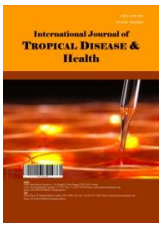

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Effects of Malaria on some Haematological Parameters among Pregnant Women in Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. The work was designed and final data interpreted by authors SON and FON, corrected the first manuscript while author BOF, recorded the experimental outcome. All the authors participated in specimen collections. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the effect of malaria parasitaemia on some haematological parameters of pregnant women in Delta State, Nigeria.
Study Design: The experiment is a vertical survey of pregnant women.
Place and Duration: The study was carried out in five designated Local Government Area in Delta State, Nigeria. They include Ughelli South, Ughelli North, Udu, Uvwie and Ethiope East Local Government Area, between January-August 2021.
Methodology: Vein puncture technique was used to obtain 5ml of blood from 1000 pregnant women in the five selected government hospital in Delta State. Malaria parasite examination was done using thin and thick blood films, following standard parasitological techniques. Haematological parameters comprising packed cell volume, hemoglobin concentration, white blood cell count, neutrophils, lymphocytes, eosinophils, monocyte and basophils were determined and CD4 count was done using flow cytometry. Socio economic data was obtained with the use of structured questionnaires. Data were analyzed using student T-test and Anova.

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Results: Out of the 1000 pregnant women examined, 624 (62.4%) tested positive for malaria. Those with secondary education had the highest prevalence where 234 (23.4%) examined and 202 (86.3%) infected. Data revealed that married women and artisans had very high infections of (70.9%) and (78.7%) respectively. There was a significant difference ($p<0.05$) in the level of PCV, HB, TWBC, Neutrophils, Lymphocytes, Eosinophils, Monocytes and CD4 count of those infected when compared to those uninfected. Women with 1+ve *Plasmodium* count had CD4 count of 712.68 ± 12.23, those with 2+ve *Plasmodium* count had CD4 count 628.88±9.11, while those with 3 +++ve *Plasmodium* count had CD4 count of 578.71±2.10.
Conclusion: *Plasmodium* parasitaemia had influenced the haematological parameters of pregnant women in the study. Pregnant women should be advice on the proper use of preventive strategies which include intermittent preventive treatment IPTp and the proper use of insecticide treated bed net.

Keywords: Malaria parasitaemia; pregnant women; haematological parameter; Delta State; Nigeria.

1. INTRODUCTION

Malaria is a life-threatening disease in sub-Saharan Africa that is most serious among pregnant women and children under the age of 5 [1] due to their compromised and low build-up of immunity respectively. Malaria is the major cause of foetal and maternal morbidity and mortality in Nigeria. Globally, it is estimated that 24 million pregnant women are infected by malaria yearly [1]. Pregnant women have a higher risk of malaria compared to non-pregnant women [2]. During pregnancy, *Plasmodium falciparum* infection in Africa is most asymptomatic, therefore, remains undetected and untreated [18]. In Nigeria, there are over 100 million people at risk of malaria every year and it is estimated that about 50% of the adult population experience at least one episode yearly [3].

Pregnant women who reside in malaria endemic zones in Nigeria are associated with high frequencies and densities of *P. falciparum* parasitaemia, with higher rates of maternal morbidity which include fever, severe aneamia, abortion, still birth, higher rate of placental malaria and low birth weight of less than 2.5kg in new-borns, caused by both prematurity and intrauterine growth retardation [4]. *Plasmodium* spp., being a parasite that affects the blood cells, induces alterations in the haematological parameters of its victims [5]. Haematological changes are some of the most common complications in malaria as they involve the blood cells [6]. Hematological alterations that are associated with malaria infection are well documented, although, there is still conflicting reports on the extent of alteration [7].

Anemia is the major effect of *P. falciparum* malaria infection, with over 500,000 pregnant women being diagnosed with severe anemia resulting from *Plasmodium* parasitaemia infection in sub-Saharan Africa [1,4]. Ekvall [8] outlined some of the mechanisms with which malaria causes anemia, some of them include; excessive removal of non-parasitized erythrocytes, immune destruction of parasitized red cells and impaired erythropoiesis as a result of bone marrow dysfunction. The severity and type of anemia can be determined by evaluating haematological parameters such as hemoglobin concentration (HB) and packed cell volume (PCV) [9]. CD4 cells also known as CD4+ T cells are white blood cells that fight infections which are compromised in the presence of viruses like the HIV Virus. CD4 count is like a snap shot of how well the immune system is functioning, such that as the disease condition progresses; the number of CD4 cell declines [11]. The normal range of CD4 cell is about 500-1,500, but when it decreases to about 200, it signifies the presence of a life threatening disease such as AIDs [11]. CD4 counts are widely used as prognostic marker to access the degree of immune impairment in HIV seropositive individuals and treatment decisions are based on this. They are also used to monitor antiretroviral therapy (ART)[12].

Pregnancy is a physiologically immunocompromised state during which alterations in T-lymphocyte subset may occur [12]. Normal pregnancy has been described as an immune-suppressed state that is induced so that the foetus would not be rejected by the mother's immune system [12], however, more recent evidence indicates that the immunology of both pregnancy and sex steroid hormones are exceedingly complex and not fully understood [13]. The foetus is then protected by the changes in Human Leukocytes Antigen (HLA) expressed in the trophoblast [8]. Therefore, if there is well-

informed alterations in blood parameters in malaria infection, it will enable the clinician to establish reliable and timely diagnosis and therapeutic interventions. It is pertinent to ascertain the influence of malaria on the haematological status of the pregnant women in the study area especially the CD4 status. This would give an overview of the immune competence of the average woman in her gravidae state.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in five (5) Local Government Area of Delta State, Nigeria. Delta State is an oil and agricultural producing State in Nigeria. It is situated in the region known as the South-South geo-political zone with a total population of about 4, 112, 445 [14]. The State has a total land area of 16, 842 square kilometers, lies approximately between $5^{0}00^{1}$ and $6^{0}45^{1}$E; and $5^{0}00^{1}$ and $6^{0}30^{1}$N. Delta State has tropical climate with two distinct seasons; the rainy season (April-October) and the dry season (November-March). Mangrove swamps are predominant in Delta State and merge with fresh water swamps to the north region of the state. The State have temperature ranging between 25^{0}C and 33^{0}C with mean temperature of 29^{0}C, it has an average relative humidity of about 79%. Delta State have 25 Local Government Areas, however, the study was conducted in five (5) selected Local Government Area namely; Ughelli North, Ughelli South, Uvwie, Ethiope East and Udu. The main occupation of the inhabitants are farming, fishing and trading.

2.2 Study Population

This study involved pregnant women (gravidae and registered for antenatal at a recognized health facility) in 5 designated Local Government Area in Delta State, Nigeria. It cuts across major Government Health Facilities.

This study include 1000 consenting pregnant women that attended antenatal clinic (ANC) in the five designated health centers and they were those that met the inclusion criteria.

2.2.1 Inclusion criterion

2.2.1a. All gravidae women (aged 18+) registered for anti-natal care in a government recognized health institution are referred to as pregnant

2.2.1b. All gravidae women (aged 18+) registered for anti-natal care in a government recognized health institution that do not express *Plasmodium* parasitaemia are referred to as uninfected pregnant women.

2.2.2 Exclusion criteria

All gravidae women (aged 18+) registered for anti-natal care in a government recognized health institution that are undertaking antimalarial treatment within 14 days before sampling are referred to as none participants

2.3 Data Collection

A total of 1000 consenting pregnant women were enrolled for the study, information on bio-data, knowledge, attitude and perception was obtained using well structured questionnaires and hospital records. 5ml of blood was collected by trained health technicians using the vein puncture technique [15]. Blood was transferred into a sterile EDTA container for malaria parasite microscopy and haematological parameters investigations.

2.4 Microscopic Examination

Thick and thin smears of blood were prepared and examined with x100 objective of the microscope as described by Cheesbrough [16].

2.5 Haematological Parameter Evaluation

Total white blood cell count (TWBC), hemoglobin level, neutrophils, lymphocytes and eosinophils were obtained manually according to the method described by Erhabor, Adias and Hart [9], packed cell volume (PCV) were determined using the micro hematocrit centrifuge in a method described by Adesina, Balogun, Babatunde, Sanni, Fadeyi and Aderibigbe [17].

2.6 CD4 Cell Determination

Three milliliter of venous blood was collected from EDTA bottles, all samples were processed using four-coloured flow cytometry on a Becton Dickins on FacsCalibur, using the multi test reagents and Trucount beads, and analyzed using multiset software, according to standard operating procedures as described by Enow-Tanjong, Atashili, Kamga, Ikomey, Akenji and Ndumbe [12].

2.7 Statistical Analysis

Data were entered into SPSS version 20 and subjected to statistical analysis using Student T test and ANOVA.

3. RESULTS

3.1 Overall Malaria Prevalence in the Study

The overall malaria prevalence in the study area was 624 (62.4%) with Central Hospital Ekpan having the highest prevalence of 142 (71.0%) followed by General Hospital Otor-Udu, 140 (70.0%), General Hospital Isiokolo had the lowest prevalence of 98 (49.0%), while Central Hospital Ughelli and General Hospital Otu-Jeremi had a prevalence of 112 (56.0%) and 123 (61.0%) respectively. Malaria prevalence was statically significant (P<0.05) C^2 = 96.627 (*P*=.00) Table 1.

3.2 Demographic Characteristics of the Respondents

Table 2 shows the demographic characteristics of the respondents in the study, Data shows that (13.8%) of the respondents had no formal education, (17.8%) had primary education, (23.4%) had secondary education, and (46.0%) had tertiary education. Out of those that had no education, 119 (86.2%) were infected, and for those that had primary education, 115 (64.8%) were infected, for those that had secondary education, 202 (86.3%) were infected while for those that had tertiary education, 194 (42.2%) were infected C^2 = 171.140, (*P*= .00).

Out of 1000 respondents, 53.9% were married while 39.7% were single and 6.0% were divorced while 0.4% were widows. Out of those that are married, 382 (70.9%) were infected, and for those that are single, 205 (51.6%) were infected, for those that are divorced, 35 (58.3%) were infected, widows had 2 (50.0%) infected C^2 = 36.774 (*P* = .00).

Out of the 1000 pregnant women that responded, 22.8% were housewives, 29.9% were traders, 25.3% were artisan while 22.0% were formally employed. Out of those that are housewives, 115 (50.4%) were infected, traders had 213 (71.2%) infected, artisans had 199 (78.7%) infected and 97 (44.1%) for those that were formally employed. C^2 83.785 (*P*=.00) (Table 2).

Table 1. Overall prevalence of malaria in the various health facilities

Locations	No. examined	No infected (%)	c^2	*P*
Ughelli	200	112(56)		
Otor-Udu	200	140(70)		
Isiokolo	200	98(49)		
Ekpan	200	142(71)		
Otu-Jeremi	200	123(61)		
Total (%)	1000	624(62.4)	96.627	.00

Table 2. Demographic characteristics of the respondents

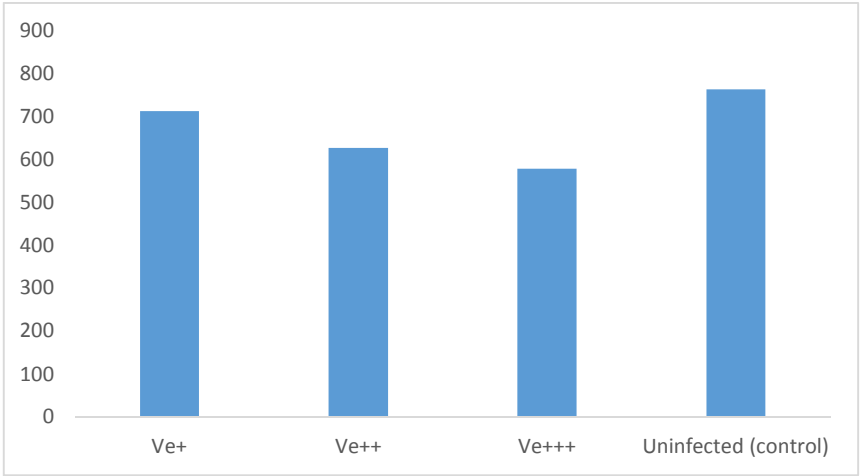
Characteristics	No. examined (%)	No. infected (%)	c^2	*P*
Education				
No Education	138(13.8)	119(86.2)		
Primary	178(17.8)	115(64.8)		
Secondary	234(23.4)	202(86.3)		
Tertiary	460(46.0)	194(42.2)	171.140	.00
Marital status				
Married	539(53.9)	382(70.9)		
Single	397(39.7)	205(51.6)		
Divorced	60(6.0)	35(58.3)		
Widow	4(0.4)	2(50.0)	36.774	.00
Occupation				
Housewife	228(22.8)	115(50.4)		
Trader	299(29.9)	213(71.2)		
Artisan	253(25.3)	199(78.7)		
Formal employment	220(22.0)	97(44.1)	83.785	.00

Table 3 shows the haematological parameters of infected pregnant women and that of the uninfected pregnant women (control). Data shows that the mean packed cell volume (PCV) amongst the pregnant women with asymptomatic *Plasmodium* parasitaemia was significantly lower than those without parasitaemia 32.36 ± 1.07% vs 37.24 ± 1.02%, (*P* = .02). The haemoglobin concentration (HB) of the infected pregnant women was 10.79± 0.36g/dl while, non-parasitized pregnant women (control) was 12.41 ± 0.34g/dl, (P = .02). The total white blood cell (WBC) count of women with parasitaemia was 7.91 ± 254.36 and was higher than the WBC count of non-parasitized pregnant women (5.41 ± 142.83) (*P*=.00). Neutrophils of the infected pregnant women was 72.64 ± 1.73 while the uninfected was 53.56 ± 1.44 (*P* = .00). Lymphocytes of the infected was 42.52±1.39 while that of the uninfected was 30.92 ± 1.40 (*P* = .00). Eosinophilia was lower in the uninfected 0.73 ± 0.16 than the infected 3.60 ± 0.48 (*P* = .00). Monocytes had 0.60 ± 0.17 for the infected pregnant women and 0.24 ± 0.09 for the uninfected pregnant women. (*P* = .01). Basophiles had 0.38 ± 0.05 for the infected pregnant women and 0.32 ± 0.12 for the uninfected pregnant women (*P* = .55). The CD_4 count of the infected pregnant women was 655.92 ± 33.127 and the non-parasitized pregnant women was 763.04 ± 26.09 (*P*= .01) (Table 3).

Fig. 1 shows the CD4 count of pregnant women in relation to the *Plasmodium* load, data shows that those with one plus (1+ve) positive malaria had a CD4 count of 712±12.23, those with two plus (2++ve) malaria had their CD4 count to be 626.88±9.11 while those with three plus (3+++ve) had their CD4 count to be 578.71±2.10. Although, they were within the normal range of 500 to 1500 cell. These values were statistically significant, when compared to the control 763.04±26.09. (*P*=.00) (Fig. 1)

Table 3. Haematological parameters of malaria patients and control

Haematological parameters	Infected ±SE	Uninfected (control) ±SE	*P*-value
PCV (%)	32.36±1.07	37.24±1.02	.02
HB (g/dl)	10.79±0.36	12.41±0.34	.02
Total WBC ($x10^9$/l)	7.91±254.36	5.41.±142.82	.00
Neutrophil (%)	72.64±1.73	53.56±1.44	.00
Lymphocyte (%)	42.52±1.39	30.92±1.40	.00
Eosinophil (%)	3.60±0.48	0.73±0.16	.00
Monocytes (%)	0.60±0.17	0.24±0.09	.01
Basophils (%)	0.38±0.05	0.32±0.12	0.5
CD4 (cell/mm^3)	655.92±33.127	763.04±26.09	0.014



v+ = indicates 1+ Plasmodium parasitaemia
v++ = indicates 2+ Plasmodium parasitaemia
V+++= indicates 3+ Plasmodium parasitaemia

Fig. 1. CD4 Count in Relation to *Plasmodium* Load in Pregnant Women

4. DISCUSSION

The prevalence of peripheral malaria infection in this study was 62.4%. This agrees with findings from other studies on malaria in Nigeria, where *P. falciparum* was the commonest species of the parasite in the infected. Usually, *P. falciparum* malaria is usually without symptoms and remains undetected and untreated even when there is the presence of parasites in the placenta [18, 1].

The study showed that secondary education holders had the highest prevalence of 86.3%, which was in line with the work of Bawa, Auta and Liadia [19]. This is not unconnected to poor knowledge of malaria and its transmission, and this may be probably because they were more exposed to mosquito bites due to poor environmental condition and lifestyles.

Married women had the highest prevalence of 70.9% while, widows had a low of prevalence 50.% which agrees with Okafor, Ezekude, Oluwole and Onigbogi [20].

Artisans had the highest prevalence of 78.7%, while formally employed had the lowest prevalence of 44.1%. The occupational environment of the women played a role in their susceptibility to the disease. Majority of the women were hair dressers and tailors who often closes late at night due to work load, and inadvertently get exposed to the bites of mosquito vectors. This is in agreement with the work of Chukwuocha, Dozie and Chukwuocha [21] and Chukwuocha, Iwuala and Dozie [22] who stated that the host agent's interaction with the environment plays a dominant role in malaria transmission.

The mean packed cell volume (PCV) and hemoglobin concentration (HB) was significantly lower in pregnant women with malaria parasitaemia than in their counterparts i.e. those without malaria. This was consistent with reports from a number of sub-Saharan African countries which indicated that the prevalence of anaemia was consistently higher among pregnant women infected with malaria than those uninfected [23,9,5]. The most common heamatological indices used for the diagnosis of anaemia were; heamoglobin concentration (HB) and packed cell volume (PCV).

In this study, the leucocytes count was significantly higher ($P<0.05$) in the parasitized pregnant women than when compared to that of the control. This is consistent with the work of Akinboye, Okonofua, Awodele, Agbolade, Ayinde, Rebecca and Haruna [24], Ogbodo, Ezeugwunne, Eze, Njoku, Oguaka, Amah, Akunneh-Warison, Ejiofor, Iheukwumere and Mbandso [25]. Obebe, Falohun, Olajuyigbe, Lawani and Ajayi [26] and Alo, Okonkwo, Anyim and Ugah [23]. White blood cells (WBC) play a vital role in the body's immune defense against diseases. The number of WBC may be reduced or increased depending on a disease condition or a reaction occurring in the body. Similarly, a previous work reported that total leukocytes count rises in early pregnancy and remains elevated throughout pregnancy [26].

Neutrophils were also significantly higher ($p<0.05$) in the parasitized women than the non-parasitized. This is in consonance with previous studies by [3,25]. Neutrophils possess prominent immune regulatory activities and are the first responders amongst the inflammatory cells to migrate towards the site of inflammation. [27]. It may also, be due to the fact that neutrophils fights against antigen since, the foetus is regarded as a foreign body [25].

Lymphocytes were significantly increased in the malaria parasitized women than that of the control set. This is also, in agreement from previous studies by Alo, Okonkwo, Anyim and Ugah [22], Rouse, Owen and Goldenberg [28]. The increase and decrease of lymphocyte counts associated with malaria may be due to reflected redistribution of lymphocytes from sequestration in the spleen [19].

Eosinophils were significantly lower in the uninfected when compared to the infected subjects, this is in line with the work of Alo, Okonkwo, Anyim and Ugah [7]. In this study, the induction of eosinophils was variously attributed to a direct responses to the parasite, or reflecting release of eosinophils following temporary bone marrow suppression. Malaria causes extensive changes in bone marrow structure and function, and eosinophils precursor are usually abundant [14]. Another factor that stimulate the production of eosinophils is high inoculation of the infective stage of *Plasmodium.*

Monocytes were higher in infected pregnant women than that of the control. This is consistent with the work of Kotepui, Phunphuech, Phiwklam, Chupeerach and Duangmano [29], [30]. The increase in the number of monocytes may be due to the major role of the monocytes in

innate immune response through the release IFNɣ in response to malaria infection [31]. monocytes protective roles include phagocytosis, cytokine production and antigen presentation [32].

There was no significant difference (P<0.05) observed in the basophils levels of the infected and the control although, there was an increase. This is in agreement with the work of Kotepui, Piwkham, PhunPhuech, Phiwklam, Chupeerach and Duangmano [31]; Eledo and Izah [32].

There was a significant reduction in the CD4 count of the infected pregnant women than the uninfected (P<0.05). This is in agreement with the work of Amadi, Eze and Chigor [10] that said that the intensity of malaria had a way of reducing the CD4 count of individuals. The reduction could be as a result of malaria infection impairing the normal functioning of the immune system. Pregnant women are susceptible to malaria during pregnancy. *P. falciparum* sequesters in the placenta. Parasitized cells in the placenta express unique variant surface antigens (VSA) and lack of immunity to these pregnancy-specific variant surface antigens explains some of the pregnancy-associated malaria susceptibility. Peripheral blood T-cell responses may be decreased in malaria and this may be due to trafficking of memory T-cells out of the circulation [13] which may also be due to the presence of other life threatening bacteria or viral infections in the study population.

The study observed that, as the intensity of malaria increased, CD4 count reduced which is in line with the work of Amadi, Eze and Chigor [10].

5. CONCLUSION

Malaria parasitaemia has a negative effect on the haematological parameter of infected pregnant women especially, in the packed cell volume which indicates anemia, the haemoblogin concentration (HB) and the CD4 count which was significantly lower (P<0.05) in the infected than the uninfected. The total while blood count (TWBC), neutrophils, lymphocytes, eosinophils and monocytes were significantly higher (p<0.05) in the infected than the uninfected. Preventive intervention measures including regular chemoprophylaxis, intermittent preventive treatment with anti-malaria, use of insecticide-treated bednets should be implemented in order to prevent the negative effects of malaria parasitaemia on the haematological parameters of pregnant women in the study population. There is need for communitybased health education to pregnant women on the need to use various WHO interventions against malaria.

CONSENT

The pregnant women also gave their consent. There is no conflict of interest amongst the authors of the work.

ETHICAL APPROVAL

The Delta State Ministry of Health and University of Port Harcourt research and ethical committee gave ethical clearance for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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