Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2025 Vol. 3(2): 1-9
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2025-0302-01*

Research Article

Effects of *Plasmodium falciparum* Infection on Blood Cell Parameters in Pregnant Women Attending Antenatal Clinics in Igabi Local Government Area, Kaduna State

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ABSTRACT

Plasmodium species are single-celled haemoprotozoans that cause malaria in humans. This study determined the effect of *Plasmodium* falciparum infection on blood cell parameters in pregnant women in Igabi Local Government Area, Kaduna State, Nigeria. A total of 210 blood samples were collected from consenting pregnant women attending antenatal care in the selected Primary Health Care Centers. The samples were examined for P. falciparum and confirmed using polymerase chain reaction. Blood cells were evaluated. Data was analysed using the IBM statistical package for social sciences (version 26). The result showed that P. falciparum infections occurred with an overall of prevalence of 31.4%. The highest prevalence was recorded in Taro-Taro, 40.0%, and the lowest in Miyetti-Allah, 21.8%. The mean packed cells volume, recorded both in malaria negative and positive individuals were 30.71% and 34.13% and haemoglobin were, 10.068g/dL and 11.234g/dL, respectively. The mean total white blood cells of the negative and positive individuals were $10.068x10^{9}$ and $11.234x10^{9,}$ respectively, which did not change significantly ($p>0.05$). The percentage mean neutrophils in non-infected pregnant women was 59.63%, which was significantly ($p < 0.05$) higher than in infected pregnant women, 56.09%. The mean lymphocytes of infected pregnant women was 37.14%, which was significantly ($p < 0.05$) higher than those without infection (34.76%). The Mean eosinophils of pregnant women infected was 0.56%, which was significantly ($p < 0.05$) higher than those who were not infected (0.24%). It can be concluded that P. falciparum infection in the pregnant women was endemic, characterized by anaemia, neutropenia, lymphocytosis, and eosinophilia.

Keywords: Blood Cells; Igabi; Infection; Kaduna State; *Plasmodium;* Pregnant Women

Citation: Abdulkadir, N., Garba, U.M., Alhaji, A.I. & Dikwa, K.B. (2025). Effects of *Plasmodium falciparum* Infection on Blood Cell Parameters in Pregnant Women Attending Antenatal Clinics in Igabi Local Government Area, Kaduna State. *Sahel Journal of Life Sciences FUDMA*, 3(2): 1-9. DOI: *https://doi.org/10.33003/sajols-2025-0302-01*

INTRODUCTION

Plasmodium species are haemoparasitic, single-celled microorganisms belonging to the genus *Plasmodium*. The genus comprises of species that cause the disease known as malaria in humans and animals (WHO, 2014). The *Plasmodium* species infection is widespread as the mosquito vector in tropical regions of the world including Africa, Central America, Island Hispaniola in the Caribbean, Amazon region of South America, part of the Arabian Peninsula, the Middle East and in parts of the South Pacific (NMIS, 2010).

Globally, *Plasmodium falciparum* and *Plasmodium vivax* account for the majority of malaria cases. *Plasmodium falciparum* is the most pathogenic and responsible for more deaths than *P. vivax, P. malariae, P. ovale* and *P. knowlesi.* (Lacey and Walter, 2022). It is assessed that around half of the grown-up adults in Nigeria are thought to get malaria at least once a year, and over 100 million people are at risk of contracting the disease each year. Statistics showed that, about 80% of the world's malaria cases were reported in sub-Saharan African as well as India: Of the 80% documented,

Nigeria bears 25% of the weight (WHO, 2018). Igbenghu and Odaibo, (2013) documented that, Pregnancy-related malaria poses a serious public health concern in Nigeria and is a top goal for the Roll Back Malaria Partnership due to the health risks to the mother, her unborn child, and the newborn. Cases of malaria in pregnancy in endemic areas are mostly asymptomatic. About 1-50% of pregnant women may have been infected with placental malaria (CDC, 2019). Malaria, a leading cause of low birth weight, maternal anemia, and prenatal mortality, is especially dangerous for expectant mothers and their unborn children.

Blood plays a very vital function in the human body. It has cellular constituents such as erythrocytes, leukocytes and thrombocytes which are essential for various functions in body physiology (Okoroiwu *et al.,* 2014). In disease condition, the cells are altered in both production and function. Blood differentials, especially the status of the white blood cells (neutrophils, eosinophils, lymphocytes, basophils and monocytes) provide clues to the kind of infection. Reports have shown that malaria could cause changes in haematological status of an individual resulting in other conditions such as anaemia and suppression of bone marrow, thrombocytopenia and leukocytosis, leukopenia, lymphocytosis, monocytosis, eosinophilia and neutrophilia (Obeagu *et al.,* 2017; Surve *et al.,* 2017). Thrombocytopenia is among the major haematological aberrations frequently observed among malaria patients and usually disappears when the malaria infection is treated (Ifeanyichukwu and Esan, 2014; Kotepui *et al.,* 2014).

The present study seeks to determine variations in blood cells parameter as may be influenced by *P. falciparum* infection in pregnant women attending antenatal care in some Primary Health Care Centres in Igabi Local Government Area of Kaduna State.

MATERIALS AND METHODS

Study Area

The study was conducted in Igabi Local Government Area (LGA), Kaduna State. It is one of the four LGAs that make up Kaduna metropolitan city, out of a total of 23 LGAs in the State. Igabi LGA is located between latitude 10^{o} 47^{1} 0^{11} North, longitude 7^{o} 46^{1} 0^{11} East and is 608 meters above sea level (Dikwa *et al.,* 2021). The LGA occupies an area of 3,222 Kilometres square (Igabi, 2020).

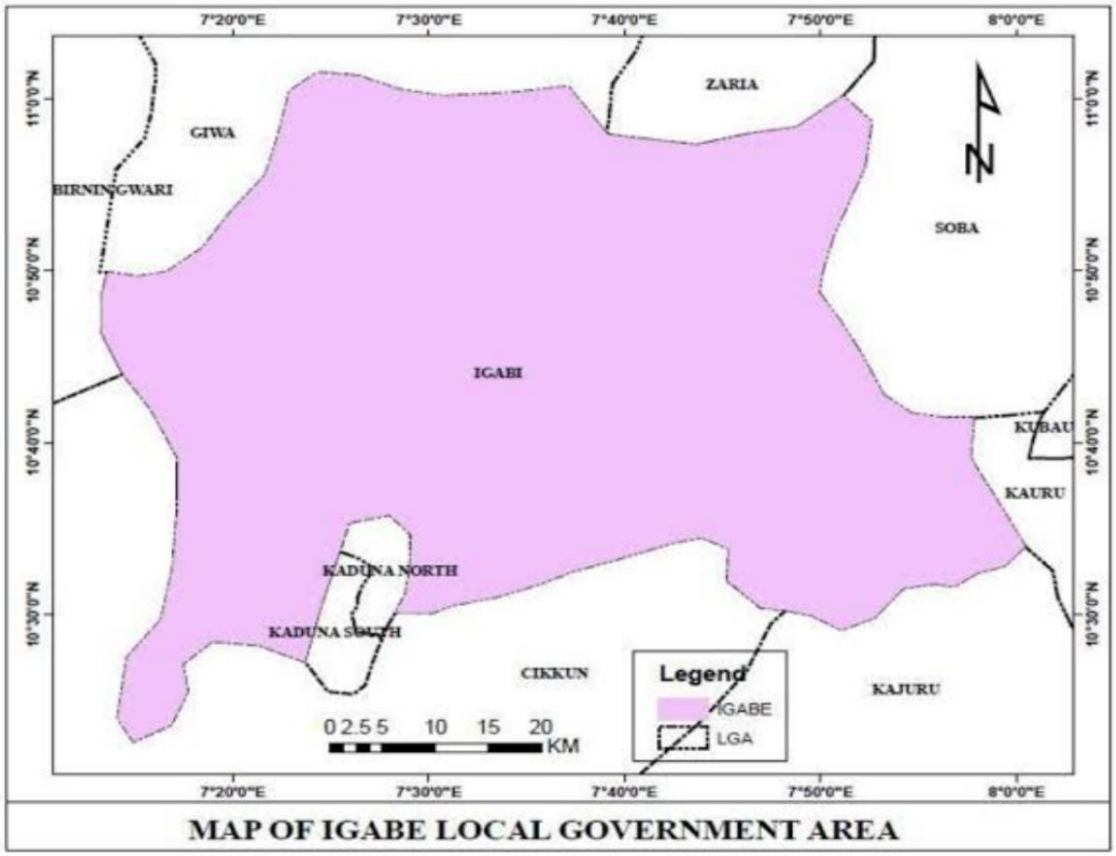

Fig 1: Map of Igabi Local Government Area, Kaduna State (Haruna *et al*., 2019)

Sampling Locations

Blood samples of pregnant women attending antenatal clinics at four (4) Primary Health Care

Centres (PHCCs) were collected. The PHCCs were: Miyetti Allah Centre in Makarfi road Rigasa, Mando Centre, Taro-Taro Centre in Rigasa and Hayin Dan-Mani Centre all situated in Igabi LGA, Kaduna State.

Ethical Approval and Informed Consent

Ethical approval; (MOH/ADM/744/VOL.1/111024) was obtained from the Health Research Ethics Committee (HREC), Ministry of Health, Kaduna State. The approval was accepted by Igabi Local Government Health Authority (ILGHA). Informed consent was obtained from the participants prior to their inclusion in the study. Participants were informed on the study in detail in English language or other local languages for those who did not understand English language.

Inclusion and Exclusion Criteria

Pregnant women who attended antenatal clinics in the sampling locations, at various gestation stages and consented to participate in the study were included**.**

Pregnant women who did not attend antenatal clinics at the sampling locations or attended but did not consent to participate in the study were excluded.

Sample Size Determination

The sample size was determined using Naing *et al,* (2022) method for calculating sample size for prevalence studies.

$$n = \frac{Z^2 \times P (1 - P)}{D^2}$$

Where n = Sample size

P = expected prevalence = 84% from WHO (2017)

Z = standard normal distribution at 95% confidence interval = 1.96.

D = Precision (allowable error) = 5% (0.05)

$$n = \frac{(1.96)^2 \times 0.84 \times (1 - 0.84)}{(0.05)^2}$$

$$= 206.52 \approx 210$$

n = 210 samples.

Samples Collection

A total of two hundred and ten (210) blood samples were collected from pregnant women attending antenatal clinics at the four different clinic sampled as follows: Fifty five(55) samples were collected from Miyetti Allah PHCC, fifty samples from (50) Taro-Taro PHCC, fifty three (53) from Dan-Mani PHCC, and fifty two (52) samples from Mando PHCC using standard method (Cheesebrough, 2010). The collection was done under the assistance of medical personnels in the health centres for four months from January to April, 2024. The blood was collected using five (5) mL syringe with 21G needle was used to aseptically withdraw 5mL of blood and transferred into an Ethylene Diamine Tetra-acetic Acid (EDTA) (Igiri *et al.,* 2018).

Sample Analysis

Rapid Diagnostic Tests (RDTs)

The RDTs was carried out using Pf (HRP-II) antigen (AdvDx^{TM}, ADVY chemical PVT Ltd, India) according to the manufacturer's instructions. The samples were placed on the pouch, then the anticoagulated blood sample was evenly mixed by gently swirling and dipping the sample dropper (5µL) into the blood sample and collected 5 µL of the blood sample. The collected blood was loaded into the sample port 1(S) of the test device. Four (4) drops (110 µL) of the buffer solution was added into the buffer port 2(B) on the test device. Reaction occurred between the blood in port 1 (S) and buffer in port 2 (B). The result was read after 20 minutes and interpreted. Appearance of only purple-coloured "C" control band indicate negative status, while two bands appearance ("Pf" test line and purple-coloured "C" control line) indicated positive status for *Plasmodium falciparum* infection.

Preparation, Staining and Examination of Blood Film

Thin and Thick blood Films were prepared using a drop of anticoagulated blood, stained with Giemsa reagent, and examined for *Plasmodium* specie microscopically at x40 and x100 (oil immersion) objective respectively (Cheesbrough 2010).

Identification of *Plasmodium falciparum*

Blood samples of the pregnant women from the four Primary Health Care Centres (Miyetti Allah, Taro-Taro, Dan-Mani and Mando) that were positive for *Plasmodium falciparum* infection from RDT and microscopy tests were pooled, and used for deoxyribonucleic acid extraction.

Deoxyribonucleic Acid Extraction

Plasmodium falciparum genomic Deoxyribonucleic Acid (DNA) was extracted from the positive blood samples of the pregnant women using Serum Viral DNA/RNA Extraction kit (XIAORUI®, Biotechnology Co. Ltd, India) according to manufacturer's instructions.

Polymerase Chain Reaction

The Polymerase Chain Reaction (PCR) was conducted as described by Abdel Hamid *et al* (2013). The protocol used for the PCR was based on genus-specific primer for *P. falciparum* (PF1 and PF2). Specie-specific primers for *P. falciparum* was used for the PCR assay. A tube containing, 25µL of the mixture of 250nm of the primer (PF1 and PF2) and 1µL of genomic DNA. The amplification and detection were performed under the following conditions; Pre-denaturation: 5 minutes at 94^{0}C, Denaturation: 30 seconds at 94^{0}C, Annealing: 30 seconds at 58^{0}C, Extension: 40 seconds at 72^{0}C, Final extension: 10 minutes at 72^{0}C for 35 cycles using GeneAMP® PCR system 9700 (Applied Biosystems, China). All PCR assays included positive control (genomic DNA from *P. falciparum*) and negative control (ultrapure water).

Agarose Gel Electrophoresis

Two grams (2 g) of Agarose powder was weighed and 100mL of TAE buffer (tris acetate) was added to it in a flat bottom flask. The mixture was placed on an oven and heated gently until the Agarose dissolved completely. The mixture was removed from the oven and 6µL of ethidium bromide was added and mixed gently. The gel containing ethidium bromide was poured into a gel cast with an inserted comb and allowed to solidify. The comb was removed and gel cast transferred to an electrophoretic tank. Using 15µL of the PCR products and 5µL of ladder, both were poured into the tank and the tank was closed and ran for 40 minutes at 400mA. The products were visualized under short UV light. The number and size of resulting DNA bands were analysed using DNA ladder of 1,500bp (Abdel Hamid *et al*., 2013).

Determination of Blood Cells Parameters

Full blood counts (FBC) including Packed Cell Volume (PCV), haemoglobin concentration, Total and differential white blood cells (WBC) counts were conducted.

Packed Cell Volume (PCV): A plain capillary tube was filled by suction pressure with well-mixed EDTA-anticoagulated blood to 2cm level from one end. The unfilled end was heat-sealed using Bunsen burner flame. The filled tube from each sampled blood was carefully loaded into one of the numbered slots (and noted against the sample label) of the microhaematocrit machine rotor with the sealed end against the outer rim gasket. The loaded capillary tubes were centrifuged for 5 minutes at 15,000g. The PCV value was read from the PCV Reader by aligning the base of the red cell column on the zero line and the top of the plasma column on the 100 line. The result was read from the scale as a percentage (Cheesbrough, 2010).

Full Blood Count (FBC*):* Using the prepared thin blood smear slides as for parasite microscopic examination, each of the slides were observed under the x100 objectives (oil immersion). The various white blood cells and platelet seen were counted using their morphological keys and their percentages determined for differential values (Gulati *et al.,* 2013).

Data Analysis

The data obtained from the study were computed using IBM Statistical Package for Social Sciences (IBM SPSS version 26) and Pearson Chi-square test was used to determine the statistically significant at P-value > 0.05.). The t-test were used to compare the samples in relation to pregnant women blood cells parameter. One-way Analysis of Variance (ANOVA) was used to compare observed values of the blood cells parameter along the different Primary Health Care centers.

RESULTS

Prevalence of *Plasmodium falciparum* infection among the pregnant women from each of the Primary Health Care Centers (PHCC).

In this study, microscopy, Rapid Diagnostic Test (RDT) method and polymerase chain reaction (PCR) were conducted on the blood samples. The PCR test confirmed *P. falciparum* as responsible for infections in the areas. The overall prevalence of *P. falciparum* in the four Primary Health Care Centers was observed to be 31.4%. The observed prevalence of infection in the various locations among the pregnant women showed that Miyetti Allah Primary Health Care Center (PHCC) had the lowest rate (21.8%), Dan Mani PHCC had 32.1%, Mando PHCC had 32.7%, while Taro-Taro PHCC had the highest rate of 40.0%.

There was no significant association, (0.250, p > 0.05) between prevalence rate of *P. falciparum* infection among the pregnant from the different Primary Health Care Centers sampled in the Local Government Area of the State.

Results of Molecular confirmation of *Plasmodium falciparum*

A total of 16 positive samples were subjected for confirmation of *Plasmodium falciparum*. Molecular analysis confirmed *Plasmodium falciparum* in all samples at 100 bp (plate 1).

Table 1: Distribution of pregnant women by their status of *Plasmodium falciparum* infection in the Primary Health Care Centers in Igabi Local Government Area

Location of Primary Health Care Centers	Number Examined	Positive cases	χ2	Df	p-value
Miyetti Allah	55	12 (21.8)	4.110	3	0.250
Dan Mani	53	17 (32.1)			
Taro-Taro	50	20 (40.0)			
Mando	52	17 (32.7)			
Overall	210	66 (31.4)			

Keys: Values in parenthesis = percentages (%), X^2 = Chi-square values and Df = Degree of freedom

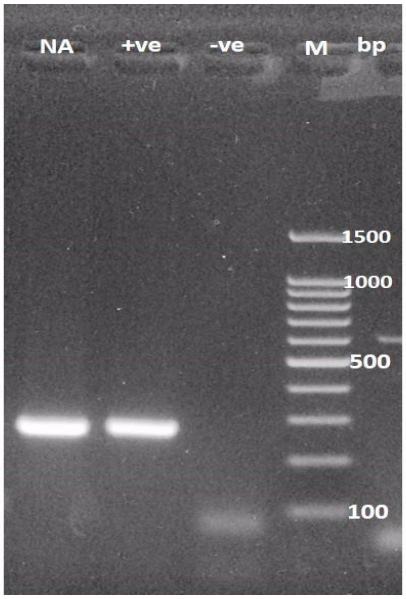

Plate 1: Amplified product of *Plasmodium falciparum* gene.
Lane NA: positive samples (100bp), Lane +ve: Positive control, Lane -ve: Negative control
Lane M: Molecular Ladder (1500 bp)

Determination of effects of *Plasmodium falciparum* infection on patients' blood cell parameters

The effects of *Plasmodium falciparum* infection on patients' blood cell parameters were determined by observed values from subjects with positive infection status (Table 2).

Mean Packed Cells Volume (PCV) level of pregnant women with *P. falciparum* infection (30.71%) was significantly lower (0.000: ($p < 0.05$). The level of Hemoglobin (HB) concentration (10.068g/dL) in pregnant women with *P. falciparum* infection were significantly ($p < 0.05$) lower.

There was no significant difference in the mean Total White Blood Cells count (WBC) of infected pregnant women ($10.068x10^9$).

The mean neutrophils count (56.09%) were significantly lower, ($p < 0.05$) of the infected pregnant women.

The mean lymphocytes (37.14%) level of pregnant women with *P. falciparum* were significantly higher ($p < 0.05$).

The mean monocytes (6.29%) level of pregnant women who were infected with *P. falciparum* did not differ significantly ($p > 0.05$).

The mean eosinophils (0.56%) level in the pregnant women infected with the *P. falciparum* were significantly ($p < 0.05$) higher (Table 2).

Determination of variations in blood cells parametre due to the *Plasmodium falciparum* infection with respect to location

Mean PCV levels of infected pregnant women showed slightly significant difference, p-value of 0.04 ($p < 0.05$) between the Primary Health Care Centers.

The highest mean PCV was observed among *P. falciparum*-infected pregnant women in Taro-Taro PHCC (32.35±5.914%a). The PCV of infected pregnant women in Miyetti Allah PHCC (31.92±2.021%a), Dan Mani PHCC (30.88±3.569%ab) and Taro-Taro (32.35±5.914%a) did not significantly differ ($p>0.05$) amongst *P. falciparum*-positive women in the three (3) centres.

The mean PCV value from the 3 centres, are significantly higher ($p< 0.05$) than mean PCV of pregnant, *P. falciparum*-positive women in Mando PHCC (27.76±6.340%b

Mean haemoglobin (HB) of pregnant women-positive for *P. falciparum* in Miyetti Allah PHCC (10.62±0.667g/dL a), Taro-Taro PHCC (10.53±1.933g/dL a) and Dan-Mani PHCC (10.19±1.126g/dL ab) were not significantly different ($p> 0.05$) across the 3 centres.

The mean HB concentration of pregnant women positive for *P*, *falciparum* in the 3 locations showed significantly higher value than for those in Mando PHCC (9.01±2.648g/dL b).

The mean WBC, Neutrophils, Lymphocytes, Monocytes and Eosinophils of the *P. falciparum*-positive pregnant women from the different Primary Health Care centers were not significantly different ($p>0.05$) (Table 3).

Table 2: Blood cell parameters of pregnant women with *Plasmodium falciparum*-infection (positive) in the Primary Health Care Centers.

Parameters	Infection status	N	Mean	Std. Dev.	Std. Error	t-value	Df	p-value
PCV (%)	Positive	66	30.71	5.226	0.643	5.312	208	0.000
HB (g/dL)	Positive	66	10.068	1.9034	0.2343	5.113	208	0.000
Total WBC (x10^9)	Positive	66	4.827	0.9403	0.1157	0.560	208	0.576
Neutrophils (%)	Positive	66	56.09	9.377	1.154	2.957	208	0.003
Lymphocytes (%)	Positive	66	37.14	8.296	1.021	2.168	208	0.031
Monocytes(%)	Positive	66	6.29	4.590	0.565	1.371	208	0.172
Eosinophils(%)	Positive	66	0.56	0.897	0.110	3.145	208	0.002

Keys: PCV= Packed Cells Volume, HB= Haemoglobin Concentration, WBC= White Blood Cell, N= Sample size, Std Dev= Standard deviation, Std Error= Standard Error and Df= Degree of freedom.

Table 3: Variations in mean blood cells parameters and standard deviations of pregnant women infected with *P. falciparum* in by the Primary Health Care Centers

PHCC	N	PCV (%)	HB (g/dL)	WBC (x10^9)	NEUT (%)	LYMP (%)	MONO (%)	EOSI (%)
Miyetti Allah	12	31.92±2.021a	10.62±0.667a	5.05±1.082a	58.58±7.280a	35.92±4.680a	5.83±3.512a	0.33±0.651a
Dan Mani	17	30.88±3.569ab	10.19±1.126ab	4.75±0.983a	54.71±9.674a	37.76±8.541a	6.71±4.985a	0.53±0.874a
Taro-Taro	20	32.35±5.914a	10.53±1.933a	4.63±0.647a	57.25±9.808a	36.90±8.397a	5.25±4.678a	0.70±1.031a
Mando	17	27.76±6.340b	9.01±2.648b	4.98±1.099a	54.35±10.068a	37.65±10.295a	7.41±4.823a	0.59±0.939a
Total	66	30.71±5.226	10.07±1.903	4.83±0.940	56.09±9.377	37.14±8.296	6.29±4.590	0.56±0.897
F-value		2.911	2.690	0.686	0.693	0.140	0.758	0.419
p-value		0.041	0.054	0.564	0.560	0.936	0.522	0.740

Keys: PHCC= primary Health Care, N= number of samples, PCV= Packed Cell Volume, HB= Haemoglobin, WBC= White Blood Cells, NEUT= Neutrophils, LYMP= Lymphocytes, MONO= Monocytes, EOS= Eosinophils. Mean values with same letter are not significantly different (P>0.05) while values with dissimilar letters are significantly different (p<0.05)

DISCUSSION

Findings from Microscopy, Rapid Diagnostic Test (RDT) kits and confirmation using Polymerase Chain reaction (PCR) on pooled positive samples showed that *P. falciparum* was responsible for infections in the areas studied.

The relatively high prevalence rate of *Plasmodium falciparum* infection among the pregnant women agreed with WHO (2014) report which says that malaria is most prevalent where there is poverty and methods of disease identification, documentation and reporting are weak. Igabi LGA is cohabited by middle class and low income earners predominantly. The report of this study is higher when compared with the record of Oluwasola *et al* (2018); who reported14.4% from Ibadan in Oyo State and Abigail *et al* (2021); with percentage prevalence of 23.5% from Zaria in Kaduna state. This strongly suggest that pregnant women in Igabi LGA have high infection burden. This may result in health complications (Obeagu *et al.,* 2017; Surve *et al.,* 2017). The lower prevalence of *P. falciparum* infection recorded among the pregnant women in the study area when compared with similar studies conducted by Foghi *et al* (2021) from Delta state who reported 62.4% prevalence and Shuaibu *et al.* (2019) who reported 60% from Zaria, Kaduna State suggests that the prevalence of infection heavily varies from place to place.

The significant drop in PCV and HB values among pregnant women with *P. falciparum* infection compared to non-infected pregnant women, indicates anemia in the infected pregnant patients. Finding from this study, the PCV level is also lower than the report from a study carried out by Garba *et al* (2015) in Sokoto, where they had 38% in PCV level among malaria patients. The decrease in PCV and HB levels may be due to some degree of haemolysis in *P. falciparum* infected patients (Garba *et al,* 2015). Also parasitized red cells are being removed from circulation by the reticuloendothelial system and spleen and low or poor dietary iron intake/utilization resulting in anaemia in pregnancy especially during in-appetence induced by fever in malaria (Obeagu *et al.,* 2017; Surve *et al.,* 2017). It can be said that pregnant women with *P. falciparum* infection in Igabi LGA experience severe effect on PCV and HB resulting in blood disorders.

Total WBC count in pregnant women with, *P. falciparum*-infection did not show significant variation from that of women without the *P. falciparum* infection. This may be because as neutrophils, dropped in infected women, the lymphocytes value rose; suggesting a relatively stable total WBC count in the pregnant women with *P. falciparum* infection ((Foghi *et al*, 2021).

Mean neutrophils count in non-infected pregnant women was significantly higher than in *P. falciparum*-infected pregnant women. *Plasmodium falciparum* infection lowers neutrophil population (Obeagu *et al.,* 2017). This is in consonance with previous studies by Ogbodo *et al* (2018) who reported that neutrophils possess prominent immune regulatory activities and are the first responders amongst the inflammatory cells to migrate towards the site of inflammation. It may also, be due to the fact that neutrophils fight against antigen since, the foetus is regarded as a foreign body. Therefore, rises at the beginning but drops later. This is suggestive of chronic nature of *P. falciparum* infection in the pregnant women in the study area.

Mean Lymphocytes count is significantly higher in pregnant *P. falciparum*-infected women than those without the infection. This is also, in agreement with previous studies by Foghi *et al*. (2012) that reported high mean lymphocytes. Lymphocytes are known to proliferate in prolonged disease situations (Bawa *et al*. 2014; Surve *et al*., 2017). The *P. falciparum* infection in pregnant women in the areas studied is therefore, predominantly chronic in nature as shown by the high level of lymphocytes (lymphocytosis).

Mean Monocytes count did not differ significantly between pregnant, *P. falciparum*-infected and pregnant, non-infected women. In blood stage infection, monocytes participate in early stage infection by production of cytokines, phagocytosis and antigen presentation in cell-mediated immune response. They drive inflammation and sequestration of infected RBC in organs such as Brain, placenta or lungs. Monocytes are therefore high and important in early stage control of *Plasmodium* infection in human (Mitchell *et al*., 2014; Eledo and Izah, 2018). The insignificant monocyte count, therefore points to an infection that is not in the early stage but points to prolonged infection. This is in contrast to the reports of Kotepui *et al* (2014) and Foghi *et al* (2021) who opined that monocytes count was higher in infected pregnant women. They may be dealing with acute infection in their areas of study.

The high mean Eosinophils count observed of *P. falciparum* infected pregnant women was highly significant in relation to that of pregnant women without *P. falciparum* infection. This is in line with the work of Foghi *et al*. (2021) who reported that the induction of eosinophils attributed to a direct response to the parasite in high parasitaemia or

reflects temporary bone marrow suppression. Malaria causes extensive changes in bone marrow structure, function and eosinophils levels. Eosinophilia (elevated eosinophil count) is a heamatologic marker for parasitic infections, an immunologic response to tissue-dwelling parasite species (Mary and Peter, 2016), seen in allergies and drugs induced hypersensitivity reactions (Chauffaille, 2010).

CONCLUSION

In conclusion, infection of *Plasmodium falciparum* has highly significant effect on the blood cells parameters in pregnant women in Igabi LGA, Kaduna State. *Plasmodium falciparum* infection is high with overall prevalence of 31.4% in pregnant women in the areas studied.

The features of changes in blood cells parameters due to *P. falciparum* infection include anaemia, neutropenia, lymphocytosis and eosinophilia. No significant variations by location (PHCC) of the *P. falciparum*-infected pregnant women with regard to neutrophils, lymphocytes, monocytes and eosinophils counts.

The findings from this study recommended that pregnant women should attend ante-natal care early, be screened for *Plasmodium* infection and evaluated for blood cells parameters, be enlightened on importance of balanced diet and iron supplements to reverse anaemia.

Conflict of Interest

Authors declared no conflict of interests.

Financial Support

The research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

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