Abstract

Background: Plasmodium parasites, which cause the acute fever sickness known as malaria, are transmitted to people through the bites of infected female Anopheles mosquitoes. Plasmodium falciparum and Plasmodium vivax are two of the five parasite species that cause malaria in humans and are also the two most hazardous.

Methods: A total of 200 subjects were recruited for this study and were grouped into pre-teen age group (1-12years) and teen age group (13-19 years), among which 50 were control and 50 were malaria infected subjects for each group. Standard laboratory procedure was employed for blood collection and analysis. Parasitological and haematological parameters such as malaria parasite, pack cell volume (PCV), white blood cell (WBC) total and differential count and platelet counts were carried out from the blood samples.

Results: From the 200 subjects recruited for this study, 88 (44%) were males and 112(56%) were females. The analysis of the haematological parameters between the two groups (preteens and teen) revealed that there was no statistical significant difference among some of the examined haematological parameters except PCV (p<0.01), total WBC (p<0.01), platelet (p<0.01) and haemoglobin (p<0.05). While for other parameters, the differences are not statistically significant; neutrophil (p>0.05), lymphocyte (p>0.05), monocyte (p>0.05), eosinophil (p>0.05), basophil (p>0.05) and platelet (p>0.05) between the preteen and teen age group.

Discussion: These changes in haematological parameters can become profound as the infection's severity rises. As a result, early diagnosis of malaria infection is necessary for proper treatment.

Keywords: *Plasmodium falciparum,* Malaria Parasite, Haemoglobin, Platelet, Monocyte.

Introduction

Plasmodium parasites, which cause the acute fever sickness known as malaria, are transmitted to people through the bites of infected female Anopheles mosquitoes. *Plasmodium falciparum* is the deadliest malaria parasite and the most frequent on the African continent (WHO, 2022). There are five parasitic species that cause malaria in humans and two of these species, *Plasmodium vivax* and *Plasmodium falciparum,* pose the greatest threat. The most common malaria parasite outside of sub-Saharan Africa is *Plasmodium vivax.* The initial signs of malaria, including fever, headache, and chills, can be mild and challenging to diagnose. They typically show 10 to 15 days after the infecting insect bite. *Plasmodium falciparum* malaria can develop to severe disease and death in less than 24 hours if untreated (WHO, 2018; Dhangadamajhi et al. 2019). *Plasmodium knowlesi*, a type of malaria that naturally affects macaques in Southeast Asia, also infects people, resulting in "zoonotic" malaria (WHO, 2022). Malaria is a serious public health concern. Families are caught in a cycle of illness, pain, and poverty due to the condition and the price of its treatment. Nearly 50% of the world's population, the majority of whom reside in sub-Saharan Africa, are currently at risk of contracting malaria and dealing with associated economic difficulties (WHO, 2022). Malaria kills a child under the age of five almost every day (WHO, 2022). Numerous of these deaths can be avoided or treated. There were 247 million cases of malaria worldwide in 2021, which resulted in 619,000 fatalities. Children under the age of five made up 77% of these fatalities. This equates to a daily toll of more than 1,000 children under the age of five.

Nearly half of the world's population was susceptible to malaria in 2020 (WHO, 2022). Infants, children under five, pregnant women, persons with HIV/AIDS, and anyone migrating to places with high malaria transmission but low immunity, such as migrant workers, mobile communities, and travelers, are at significantly increased risk of contracting malaria and developing severe disease (WHO, 2022). Any disease condition, including endemic illnesses like malaria that might have an impact on human health and appear in a variety of clinical manifestations, is likely to have an impact on changes in haematological parameters. In the tropics, malaria is one of the leading causes of death. In 2010, there were around 219,000,000 cases recorded worldwide (WHO, 2016). One of the most typical malarial consequences is hemorrhaging, and this plays a significant part in the pathogenesis of malaria (Bakhubaira, 2013). The main cell types that are affected by these alterations include leucocytes, thrombocytes, and red blood cells (RBCs) (Van-Wolfswinkel et al. 2013; Naing and Whittaker, 2018). Patients with malaria typically have significantly higher monocyte and neutrophil counts compared to

non-malaria infected patients (Sakzabre et al. 2020), while patients with malaria typically have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs, and Hb levels (Sakzabre et al. 2020; Watson et al. 2021). Thrombocytopenia is the most typical consequence from malaria infection (Kotepui, 2014; Kho et al. 2018; Kotepui et al. 2020). People with platelet counts below 150,000/L are 12–15 times more likely to contract malaria than people with platelet counts above 150,000/L (Kho et al., 2018). Findings have demonstrated a correlation between the ratio of monocytes to lymphocytes and the probability of developing clinical malaria over time (Warimwe et al. 2013). The majority of the five million people in Thailand who live in high malaria transmission areas reside around the border with Burma (Fuhad et al. 2020). Clinical diagnosis is widely performed to determine the presence of malaria, especially in these areas. Fever and other signs and symptoms are known to be sensitive indicators of malaria infection, but they lack specificity and positive predictive values, particularly in regions where malaria is less common (Awoke and Arota, 2019). Additionally, it may be challenging to differentiate between the signs and symptoms of the disease from other viral or bacterial infections (WHO, 2013). The most frequent test and gold standard for identifying malaria infection is often microscopic slide examination of peripheral blood (Baird, 2013). In smear examinations, it is time-consuming and demands technical knowledge. It is widely known that malaria infection causes haematological abnormalities such as leucocytosis and thrombocytopenia. People who live in malaria-endemic areas may find it simple and helpful to obtain these haematological changes, which have diagnostic value. The enormous burden of sickness in early children has largely eclipsed the issue of malaria in adolescents. Although a large number of teenagers are at risk of contracting malaria, little is known about the disease's prevalence and effects in this age range. According to studies, even in regions with steady malaria transmission, malaria is a common cause of clinical disease in adolescents and a preventable cause of mortality. Due to immunological and hormonal variables, younger adolescents may be more at risk than older adolescents (Lalloo et al. 2006). Comparing the haematological parameters in malaria patients of the children and adolescent age groups at State Hospital, Ijebu-Ode, Ogun State, was the aim of this study.

Materials and Methods

Study Area

This study was conducted in Ijebu-Ode, Ogun State, Nigeria.

Study Population

This study was carried out among children between the ages of 1 year to 12 years and teens who are between the ages of 13 years to 19 years in Ijebu-Ode, children with malaria infected were recruited from State Hospital, Ijebu-Ode, while control samples (malaria non-infected) were recruited randomly from subjects outside the hospital who were tested to be negative to malaria. 200 sample size of participants of children were selected. The participants were divided into two; preteen (age 1-12) and teen (13-19). 50 participants were selected for control in each group of the participant. The participants were selected within the target population.

Research Design

This is a cross-sectional descriptive study of comparative assessment of haematological parameters in malaria patients of children and teens age group attending State Hospital, Ijebu-Ode Ogun State.

Ethical Consideration

The protocol for this study was sought and approved by the ethics and research committee of Lead City University, Ibadan, Oyo State, with the approval number LCU-REC/22/072 dated 31st January, 2022.

Inclusion criteria

- Children and teens attending State Hospital, Ijebu Ode, Ogun State.
- Children between the ages of 1year to 12years and teens between the ages of 13-19years
- Both male and female subjects
- Those that consented to participate in the study.

Exclusion criteria

- Subjects younger than 1 year old and older than 19 years old
- Those that did not consent to this study
- Sickle cell patients

Sampling Technique

The blood samples from children and teens attending State Hospital Ijebu-Ode, were selectively collected for laboratory tests to determine malaria infection as the evidence of infection before proceeding to haematological parameters.

Sample Collection, Processing and Analysis for malaria parasite

A venous blood sample of 2mls was collected into EDTA bottle from each of the children/teens using needle and syringe. Standard and careful laboratory procedures were adopted in collecting blood samples from the subjects. Thick and thin blood films of the blood samples were made on clean dry grease free slides, labelled and allowed to dry. The thin films were fixed with methanol to avoid lysis, allowed to dry for 30 seconds. The thin and thick films were stained with freshly prepared 10% working solution of Giemsa stain for 8-10 minutes. The stain is gently flush by dropping clean water over it and allowed to dry. The films were examined under the light binocular microscope using x100 objective lens, with a drop of oil immersion (Ochei and Kolhatkar, 2005)**.** The presence of malaria parasite in either of the films is regarded as positive, either with scanty, one plus (+), two pluses (++) or three pluses (+++).

Procedure for Packed Cell Volume Estimation

Micro-haematocrit method for packed cell volume estimation: this involves the use of micro haematocrit centrifuge to spin blood in a capillary tube at 12,000rpm for 5minutes and read using the micro-haematocrit reader.

The capillary tube was filled to about three quarter (¾) with well mixed EDTA anticoagulated blood. The unfilled end was sealed using a sealant material. The capillary tube was carefully placed in one of the numbered slots of the microhaematocrit centrifuge with the sealed end against the rim gasket (to prevent breakage). The inner lid was carefully positioned to avoid dislodging of the tube and centrifuged at 12,000rpm for 5minutes. Immediately after centrifuging, the packed cell volume was read.

The PCV was read using micro-haematocrit reader with the base of the red cell column (above the sealant) on the 0 line and the top of the plasma column on the 100 line. The PCV was read from scale. The reading point was the top of the red cell columns just below the buffy coat layer (consisting of white blood cell and platelet).

Procedure for Total White Blood Cell Counts

The method for total white blood cell counts involved the use of Neubauer counting chamber and Turks solution using the microscope which is the manual method of counting. About 0.38ml of diluting fluid was measured and dispensed into a small container or tube, 0.02ml of well mixed EDTA anticoagulated venous blood was added and mixed. The counting chamber was assembled, the center grid area of the chamber and the haemocytometer cover glass was completely clean and dry. The cover glass was slide into position over the grid area and it was pressed down on each side until rainbow colors (Newton's rings) are seen. The solution was remixed by using capillary tube. At an angle of about 45º, the grid area of the chamber was filled with the solution, the filled chamber was left undisturbed for 2 minutes to allow the white cells to settle. To prevent drying of the fluid, the chamber was placed in a Petri-dish on dampen tissue and it was covered with a lid. The underside of the chamber was dried and it was placed on the microscope stage using x10 objective lens with condenser iris closed, the ruling was focused and the white cell. It was focus until the white blood cells appear as small black dot. The cells were counted in the four large corner squares of the chamber

Statistical Analysis

Statistical analysis for Social Sciences (SPSS) version 25 was the statistical package used in analyzing all data obtained. Frequency table, Bar chart and Pearson Chi-Square were used to compare the means of the different analytes at $p < 0.05$ statistical significance.

RESULT

Table 1.0: frequency distribution according to gender and malaria status.

		Frequency (%)
Gender	Male	88 (44)
	Female	112 (56)
	Total	200 (100)
Malaria status	Negative	100 (50)
	Positive	100 (50)
	Total	200 (100)

This shows the frequency and the percentage frequency of the study subjects based on gender and the malaria status. From the 200 participant, it is seen that females constituted the larger percentage of the study (Table 1.0).

Table 2.0: Mean value and standard deviation of haematological parameters across groups

Parameters	**Preteen (1-12yrs) (mp -ve) n=50**	**Preteen (1-12yrs) (mp +ve) n=50**	**Teen(13-19yrs) (mp -ve) n=50**	**Teen(13-19yrs) (mp +ve) n=50**	***p*-value**
Age Mn±SD	6.48 ±3.530	6.12 ±2.974	15.92 ±1.627	15.74 ±1.736	0.023
haemoglobin Mn±SD	10.84 ±1.9104	10.59 ±2.2439	11.84 ±1.616	11.20 ±1.8066	0.031
PCV Mn±SD	32.52 ±5.726	31.78 ±6.741	35.50 ±4.841	33.58 ±5.406	0.001
Total WBC Mn±SD	8683.80 ±4728.266	10152.80 ±5873.058	7156.40 ±3104.843	8157.80 ±6495.080	0.001
Neutrophil Mn±SD	57.78 ±14.966	53.68 ±10.374	53.18 ±12.676	55.94 ±10.135	0.678
Lymphocyte Mn±SD	33.50 ±12.981	37.20 ±10.319	38.02 ±11.741	34.00 ±11.308	0.878
Monocytes Mn±SD	6.74 ±3.294	6.74 ±3.932	6.58 ±2.726	6.70 ±3.358	0.678
Eosinophil Mn±SD	1.96 ±2.040	2.36 ±2.164	2.16 ±2.132	2.44 ±2.681	0.455
Basophil Mn±SD	0.02 ±0.141	0.02 ±0.141	0.04 ±0.283	0.10 ±0.364	0.344
Platelet Mn±SD	256.64 ±129.332	211.96 ±123.757	243.10 ±124.215	180.22 ±80.394	0.001

This shows the mean value and standard deviation of haematological parameters across groups. The groups include Preteen 1-12 years (mp -ve), Preteen 1-12 years (mp +ve), Teen 13-19 years (mp -ve), Teen 13-19 years (mp +ve). Each group consist of 50 subjects (N) (Table 2.0).

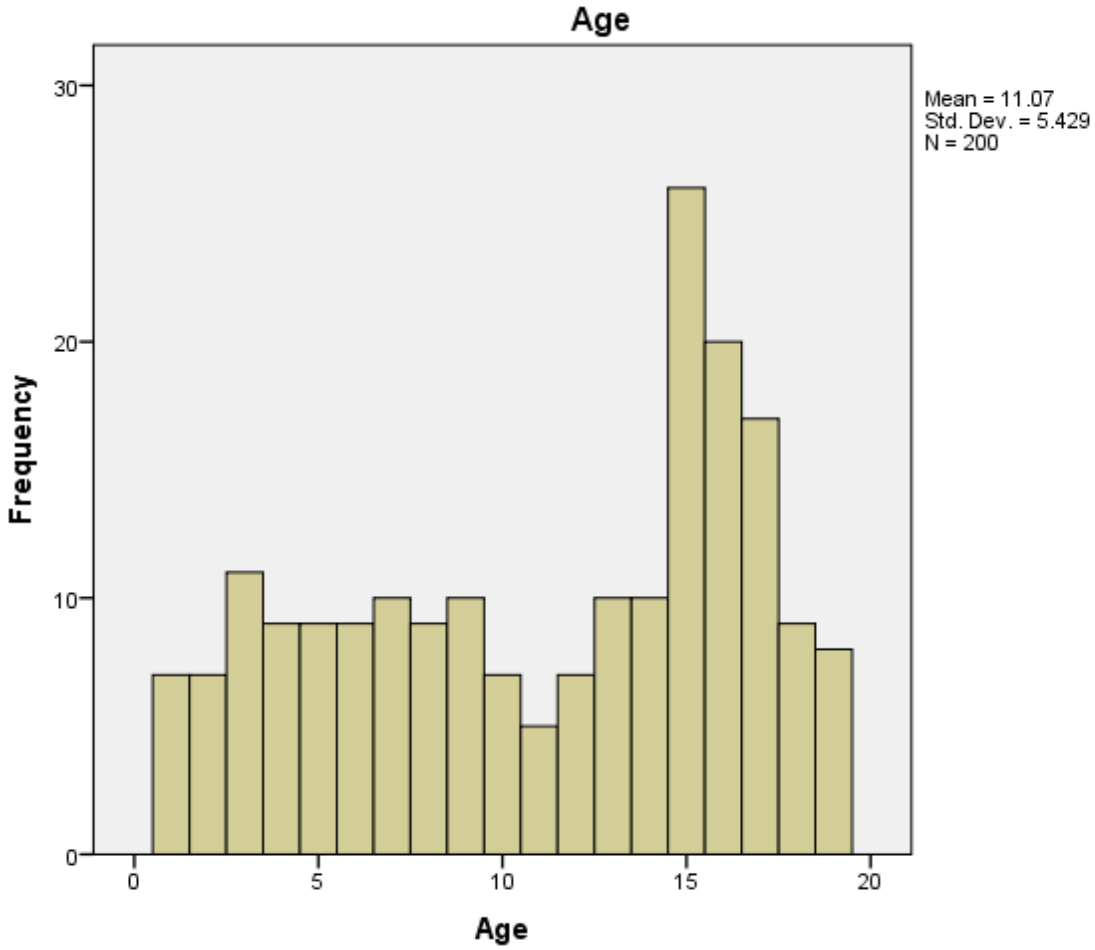

Figure 1.0: Age Frequency of the study

The above chart shows the age frequency of the subjects with age 15 occurring most.

Table 3.0: Mean and standard deviation of haematological parameters for preteen subjects with malaria parasite.

Malaria parasite		Packed cell volume	Total WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Platelet	*p*-value
	N	38	38	38	38	38	38	38	38	
	% of Total N	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	76.0%	0.677
1+	Mean	31.61	11665.79	55.55	35.34	6.21	2.05	.00	141.13	
	Std. Deviation	6.352	9003.769	15.076	14.964	3.042	1.845	.000	44.734	
	N	10	10	10	10	10	10	10	10	
	% of Total N	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	20.0%	
2+	Mean	17.30	18480.00	55.10	36.40	8.50	1.00	.00	147.40	
	Std. Deviation	8.795	11200.74	19.105	15.650	6.096	1.247	.000	61.815	
	N	2	2	2	2	2	2	2	2	
	% of Total N	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	4.0%	
3+	Mean	23.00	9200.00	64.00	24.50	10.00	1.50	.00	114.00	
	Std. Deviation	8.485	2828.427	4.243	3.536	7.071	.707	.000	36.770	

This shows the mean and standard deviation of haematological parameters for preteen subjects that are positive to malaria parasite (1+, 2+ and 3+). There are changes in the haematological values of 1+ parasitemia, 2+ parasitemia and 3+ parasitemia. Although the changes were not statistically significant ($p>0.05$). Out of the 50 subjects that are malaria positive in the age group of 1-12 years, 38 of the subjects were certified with 1+ parasitemia, 10 were certified with 2+ parasitemia and 2 were certified with 3+ parasitemia. (Table 3.0).

Table 4.0: Mean and standard deviation of haematological parameters for teen subjects with malaria parasite.

Malaria parasite		Packed cell volume	Total WBC	Neutrophil	Monocyte	Lympho cyte	Eosinophil	Basophil	Platelet	*p*-value
1+	N	44	44	44	44	44	44	44	44	0.455
	% of Total N	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	88.0%	
	Mean	32.93	10315.45	65.95	6.20	25.84	2.23	.00	123.48	
	Std. Deviation	6.711	7645.991	14.338	3.651	13.362	2.133	.000	32.205	
2+	N	6	6	6	6	6	6	6	6	
	% of Total N	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	12.0%	
	Mean	35.17	7050.00	69.50	8.50	21.33	.67	.00	98.33	
	Std. Deviation	4.215	4332.090	15.110	5.541	12.770	.816	.000	38.229	

This shows the mean and standard deviation of haematological parameters for teen subjects with malaria parasite (1+ and 2+). There are changes in the haematological value of 1+ parasitemia and 2+ parasitemia. Although the change was not statistically significant ($p>0.05$). out of the 50 subjects that are malaria positive in the age group of 13-19, 44(88%) of the subjects were certified with 1+ parasitemia while 6(12%) of the subjects were certified with 2+ parasitemia (Table 4.0).

Discussion

In sub-Saharan Africa, particularly Nigeria, malaria is a major health concern that can be prevented and treated (WHO, 2018; WHO, 2022).

Plasmodium falciparum is the most prevalent malaria parasite in the WHO African region, accounting for 99.7% of estimated malaria cases in 2017, *Plasmodium vivax* is the predominant (74.1%) parasite in the WHO region of the Americas (WHO, 2018). *Plasmodium vivax* contributed 4% of the total global cases in 2015, but outside Africa the proportion was 41% among all malaria infections. Its high burden of disease is maintained in part due to dormant liver stage parasite forms known as hypnozoites which can induce clinical relapse episodes (Baird, 2013).

In this study, 200 subjects were recruited, out of which 88 (44%) were males and 112 (56%) were females. The females constituted the larger percentage of the study. The subjects were divided into pre-teen age group (1-12years) with a mean age of 6.3 years (SD= 3.25years) and teen age group (13-19 years) with a mean age of 15.83 years (SD=1.68years), among which 50 were control and 50 were malaria infected subjects for each of the two groups. The analysis of the haematological parameters between the two groups (preteens and teen) revealed that there was no statistical significant difference among some of the examined haematological parameters except haemoglobin (p<0.05), platelet (p<0.01), PCV (p<0.01), and total WBC (p<0.01). While for other parameters, the differences are not statistically significant; neutrophil (p>0.05), lymphocyte (p>0.05), monocyte (p>0.05), eosinophil (p>0.05), basophil (p>0.05) and platelet (p>0.05) between the preteen and teen age group (table 2.0).

Thrombocytopenia was the most common haematological abnormality observed in malaria subjects, both pre-teen and teen age groups (Table 2.0). This is in tandem with Abro et al. 2005 who carried out a study on malaria and “haematological changes, the results indicated that 83% of the subjects recruited for the study had thrombocytopenia. Malaria, which is frequently accompanied by palpable splenomegaly and circulating immune complexes (Khan et al. 2012), is thought to promote platelet destruction and shorten the lifespan of the cells, which is thought to be the cause of thrombocytopenia. Reduced platelet synthesis due to an increase in megakaryocytes in the bone marrow may result in thrombocytopenia in malaria patients

(Kotepui, et al. 2014). The immune system's destruction of platelets has been theorized as the cause of the thrombocytopenia seen in malaria infection. Patients with malaria have higher blood levels of immunoglobulin G (IgG), which binds to malaria antigens that are attached to platelets and causes more thrombocytes to be destroyed (Maina et al. 2010). According to one study, aggregated platelets are mistakenly counted by analyzers as a single platelet, which results in pseudo-thrombocytopenia (Kotepui, et al. 2014).

Due to its considerable decrease when compared to the control, anemia was the second most often seen haematological abnormality in malaria-infected participants. This suggests that anaemia and P. falciparum infection are more frequently linked. This is in line with research by Jain et al. 2005, in which anaemia was found in 66 patients (94.28%), of which 37 (56.06%) had Plasmodium falciparum infection. According to Abro et al. 2008 who carried out a study on malaria and "haematological changes, the results indicated that 64% of the subjects recruited for the study had anaemia, 24% lymphopenia and 10% monocytosis. Also, the findings of Bawal et al. 2018 which observed a reduced PCV among children infected with malaria parasite in Kisumu, western Kenya. Anaemia is brought on by high parasitaemia, especially when *P. falciparum* is present. *P. falciparum* causes increased red blood cell death. This could be the result of parasitized red blood cells being hemolyzed, the clearance of parasitized red blood cells being expedited, or inefficient erythropoiesis (Absar, 2012). A number of variables, including mechanical destruction of the parasitized red blood cells, decreased RBC synthesis in the bone marrow, and phagocytosis of parasite-infected RBC, have been proposed to play a role in the complex and poorly understood pathophysiology of anemia in malaria (Maina et al. 2010). One of the most frequent side effects of malaria, particularly in young children and pregnant women in areas with high transmission rates, is anemia. (Ugwu et al. 2014).

According to this study, both *P. falciparum* infected groups had a little lower Hb level than those who weren't infected (Table 2). This is in line with the findings of Maina et al. 2010, who noted a decrease in the level of hemoglobin among P. falciparum-infected kids residing in western Kenya. This is also consistent with a study by (Bawah et al. 2018), which discovered a

substantial rise in the haemoglobin and WBC count in children under five who had malaria compared to the control in a cross-sectional study at the HO Municipality of Ghana.

This study revealed that there was significant difference in total white blood cell count in malaria-infected children (teen and preteen age group) when compared with their controls. This is in tandem with the findings of Maina *et al.* 2010, who revealed significant increase in WBC count in children infected with malaria when compared to the control in a cross-sectional study among children in Kisumu, western Kenya, also with the study of Francis et al. 2014 who observed a significant difference the WBC counts of malaria infected subjects when compared with the control. White blood cell alterations caused by malaria can vary based on host immunity, parasitemia, and co-infection levels. Neutrophils, macrophages, and natural killer (NK) cells are examples of effector cells that are stimulated as part of the body's immunological response to infections (Vivier et al. 2011).

Conclusion

This study established that there are changes in haematological profile of malaria infected children (pre-teen and teen age group) when compared with non-infected children. Although some of the haematological profile changes are not statistically significant, while some are significant such as the platelet. The common thing that was noticed are thrombocytopenia, reduced packed cell volume and reduced total white blood cell.

Therefore this study concluded that malaria tends to cause reduce platelet, packed cell volume and total white blood cell count.

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