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Effect of Malaria Infections on the Haematologic and Anthropometric Indices of Pregnant Women in Six Villages in Northern Nigeria

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ABSTRACT
Malaria presents a formidable global health challenge and is responsible for the deaths of millions of children annually. This cross-sectional descriptive study evaluates the effect of malaria infections on the haematologic and anthropometric indices of pregnant women in six villages in the Federal Capital Territory, Abuja, Nigeria. A total of 212 pregnant women (ages between 15 and 40 years) attending antenatal clinics at the Primary Health Centres of these communities were selected for the study. Using microscopy, malaria was diagnosed in the blood samples of the pregnant women and a complete haemogram testusing an automated haematological analyzer was performed. Anthropometric measurements of the participants such as the height, weight, and body mass index (BMI) were obtained. Through a structured questionnaire, the socio-demographic data were also collected. The overall prevalence of malaria was 61.79%. There was a significant ($p < 0.05$) association between malaria and the ages of the pregnant women; and also, between malariaand the trimesters of pregnancy. Haematological parameters such as the packed cell volume, haemoglobin, and neutrophil count were significantly ($p < 0.05$) lower in malaria while the white blood cell and lymphocyte counts were significantly higher. Malaria parasite density revealed that white blood cells, packed cell volume, and haemoglobin were significantly ($p < 0.05$) affected. The BMI reveals that no participant was underweight. There was no significant association ($p > 0.05$) between the BMI of infected and non-infected patients. This study suggests that malaria in pregnancy further increases the burden of anemia in rural women.

Keywords: Malaria, Pregnant women, Anthropometric Indices, Body Mass Index, Haemoglobin.

I. INTRODUCTION

Malaria remains one of the most challenging public health issues being faced by developing countries. Malaria is a parasitic disease transmitted through the bite of the sporozoite-bearing female Anopheles mosquito. The malarial parasite, Plasmodium exists in four forms viz: Plasmodium falciparum, P. vivax, P. malaria, and P. ovale. P. falciparum is the most prevalent and it is responsible for 99.7% of estimated malaria cases in 2018[1]. In the South-East Asia Region, P. falciparum is responsible for 50% of the malaria cases, while in the Eastern Mediterranean Region and Western Pacific Region, it is responsible for 71% and 65% respectively[1]. According to the 2019 World Malaria Report of the World Health Organization (WHO), the African Region had the highest malaria cases (213 million or 93%) in 2018, with six countries in sub-Saharan Africa accounting for more than half of all the global cases of malaria: Nigeria (25%), Democratic Republic of Congo (12%), Uganda (5%), and Côte d'Ivoire, Niger, and Mozambique (4% each)[1]. In malaria-endemic regions particularly in sub-Saharan Africa, high rates of malaria-associated morbidity and mortality occur amongst children under the age of five,[2] closely followed by pregnant women[3]. This can be attributed to high rates of malaria in pregnant women[4]. In pregnant women, malaria leads to miscarriage, premature birth, Intra-Uterine Growth Restriction (IUGR), stillbirths maternal, and fetal anaemia[5]. Malaria in pregnancy also leads to low birth weight in infants. According to the WHO, West Africa accounted for the highest number of pregnancies exposed to malaria infections that resulted in low birth weight amongst newborns in 2019[1]. Interventions for pregnant women living in malaria-endemic areas in Africa include insecticide-treated bed nets (ITNs), intermittent preventive treatment with sulfadoxine-

pyrimethamine in pregnancy (IPTp), and effective case management. These have led to reductions in the occurrence of anemia in pregnant women and reduced placenta parasitemia[6]. A major consequence of maternal malaria is the low birth weight (LBW) amongst infants and newborn and neonatal death. Malaria doubles the risk of LBW amongst primigravidae and secundigravidae[7]. Congenital malaria often results from the transplacental transmission of malaria parasites(mostly P. falciparum) from a pregnant mother to her foetus resulting in low birth weight amongst infants.[8,9]Additionally, Falade et al., (2010) significant symmetric fetal growth restriction which was attributed to congenital malaria[10]. The incidence of congenital malaria greatly varies. In malaria-endemic areas, thereported incidence of congenital malaria varies from 8 to 33%.[2]Several studies carried out in parts of sub-Saharan Africa place the prevalence of congenital malaria between 10.8% and 54.2%[11]. Prevalence of congenital malaria in parts of Nigeria includes 0.7% in Sagamu,[12], 32.48% in Enugu,[13] 5.1% in Ibadan,[14] and 2.82% in Jos[15]. Congenital malaria also affects the anthropometric indices in infants. The evaluation of theanthropometric indices in pregnancy is recommended and it helps identify the risk of intrauterine growth retardation (IUGR) and low birth weight[16]. Anthropometric indices also indicate the nutritional status of pregnant women[17]. Common anthropometric indices in pregnant women include mid-upper arm circumference, calf circumference, waist circumference, waist to hip ratio, weight, height, and body mass index (BMI). Malaria and malnutrition in pregnant women often cause low BMI. This exacerbates the changes in their anthropometric measurements[18]. Studies have shown associated low BMI and low mid-upper arm circumference (MUAC) to high placental parasite loads[19]. Malariamay also contribute to protein-energy malnutrition through several mechanisms triggered by augmented levels of inflammatory cytokines, including anorexia and the induction of a catabolic response[20]. Malaria infections occurring during the first trimester in pregnant women are associated with more severe fetal and placental consequences than those occurring later in pregnancy. This causes changes in blood cell counts, red blood cells (RBC), leukocytes, and thrombocytes. Hematological changes such as anemia, thrombocytopenia, and leukocytosis or leucopenia that occur in the course of malaria infection are well recognized. These changes can be attributed to variations in malarial endemicity, blood disorders, nutritional status, demographic factors, and malaria immunity[21]. Haematological indices are a reflection of the health status of a pregnant woman. Pregnancy is often characterized by alterations in the haematological indices. The haematological indices include WBC, PCV, platelets, haemoglobin, neutrophils, lymphocytes, monocytes, eosinophils, basophils, MCV (Mean Cell Volume), MCH (Mean Cell Haemoglobin), and MCHC- (Mean Cell Haemoglobin Concentration). Anaemia is the most common abnormality associated with significant changes in the haematological indices of pregnant women, especially in low medium-income countries like Nigeria[22]. Several authors such as have identified significant effects in the hematological indices of pregnant women infected with the malaria parasite (P.falciparum) compared to healthy pregnant mothers in different parts of Nigeria[23–25]. This study is to evaluate the effect of malaria infection using haematologic and anthropometric indices in pregnant women in selected rural communities of FCT, Abuja, Nigeria.

II. MATERIAL AND METHODS

2.1 Study area

A cross-sectional study was carried out to assess the prevalence of malaria among pregnant women in Chibiri, Shetuko, Chukuku, and Kiyi villages located in Kuje Area Council (KAC) and Lugbe and Iddo villages located in Abuja Municipal Area Council (AMAC). These areas have inadequate waste disposal facilities and a portable water supply. Their staple foods include rice, cassava, yam, and maize.

2.2 Data Collection Procedure

2.2.1 Questionnaires

A structured questionnaire was used to obtain socio-demographic information, present and past history in pregnant women, environmental-related factors, and dietary habits. The questionnaire was developed in English. The community nurses who can speak the local language and Hausa were used after obtaining training on data collection procedures for this particular study to attain standardization and maximize interviewer reliability. The data collectors were regularly supervised by the principal investigator for proper data collection.

2.2.2 Sample Collection and Evaluation

Five milliliters of blood were collected by venipuncture using an aseptic technique from each participant into two EDTA bottles. One part was used for the determination of haematological

parameters while the other was used for the diagnosis of malaria.

2.2.3 Ethical Clearance

Ethical authorization for this study was issued by the Federal Capital Territory Health Research Ethics Committee (FHREC), Abuja, Nigeria with protocol approval number FHREC/2014/01/67/11-11-14.

2.3 Diagnosis of Malaria

2.3.1 Malaria Microscopy

Thick and thin films for the diagnosis of malaria were prepared on different slides. For the thick film,12ul of blood was taken with an adjustable micropipette (P20 Pipetman,Gilson) and spread over a diameter of 15mm, 2ul of blood was used to make the thin film. The slides were made in duplicates and labeled appropriately. The thin film end of the slide was fixed by dipping the prepared film in absolute methanol for one to two seconds, both films were allowed to dry and subsequently stained with 10%Giemsa at pH 7.2 for 30minutes.The slide was read under a x100 oil immersion objective lens. The number of asexual parasites was counted against 200 leucocytes. Before a smear was considered negative, 200 high power fields had been examined[26].

2.3.2 Determination of Haematological Indices

2 ml of blood was taken in an ethylene diamine tetraacetic acid (EDTA) bottle and immediately analyzed for a complete haemogram using an automated haematology analyzer. The Sysmex KX21N is an automated blood cell counter used for in vitro diagnostic in clinical laboratories. It is a compact, fully automated haematology analyzer with simultaneous analysis of 18 parameters in whole blood mode and capillary blood mode. This analyzer counts blood cells as routine in a few minutes. The test was performed as stated in the manufacturer's instructions (Sysmex Corporation, 2006) haematological parameters like White Blood Cell count (WBC), Haemoglobin level (Hb) and Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean corpuscular Hemoglobin (MCHC), Mean Corpuscular Haemoglobin Concentration (MCHC), absolute RBC count, Platelet count were determined using the automated cell counter (Sysmex Corporation, 2006).

2.3.3 Determination of Anthropometry Parameters

Anthropometric consisted of weighing the pregnant women without shoes using the weight measurement, measured precision was 0.1 kilogram. Then a meter rule measure was placed vertically against the wall, the women stood up against the tape measure vertically without shoes, and height was measured with 0.1centimetre precision. Height and weight were taken and their body mass index (BMI) was calculated to determine the weight status of these women (using the formula weight in kg).

2.3.4 WBC Count (Manual)

The total leukocyte count determination was done using the improved Neubauer chamber, as described in detail by Baker and Silverton[27].Briefly, 20 µL of blood was taken with an adjustable micropipette (P20 Pipetman, Gilson) and mixed with 380 µl of Turk's solution (2% Acetic acid tinged with gentian violet) and was taken with an adjustable micropipette (P1000 Pipetman, Gilson) to give a final 1:20 dilution. The red blood cells were lysed leaving the leukocytes. The leukocytes were counted using the New Improved Neubauer Chamber.

2.3.5 Determination of Haemoglobin Concentration (manual)

The haematocrit level was determined by filling a capillary tube up to 75% with well-mixed anti-coagulated blood, sealed at one end with Cristaseal, and spun at 3,000 rpm for five minutes in a Hawksley Haematocrit Reader (Hawksley, England).

The hemoglobin was calculated from the result gotten from Packed Cell Volume by dividing the result by three and the benchmark for anaemia (Hb<11g/dl) will be used[28].

III. RESULTS

A total of 212 pregnant women were enrolled in this study from six selected villages: Chukuku, Chibiri, Kiyi, Shetuko, Lugbe, and Iddo in the Federal Capital Territory, Abuja. The age range of the pregnant women was between 15-40 yrs. Thirty-one samples each were collected from Chibiri, Kiyi, and Lugbe respectively. Table 1 shows the number of participants in each of the villages.

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Table 1: Geographical, age, and Trimester Distribution of Malaria in Pregnant Women

Location	Number Examined	Infected Persons (%)	Non-infected Persons (%)
Chukuku	31	22 (71%)	9 (29%)
Chibiri	32	17 (53%)	15 (47%)
Iddo	31	24 (77%)	7 (23%)
Kiyi	33	20 (61%)	13 (39%)
Lugbe	54	39 (72%)	15 (28%)
Shetuko	31	14 (45%)	17 (55%)
	212	**136 (64%)**	**76 (36%)**
Age			
15-20		21%	17.80%
21-25		37%	26.70%
26-30		26.10%	40%
31-36		11.80%	14.40%
36-40		4.20%	1.10%
Trimester			
1st		17 (14.3%)	0 (0.0%)
2nd		75 (63.0%)	12 (13.2%)
3rd		27 (22.7%)	79 (86.8%)

From Table 1, there was a high prevalence of malaria infection in the six villages where a majority had above 50% of infection. Iddo village had the highest prevalence with 77%, the least rate of prevalence was seen in Shetuko which was 45%. The average prevalence of malaria in all six villages was 63%. Concerning malaria and the age of pregnant women (x^2 =8.994, p-value = 0.041), there was a significant ($p < 0.05$) association between the ages of the pregnant subjects and malaria.Table 1 also shows that all the people at various trimesters especially 2nd and 3rd were susceptible to malaria infection (x^2 = 85.924, p-value = 0.000). There was a significant (p values < 0.05) association between the occurrence of malaria and the trimesters of pregnant women.

Table 2: Prevalence of malaria in the six villages across the age group
MP Status

Age	Positive		Negative		Total	
	Freq	%	Freq	%	Freq	%

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15-20	15	7.04	19	6.96	34	15.90
21-25	58	27.36	31	14.62	89	41.98
26-30	38	17.92	19	8.96	57	26.89
31-35	17	8.01	11	5.16	28	13.21
36-40	3	1.41	1	0.47	4	1.89
Total	**131**	**61.74**	**81**	**38.17**	**212**	**100**

% - percentage, Freq – Frequency

Table 2 reveals the prevalence of malaria infection among the participants. Participants in age bracket 21-25 had the highest rate of malaria infection (27.36%). The age range 35-40 had the least of people 3(1.41%) that were infected. The total percentage of participants who were positive for malaria parasite represents 61.79%

Table 3: Effect of Malaria on Haematological Parameters

Parameters	Malaria +ve`	Malaria –ve	p-value
WBC(x10^{3}ul)	8.0439±0.15	7.32±0.15	0.002
PCV (%)	28.40±0.27	29.91±0.39	0.006
Hb(g/dl)	9.2338+ 0.34	9.7667±0.40	0.032
Neut (%)	65.81±0.61	62.80±0.72	0.002
Lymph (%)	26.50±0.54	28.88±0.63	0.004
Mono (%)	4.76±0.26	4.70±0.27	0.883
Eosino (%)	3.22±0.15	3.44±0.16	0.312
Baso (%)	0.02±0.01	0.03±0.02	0.502
Plt(x103ul)	377.91±25.03	322.03±21.05	0.103
MCV (fl)	86.61±1.69	86.45±1.85	0.948
MCH (pg)	34.28±1.43	33.78±1.54	0.813
MCH conc(g/l)	30.46±0.18	30.09±0.21	0.178

p values less than 0.05 are statistically significant.

Key: +ve – positive, -ve-negative, WBC-White Blood Cells, PCV- Packed Cell Volume, Hb-Haemoglobin, Neut- Neutrophils, Lymph-Lymphocytes, Mono-Monocytes, Eosino-Eosinophils, Baso-Basophils, Plt- Platelets, MCV-Mean Cell Volume, MCH- Mean Cell Haemoglobin, MCHC- Mean Cell Haemoglobin Concentration.

Table 3 shows the effect of malaria on haematological parameters in infected women. Subjects infected with malaria parasites were significantly lower in Packed Cell Volume, Haemoglobin, and Lymphocyte when compared to non-infected subjects. Also, White Blood Cells and Neutrophil counts were significantly higher when compared to non-infected subjects.

Table 4: Effect of MPD on Haematological Parameters

Parameters	Mean	r-value	p-value
WBC(x10^3/ul)	7.73	0.321	0.000
PCV(%)	29.05	-0.213	0.002
Hb(g/dl)	9.65	-0.165	0.017
Neut (%)	64.51	0.038	0.582
Lymph(%)	27.53	-0.071	0.306
Monocytes(%)	4.73	0.085	0.219`
Eosino(%)	3.32	0.118	0.089

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Baso(%)	0.02	0.045	0.523
Plt(x103ul)	353.88	0.048	0.492
MCV(fl)	86.54	0.023	0.746
MCH(pg)	34.06	-0.088	0.208
MCHconc(g/l)	30.30	0.030	0.670
MPD	601.13		

r values that are negative show negative correlation while r values that are positive show positive correlation p values less than 0.05 are statistically significant.

Table 4 shows the impact of Malaria Parasite Density (MPD) on haematological parameters. Pearson's correlation analysis showed the parameters. White Blood Cells, Packed Cell Volume, and Haemoglobin were significantly ($p<0.05$) affected by the Malaria Parasite Density.

Table 5: Effect of Malaria on BMI of the Participants

Infected Persons	24.57±0.45	**Non-infected persons**	25.63 ± 0.41	**p-value**	0.086	

Participants' BMI (kg/m2)	**Under weight (<18)**		**Normal weight (18-24)**		**Over weight (24.5-29)**		**Obesity (>30)**		**Total**	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Chukuku	0	0	15	48.4	13	41.9	3	9.7	31	100
Chibiri	0	0	20	62.5	9	28.1	3	9.4	32	100
Iddo	0	0	9	29	15	48.4	7	22.6	31	100
Kiyi	0	0	22	66.7	10	30.3	1	3	33	100
Lugbe	0	0	16	29.6	22	40.7	16	29.6	54	100
Shetuko	0	0	15	48.4	10	32.3	6	19.4	31	100
	0		**97**		**79**		**36**		212	

Table 5 shows the effect of malaria on the Body Mass Index (BMI) of pregnant women (The p-value was 0.086). The result revealed that there was no significant ($p > 0.05$) association between the body mass index of infected (25.63+0.41) and non-infected (24.57+0.45) patients with malaria. The BMI of the participants reveals that none of them was underweight. Lugbe and Iddo recorded the highest number of people that were obese with 29.6% and 22.6% respectively. The overall percentage of the people that were normal, overweight, and obese was 45.75%, 37.26%, and 16.98% respectively.

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Table 6: Demographic Factors of the Participants and the Presence of Malaria

Factors		Participants (%)	Malaria +ve (%)
Educational qualification	Tertiary	2.02	8.60
	Secondary	43.87	16.09
	Primary	41.51	23.64
	None	7.08	13.60
Marital Status	Married	91.04	35.40
	Single	8.02	27.56
Gravidae	primigravidae	45.25	29.43
	secungravidae	32.21	22.12
	multigravidae	22.54	10.80
Use of mosquito nets	Yes	35.85	21.70
	No	64.15	39.62
Use of toilet facilities	water system	50.47	10.79
	Pit	22.17	28.35
	open defecation	27.36	23.42

IV. DISCUSSION

With a 64% average prevalence in the six villages (Table 1) and 61.74% frequency of infection (Table 2), this study has demonstrated the high endemicity of malaria among pregnant women living in the study area. Consequently, malaria is a public health risk among pregnant women in these six villages. Specifically, the prevalence of malaria is holoendemic (more than 75%) in Iddo, hyperendemic (between 51–75%) in Lugbe, Chibiri, Kivi, and Chukuku, and mesoendemic (between 11–50%) in Shetuko[29]. The variability in endemicity seen in these locations could be attributed to several factors such as the season, altitude, and environmental factors (poor drainage, presence of stagnant water, etc) that encourage the breeding of the vector[30].

Another factor is the prevailing community social and health care practices such as the use of mosquito nets while sleeping and type of toilet facilities. Data from Table 6 suggests that the prevalence of malaria increased with the non-usage of mosquito nets and the use of pit latrines and the practice of open defecation which aids mosquito breeding.

The malariometric data suggests there was a significant ($p < 0.05$) association between malaria and the age of the pregnant women with younger women more at risk (Table 1). This finding is similar to that of Mcgregor (1983)[31]. Younger women are more susceptible to infection (Table 2) because many of the younger women (15-30) were in their primigravidae and secundigravidae (Table 6). Older women are less prone to malaria and studies have shown that young women are still in the process of acquiring immunity[32].

It has been suggested that the development of pregnancy-associated immunity is greater in older women than younger women due to a higher number of pregnancies[33]. In pregnancy, antibodies that inhibit the adherence of placental parasites to chondroitin sulfate A are produced. Primigravidae are less likely to have these anti-cytoadherent antibodies than multigravidae due to a lack of specific immune response to the placental parasites[21]. Primigravidae and secundigravidae

women have no or low level of immunity against the malaria strain and hence suffer the consequence[32]. The findings of Fried et al., (1998) showed that multigravida mothers cumulatively develop malaria antibodies that block the adhesion of parasites to CSA receptors in the placentae in subsequent pregnancies[34].

The susceptibility of pregnant women to malaria parasite infection is well established and it has been associated with several pathologies such as maternal anaemia,stillbirth, miscarriage, premature birth, and maternal death[31]. However, pregnancy is a dynamic interplay between hormonal, hematological, and immunological factors [35]. The significant ($p > 0.05$)association between the occurrence of malaria and the trimesters of the pregnant women is due to their changing immunological status at these times. As reflected in the result, immunity against malaria parasites is high in the first trimester (Table 1). This is because of the pro-inflammatory status of pregnancy. Due to implantation, placentation, and other associated events in the first trimester, the body reacts as it would to an open wound with a strong inflammatory response[36]. During this period, there is a mobilization of pro-inflammatory cells such as macrophages, dendritic cells, and natural killer cells and they infiltrate and accumulate at the point where the trophoblast cells meet the decidua[37,38]. In the second trimester, the pregnancy assumes an anti-inflammatory status and this explains, the reduced immunity and increased parasitemia seen in Table 4[36]. Also, to encourage parturition, a reactivation of the pro-inflammatory status is required. As pregnancy approaches term, there is an infiltration of the immune cells into the myometrium in preparation for the contraction of the uterus[39,40]. This rise in immunity explains the lowered parasitemia in the third trimester (Table 1).

Haematological parameters of infected pregnant women showed significant ($p < 0.05$) difference in total WBC count, PCV, HB concentration, neutrophil count, and lymphocyte count when compared to those of non-infected participants (Table 3). In a similar vein, white blood cells, packed cell volume, and haemoglobin were significantly ($p<0.05$) affected by the Malaria Parasite Density (Table 4). This finding is consistent with previous studies which observed that anemia (lower PCV and Hb values) is one of the most frequent complications related to pregnancy[41]. Anaemia in pregnancy is defined as those having haemoglobin concentration less than 10g/dl as this is the usual benchmark in most hospitals in Nigeria[42]. Though the WHO benchmark for anemia is PCV less than 33%, in developing countries like Nigeria only PCV values lower than 30% are worthy of further investigation and treatment[43,44]. The results from Table 3 show that all the subjects of the study had a mild anemic status[24].

From Table 3, the neutrophil and lymphocyte counts were the most important significant ($p<0.05$) leucocytic changes associated with malaria infection[45]. The Neutrophil count was significantly ($p<0.05$) higher than those of non-infected participants. The neutrophilia is because, during malaria infection, neutrophils are activated from the bone marrow by several mechanisms to destroy infected red blood cells (RBC) [46]. Their removal from peripheral blood could also be suppressed[45].Neutrophils with malaria pigment could also serve as markers of the severity of the disease[46].

The Lymphocyte count was significantly ($p<0.05$) decreased in patients with Plasmodium falciparium infection as compared to those inthe non-malaria group. The observed decrease in lymphocyte count may be due to the redistribution of lymphocyte cells to sites of inflammation with the sequestration to the spleen[47,48]. The mean values of all other haematologic parameters other than PCV, Hb, WBC, Lymphocytes, and Neutrophils were not significant ($p<0.05$) when infected and non-infected women were compared. This compares favorably with the findings of other workers in Jos and Ibadan, Nigeria[47,48].

Table 5 reveals that none of the participants was underweight and it also reveals that there is no association between malaria and the BMI of pregnant women. However, empirical data reveal that an increased parasitemia harms the nutritional status of the patient and consequently the BMI[49]. In malaria-endemic regions, ahigh BMI might contribute to anemia in pregnancy[50].

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V. CONCLUSION

There was a high prevalence of malaria in the villages under study (Chibiri, Shetuko, Chukuku, Kiyi, Lugbe, and Iddo). The study showed that there was an association between the age of the pregnant women and malaria infection in that the younger women recorded more cases of malaria. The rate of malaria infection was found to

be higher in the second and third trimesters compared to the first trimesters. Primigravidae and Secundgravidae seemed to be at a greater risk as the parasitemia level was higher than in multigravidae. There was significantly lower packed cell volume, haemoglobin, lymphocyte, and higher white blood cells and neutrophil level in women with malaria compared to women without malaria.

It has been shown also in this study that there was no association between malaria and body mass index (BMI). Malaria in pregnancy further increases the burden of anaemia for women especially those in rural farming communities. Malaria-induced anaemia in pregnancy may also be aggravated by the low nutritional status of pregnant women whose staple food was mainly carbohydrates.

Conflict of Interest: The authors declared there are no conflicts of interest.

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