile of Patients in Parts of Ondo State, South West Nigeria**

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Authors' contributions

This work was carried out in collaboration among all authors. Author OAO conceived the study. Authors TAO, IAS-O and OAO designed the research work. Authors OAO did the sample collection. Author OAO and IAS-O carried out the laboratory work. Author OAO conducted the data analysis. Author OAO wrote the first draft of the manuscript. Author IAS-O reviewed the manuscript. Authors TAO and IAS-O reviewed and approved the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Malaria parasite induces significant devastating changes in blood cell components causing enormous destruction of red blood cells. This study seeks to investigate the various haematological changes that could result from falciparum malaria infection in man. Haematological parameters of out-patients visiting selected health facilities in Akure North and South Local Government Areas in

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Ondo State from January to October 2022 were evaluated in this cross-sectional study. Venous blood samples were collected in EDTA (ethylenediamine tetra-acetic acid) and plain bottles from febrile patients visiting these hospitals and were examined for different haematological parameters. Thick and thin blood smears were made for all enrolled patients and stained smears were examined microscopically for *Plasmodium falciparum* malaria parasites. One thousand four hundred and three (1403) patients were enrolled in this study, this was made up of 369 (26.3%) males and 1034 (73.7%) females. Findings indicate the following parameters: Packed Cell Volume (PCV), Neutrophil counts and Lymphocyte counts were significantly associated with malaria diagnosis as they were either significantly increased or reduced as a result of *P. falciparum* infection. The average PCV of patients infected with *Plasmodium falciparum* (32.22 %) was significantly ($P < .001$) lower than uninfected patients (35.46 %). Also mean Lymphocytes count obtained from infected patients (40.29%) was significantly ($P < .001$) lower than uninfected (46.06%). An opposite trend was however observed in the neutrophil counts, as infected patients (58.54%) recorded significantly ($P < .001$) higher neutrophil counts than uninfected patients (52.58%). Malaria induced notable significant alterations in some of the haematological parameters evaluated in the study, patients with microscopic confirmation of malaria parasites showed significant decrease in the PCV and lymphocytes levels, while a significant increase in neutrophil count was recorded in response to *Plasmodium falciparum* malaria.

Keywords: Malaria; parasite; blood; packed cell volume; neutrophils; lymphocytes.

1. INTRODUCTION

The *Plasmodium* parasite is a blood pathogen that causes different haematological manifestations, targeted at not only the red blood cells, but white blood cells and platelets [1]. Changes in differential blood counts in man are likely presumed as outcomes from diseased conditions in man. Malaria remains a major cause of health problem with enormous devastating consequences in tropical countries including Nigeria. In 2022, studies revealed that there were 249 million estimated cases of malaria reported in 85 malaria endemic countries, with 608,000 deaths, mostly children in sub-Saharan Africa [2]. WHO also reported that in Nigeria, malaria accounted for 26.8% of the total number of cases recorded, with 33.1% of the deaths representing nearly half of all cases reported globally, far more than any country. Malaria in man can be caused by any of the several species of the human *Plasmodium* parasites that may occur as mono infection or as mixed infections in regions of endemicity. In Nigeria, malaria remains a major health problem, with *Plasmodium falciparum* as the predominant specie responsible for over 90% of the malaria cases [3,4]. The most severe forms of malaria, is caused by *Plasmodium falciparum*, it accounts for most of the morbidity and mortality associated with the disease, while other species of *Plasmodium* rarely produce serious complications, debilitating diseases or deaths.

Malaria microscopy remains the gold standard for laboratory confirmation, not just because of its

high sensitivity and specificity, but because it also allows for species identification and parasitaemia quantification. Malaria is characterized by release of inflammatory mediators from innate immune cells, this early interaction between the parasites and these inflammatory mediators has been shown to be important in controlling parasitaemia and subsequently elimination of infection [5].

Haematologic alterations characterize malaria and are related to the overt biochemical changes that occur during the asexual stages of the life cycle of the malaria parasite [6]. *Falciparum* malaria can lead to significant alterations in the haematological parameters of infected persons, parameters such as red blood cell, white blood cell and platelets counts [7]. Patients with parasitological confirmed malaria infection have been reported to have significantly lower counts of leukocytes, lymphocytes, eosinophil, platelets and Packed Cell Volume (PCV), and also significantly higher counts of neutrophils and monocyte [8,9]. *Falciparum* malaria can lead to haematological abnormalities such as anaemia and thrombocytopaenia, which has been investigated to play major roles in its pathogenesis, and results in severe complications of the disease [8]. Malaria induced anaemia is one of the most common complications frequently seen in pregnant women and children in countries of high endemicities including Nigeria and If untreated, malaria can progress rapidly to severe state or death within hours or days. Complications such as thrombocytopaenia, marked by platelets count

of less than 150,000/ μ L seems to be the most commonly observed in severe malaria patients [10,11]. Diagnostic predictions of these haematological alterations may be easily obtained and useful in people living in malaria endemic countries, including Nigeria.

Alterations in the values of these haematological parameters of patients coupled with parasitological confirmation of malaria, will avail clinicians to establish very early effective therapeutic interventions to prevent major complications in nonendemic and endemic countries including Nigeria. This includes guidance in diagnosis, intensification of treatment, ultimately resulting in reductions and preventions of morbidity and deaths that might be possible outcomes of malaria complications. We hypothesize that haematological ratios could be more useful in clinical routine practices in the diagnosis and treatment of malaria infection.

2. MATERIALS AND METHODS

2.1 Study Area

This is a cross-sectional study which was carried out in five selected health facilities in major towns of Akure North and South Local Government Areas (LGAs) of Ondo state. These include: University of Medicine Teaching Hospital Akure (UMTH), Mother and Child Hospital Akure (MCHA), Community Health Centre Akure (CHCA), Basic Health Centre Oba-Ile (BHCO) and General Hospital Iju (GHI). (Fig. 1).

These LGAs lie between longitudes 5°06" and 5°38"E; and latitudes 7°07" to 7° 37"N in the South western part of Nigeria [12]. The area normally experiences warm humid tropical climate with two distinct seasons, these are the rainy and dry seasons. It receives an annual rainfall of over 1500mm with a short break in August. The average temperature is about 22°C during harmattan (December to February) and 32°C in March [13].

2.2 Ethical Consideration and Inclusion Criteria

The ethical approval of the research work with Number: OSHREC 10/9/2021/377 was obtained from The Health Research Ethics Committee of Ondo State Ministry of Health, Akure. Institutional permission and approval were in addition sought and obtained from the Local Ethics committees of each health facility. The purpose of the study

was explained to each patient after which they were required to sign the consent or assent forms before being recruited to participate in the research. Patients aged six months and above were then assigned unique identification numbers (Code). They were recruited based on defined inclusion criteria which were presentation of fever at the time of visit to the hospital and/or history of fever within the last 48 hours. Exclusion criteria for the study were: presence of any danger signs of complicated/severe malaria, patients presenting with severe /chronic disease and refusal of any patient/guardian to give written consent to be enrolled in the study.

2.3 Collection of Questionnaire Data

Case Report Forms (CRFs) were employed to capture demographic characteristics and clinical history of patients. Information such as age, sex, temperature at point of presentation, symptoms presented, medications used/taken, frequency of use of bed nets etc were filled for each patient.

2.4 Blood Sample Collection

Human venous blood samples were collected from January to October, 2022, spanning both the dry season and the raining season known to be periods of high transmission. Blood samples were collected from febrile patients visiting the Out Patients Departments (OPD) of the five selected health facilities in the study area into labelled EDTA bottles and plain bottles from each patient by well-trained phlebotomist or pediatricians at the different health facilities. Good clinical and laboratory practice were thoroughly adhered to in the course of the study. One thousand four hundred and three patients that had complete full blood count and this includes PCV, WBC, Neutrophil, Lymphocyte, Eosinophil, Monocyte and Basophil counts results, were enrolled in the study, while patients with incomplete haematological results were excluded from this study.

2.5 Determination of Sample Size

A minimum of 384 samples was collected from each LGA in accordance with Raosoft sample size calculator [14] at 5% margin of error and 95% confidence interval.

2.6 Microscopy

Duplicate slides with thick and thin smears of patient's blood sample were prepared for each

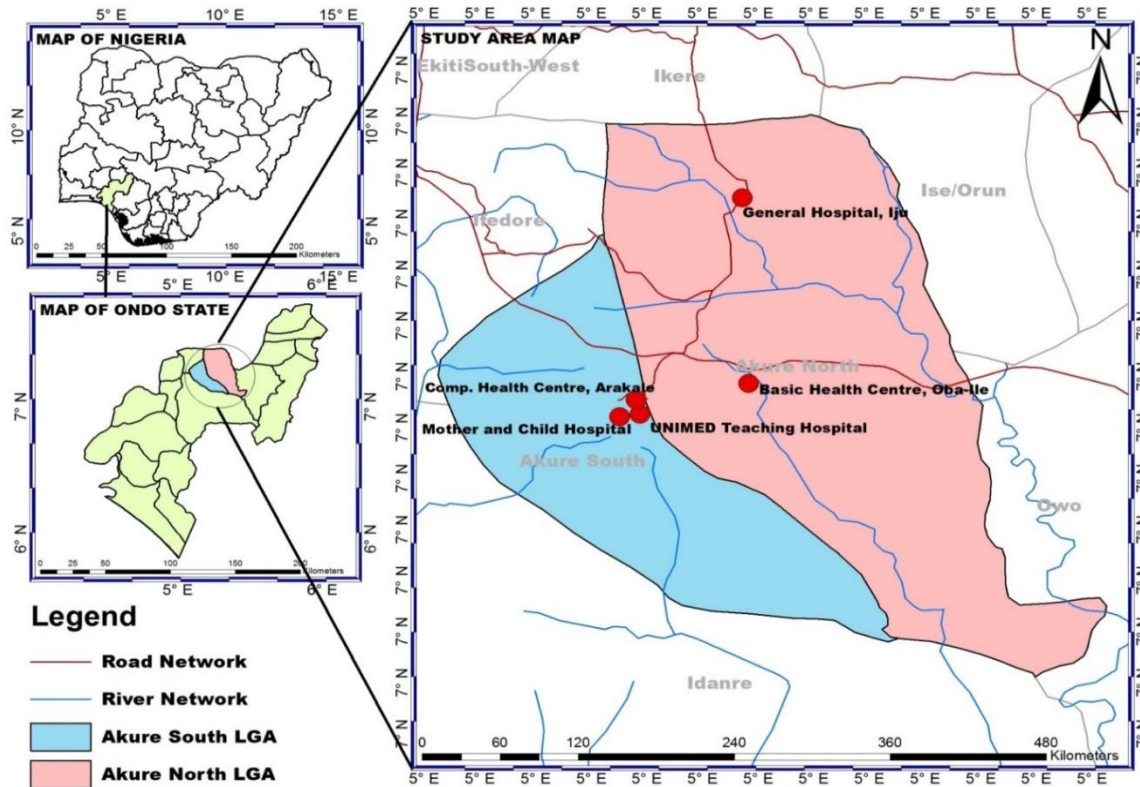


Fig. 1. Map Showing Selected Health Establishments in the Study Areas

patient, dry slides were stained with 3% Giemsa's stain pH of 7.0-7.2 for one hour [15], examined under light microscope using oil immersion objective lens x 100 and the results documented for presence or absence of malaria parasite, stages present and Plasmodium species. A slide was interpreted/reported as negative when none of the asexual forms (i.e., trophozoites or schizonts) or sexual stage (gametocyte) of the parasite was observed after 200 high power fields have been examined. Each study slide was read independently by two expert microscopists, mean parasitaemia was computed and discordance of <20% was ensured, however, parasitaemia counts >20% discordance necessitated a 3rd read. The parasitaemia levels were categorized as low (<1000 parasites / μ L), moderate (1,000 -9,999 parasites / μ L) and severe (\geq 10,000 parasites / μ L).

2.7 Haematological Measurements

Glass capillary tubes were filled with EDTA blood to $\frac{3}{4}$ of its lengths after which they were centrifuged in a microhaematocrit centrifuge at 12 000g for 5 minutes and examined for packed cell volume and results documented in percentages (%). Determination of White Blood

Cell count was done using Turks solution to dilute the blood samples in the ratio 1:20, diluted blood was added to the Neubauer chamber, microscopically examined and white blood cells were counted. For differential WBC counts, thin blood smears were prepared and stained with Leishman stain and afterwards examined microscopically using procedures as enumerated [16].

2.8 Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 23. Data were presented as frequencies, percentages, means, standard deviations. Categorical data were compared using Pearson Chi-Square test. Differences between continuous variables were tested using the Kruskal-Wallis test and the Mann -Whitney test, association between two continuous data variables were assessed by Spearman's correlation. *P*-values less than .05 were considered statistically significant.

3. RESULTS

A total of one thousand four hundred and three patients were enrolled in the study, this includes 369 (26.30%) males and 1034 (73.0%) females,

average age of study population was 30.11, the modal group for the study was age group 21-30 years with a representation of 26.59% of the total number enrolled, the least number enrolled was from the elderly group of ages 61 years and above with an enrollment of 5.70%. A total of 178 (12.69%) had parasitological confirmation of malaria by microscopy (Table 1).

Table 1. Demographic characteristics of patients in Akure North and South Local Governments of Ondo State, South West Nigeria, enrolled in the study

Characteristics	Number (%)
Gender	
Males	369 (26.30)
Females	1034 (73.70)
Age: Mean (SD), years	30.11 (17.60)
Age groups	
≤1-10	237 (16.89)
11-20	162 (11.55)
21-30	373 (26.59)
31-40	303 (21.6)
41-50	152 (10.83)
51-60	96 (6.84)
≥61	80 (5.70)
Malaria prevalence	
Number positive	178 (12.69)
Number negative	1225(87.31)

Table 2. Diagnostic haematological markers for *P. falciparum* malaria in patients attending selected health facilities in Akure North and South LGAs of Ondo State, Nigeria

Haematological Parameters	Levels	Positive N=178(%)	Negative N=1225(%)	P value
PCV (%)	Very Low (<15%)	3(1.7)	9(0.7)	<.001
	Low (16-35%)	123(69.1)	658(53.7)	
	Normal (36-45%)	47(26.4)	497(40.6)	
	High (>45%)	5(2.8)	61(5.0)	
WBC (cells/μL)	Low (< 4,000 cells/μL)	54(30.3)	327(26.7)	.46
	Normal (4,000-11,000 cells/μL)	108(60.7)	802(65.5)	
	High (> 11,000 cells/μL)	16(9.0)	96(7.8)	
Neutrophils (%)	Low (< 40%)	24(13.5)	288(23.5)	.001
	Normal (40 – 75%)	124(69.7)	813(66.4)	
	High (>75%)	30 (16.9)	124(10.1)	
Lymphocytes (%)	Low (<20%)	17(9.6)	58(4.7)	<.001
	Normal (20-45%)	97(54.5)	542(44.2)	
	High (>45%)	64(36.0)	625(51.0)	
Eosinophils (%)	Low (<2%)	173(97.2)	1169 (95.4)	.32
	Normal (2-6%)	4 (2.2)	53 (4.3)	
	High (>6%)	1 (0.6)	3 (0.2)	
Monocytes (%)	Low (< 2%)	121 (68.0)	818 (66.8)	.77
	Normal (2-10)	57 (32.0)	404 (33.0)	
	High (> 10%)	0 (0.0)	3 (0.2)	
Basophils (%)	Normal (0-1 %)	177 (99.4)	1220 (99.6)	.56
	High (> 1%)	1 (0.6)	5 (0.4)	

Key: WBC: White Blood Cell; PCV: Packed cell volume

Table 3. Comparison of Mean haematological profile Indices between *Plasmodium falciparum* infected and uninfected patients in Akure North and South Local Government areas of Ondo State, South West Nigeria

Indices	<i>P. falciparum</i> infected patients (n=178) Mean ± SD	<i>P. falciparum</i> malaria negative patients (n=1225) Mean ± SD	P values
Total WBC (cells /µL)	6138.76 ± 4590.55	6235.10 ± 4534.90	.20
PCV (%)	32.22 ± 6.74	35.46 ± 6.27	<.001
Neutrophils (%)	58.54 ± 17.47	52.58 ± 17.11	<.001
Lymphocytes (%)	40.29 ± 17.40	46.06 ± 16.92	<.001
Eosinophils (%)	0.13 ± 0.87	0.15 ± 0.81	.30
Monocytes (%)	0.98 ± 1.74	1.12 ± 1.93	.55
Basophils (%)	0.01± 0.15	0.01± 0.14	.80

Key: WBC: White Blood Cell; PCV: Packed cell volume

Table 4. Mean Haematological Parameters at different parasitaemia levels recorded from patients at Akure North and South LGAs, Ondo State, Nigeria.

Indices	Low (1-999 p/µL) (n=78)	Moderate (1000 - 9999 p/µL) (n=34)	Severe (≥ 10000 p/µL) (n=66)	P value
Total WBC (cells /µL)	5221.80 ±3490.75	7361.77 ±7406.18	6615.15 ±3599.97	.001
PCV (%)	32.99 ±5.89	32.97 ±7.81	31.15 ±7.15	.50
Neutrophils (%)	55.64 ±16.36	57.12 ±16.43	62.94 ±18.47	.02
Lymphocytes (%)	43.00 ±15.87	41.76 ±16.98	36.12 ±18.62	.03
Eosinophils (%)	0.03 ±0.23	0.12 ± 0.69	0.27 ±1.31	.48
Monocytes (%)	1.21 ±2.10	1.06 ±1.65	0.67 ±1.23	.39
Basophils (%)	0.00 ±0.00	0.00 ± 0.00	0.03 ±0.25	.43

Key: WBC: White Blood Cell; PCV: Packed cell volume

Pearson Chi-Square test was used to test for diagnosis in relation to the haematological parameters of patients in the study, a significant *P*-value of less than .05, was recorded in PCV, Neutrophil counts, and Lymphocyte counts (Table 2).

Categorical mean values between infected and uninfected were compared by Mann Whitney U test, *P*-values were presented, values less than .05 are marked in bold, patients with parasitological confirmation of malaria had a significant reduction in the PCV count, also the mean neutrophil counts was significantly higher in patients infected with malaria as compared with uninfected patients, on the contrary, the mean Lymphocyte count was higher in negative patients as compared with positive, with *P*-values of <.001 (Table 3).

Mean haematological values were tested with Kruskal Wallis test along the three parasitaemia levels. WBCs, Neutrophil, and Lymphocyte counts recorded statistical difference across the parasitaemia levels. Mean neutrophil counts was

highest in high parasitaemia category, the reverse was observed in Lymphocyte counts as the mean lymphocyte counts was highest in low parasitaemia category and lowest in severe parasitaemia category. Mean PCV levels was lowest in high parasitaemia group and highest in low parasitaemia group (Table 4).

Spearman correlation was used to measure strength of relationship between haematological parameters, age and parasitaemia of patients with parasitological confirmation of malaria. A negative slope was observed between parasitaemia and PCV while a large significant positive relationship was observed between the parasitaemia levels and WBC (*P*= .31, rho value = -.076 and *P* = < .001, rho value = .340 for PCV and WBC respectively) as reflected in Fig. 2 A1 and A2. A large positive significant relationship reflected in a positive slope was observed between parasitaemia and neutrophils of patients, while a negative slope was observed in the relationship between parasitaemia and lymphocytes (A3 and A4). In A5 and A6, large negative significant relationship which was

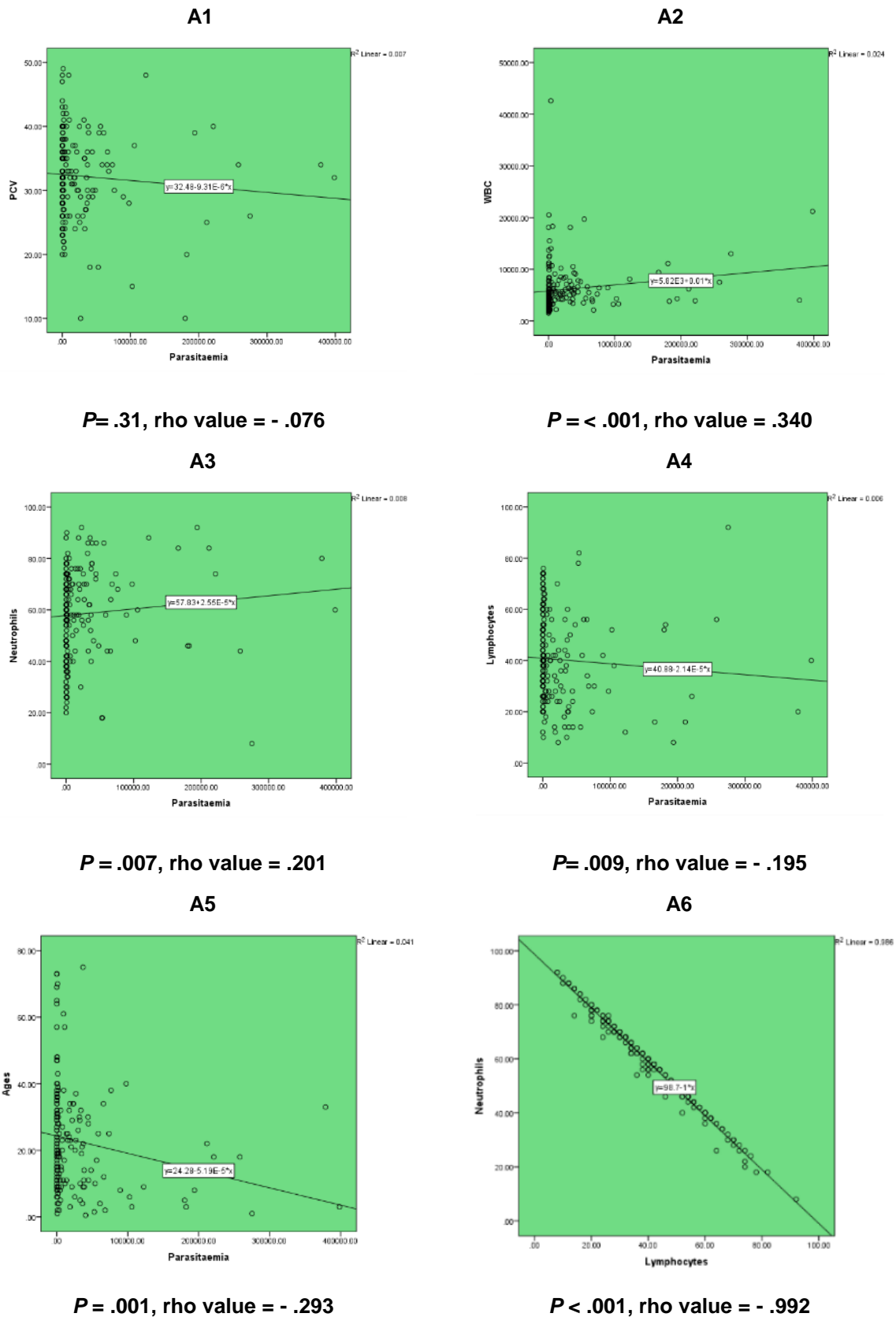


Fig. 2. Graphical Correlations of Different Parameters in Patients Infected with *Plasmodium falciparum* in Akure North and South Local Governments of Ondo State, Nigeria

reflected with a negative slope was recorded between the age and parasitaemia levels of patients infected with *Plasmodium falciparum*, while the neutrophils and lymphocytes of patients infected with *P. falciparum* showed an extreme high negative significant relationship (Fig. 2).

4. DISCUSSION

The study recorded a malaria prevalence of 12.69% in patients attending the selected health facilities in Akure North and South LGAs of Ondo State. Malaria prevalence recorded was lower than previously reported researchers [17,18] that worked in other rural parts of Ondo State Nigeria. This can be explained as part of interventions by organizations around the world to extensively control and eliminate the vector. Also, the study was conducted in an urban setting that has afforded a greater populace knowledge of the prevention and also risks factors of malaria infection as compared with the above listed authors who worked within the same state but a rural setting.

The PCV of patients with malaria was significantly lower to uninfected patients. Malaria parasite tends to cause severe destruction of red blood cells and also causes cytoadherence causing infected and noninfected red blood cells to be removed rapidly from the blood stream resulting to significant reduction of red blood cells in man. Anaemia which is a PCV of less than 15% is a manifestation of severe malaria and is frequently observed in young children and pregnant mothers, this is due to their relatively immature/ weak immune system. *Plasmodium falciparum* malaria, if untreated within 24 hours, can progress to severe malaria and death. Severe malaria can cause multi-organ failure in adults, while children frequently suffer from severe anaemia, respiratory distress or cerebral malaria.

In this study, anaemia was reported in a greater percentage of positive patients as compared with negative, more positive patients had significantly lower PCV values than uninfected, anaemia in malaria negative individuals could be as a result of bacterial infection e.g *Salmonella typhi*. Mean PCV values were also significantly lower in infected patients as compared with uninfected. Infected red blood cells display a reduced deformability and altered surface characteristics which usually leads to the RBCs being filtered and cleared by the spleen [19]. Decreased erythrocyte production, destruction of uninfected

red cells in addition to cytoadherence a process whereby infected RBCs cling to uninfected RBCs, through sequestration and rosetting in capillary and deep capillary of various organs and are filtered by the spleen are all major causes of malaria anaemia [20].

This study showed a fairly lower mean values of WBC count of infected patients but no significant difference in total WBC in malaria infected patients as compared to uninfected, this is also reported [6,8]. Similarly, lower WBC counts in malaria infected patients was reported [21,22] as compared with uninfected patients as leukocytes are drawn away from the peripheral circulation to the spleen and other marginal pools. Leucocytopenia which is low leucocytes (WBCs) count among patients was recorded in 30.3% of the positive patients and 26.7% of uninfected patients, while 60.7% and 65.5% of malaria positive and negative patients respectively recorded normal WBC count which confirms with previous authors that have reported that malaria induces low to normal WBC [23].

In this study, we report that malaria tends to induce significant increase in neutrophils as more neutrophils are recruited to sites of inflammation, splenic localisation to combat the infection. A greater percentage of positive patients recorded high neutrophils count as compared to uninfected patients. Higher Mean values of neutrophil count was obtained in positive patients as compared with neutrophil counts of uninfected. This is also similarly reported [23] and contrast to some other researchers [24] that reported a lower neutrophil count in infected patients.

Lymphocytopenia, defined as low lymphocytes count is reported to be accompanied by an increase in neutrophil count. This is interpreted as a sign of systemic inflammation and stress, which is frequently observed in many infectious and non-infectious diseases. Previous researchers have reported that mean monocytes, neutrophils were significantly higher in malaria patients compared with patients without malaria, but all within normal range [19,23].

Parasitaemia and Leucocyte counts were positively correlated. Association of lymphocyte count and malaria severity has been previously controversially reported. Malaria induces Lymphocytopenia. Significance difference in correlations were also recorded, the age of patients showed a significant negative relationship to parasitaemia, showing a negative

slope as a patient's advances in age the parasitaemia drops. The neutrophils – lymphocyte recorded extremely high significant correlations. This study further shows significant haematological correlation in patients presenting with malaria and the need to utilize the knowledge of these significant changes in addition with parasitological confirmation in diagnosis and treatment of malaria infection. Findings from researchers, further confirm that children are most susceptible to malaria infection [6,25] General clinical symptoms such as cough, headache, fever, chills, anorexia, malaise, vomiting diarrhoea are not sufficiently enough and are not significantly associated with a malaria diagnosis this is because these symptoms can be mimicked by other infectious and noninfectious diseases such as typhoid, pneumonia, and other bacterial infections, therefore the continued use of clinical presentation as a basis of treatment will likely increase degree of overtreatment and inevitably failure to treat alternative diseases which could lead to high morbidity and mortality.

Limitation to this research work is dearth of information on the prevalence of some other bacterial, viral, and other parasitic infections that could significantly impair the haematological parameters in the enrolled patients. Also, the knowledge of the micronutrient deficiencies and genetic background of patients which could also possibly impair on the haematological indices of the enrolled patients in this research is also lacking

5. CONCLUSION

In conclusion, the association of haematological parameters and diagnosis of malaria was established. Patients infected with *Plasmodium falciparum* showed important haematological changes; PCV, neutrophils and lymphocytes showed the most significant changes in relation to parasitological confirmation of malaria. Patients infected with *Plasmodium falciparum* malaria living in Ondo State, South-West Nigeria, a malaria endemic country recorded significant lower PCV, lower lymphocyte counts and higher neutrophil counts compared with uninfected patients. *Plasmodium falciparum* malaria is a medical emergency, if untreated within 24 hours the disease can progress to severe illness such as multiorgan failures, severe anaemia, respiratory distress or cerebral malaria and death. Knowledge and utilization of these significant haematological changes in

combination with parasitological confirmation will further improve malaria diagnosis, enhance better quality of life, enhance quality of treatment thus reducing morbidity and mortality as a result of malaria infection in endemic countries of the world including Nigeria. There is also need to improve the quality of malaria microscopy in our health facilities in order to avoid overtreatment that could result to development of resistance to presently available drugs of treatment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

CONSENT

Signed written consent was provided by all adult participants and parents /guardian of children.

ETHICAL APPROVAL

The ethical approval of the research work was first obtained from The Health Research Ethics Committee of Ondo State Ministry of Health, Akure. Protocol Number: OSHREC/10/9/2021/377, Committee Assigned Number: NHREC/18/08/2016. Permission and approval were also obtained from the Local Ethics committees of each health establishment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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