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# Plasmodiasis Among Pregnant Women In Enugu, Nigeria: Prevalence And Risk Factors

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## Abstract

Malaria is a major public health problem in endemic areas especially during pregnancy. The aim of the study was to determine the prevalence and risk factors of plasmodiasis among pregnant women attending antenatal care in Enugu State, Nigeria. Pregnant women are vulnerable to malaria infection. Information on their infection status will go a long way in determining the effectiveness of some of the interventions put in place for the control.

**Methods:** This is a cross sectional study conducted from January to September 2018. Five hundred and forty eight pregnant women who gave their consent were included in the study. For each participant, demographic was obtained using structured questionnaire. Blood samples were collected; thick and thin films were made and stained with Giemsa stain using parasitological standard procedures. The blood films were examined using oil immersion objective. Hemoglobin concentration was measured. Data was analyzed using GraphPad Prism version 7.0.

**Result**:  Two hundred and ninety-four were found to be positive for parasitaemia giving a prevalence of 53.7%. Pregnant women between the ages of 20-24 (78.7 %, 635 parasites/ ul) had the highest prevalence and the least was 40-44 (28.6 %, 355parasites/ ul). Demographic variables that significantly associated with increased risk of malaria infections were age (OD= 1.61, 95% C.I. 1.5-2.5), level of education (OD=16.8, 95% C.I. 6.030-47.01), area of residence (OD= 5.6 95% C.I. 3.72-8.61), and gravidity (OD=8.0 95% C.I. 4.97-12.96). Non-use of insecticide treated nets (ITNs), insecticide sprays and IPTp-SP were associated with increased risk of plasmodiasis.

**Conclusion**: Malaria has continued to be a serious challenge for pregnant women in Enugu due to inadequate environmental conditions, low level of education and non-conformity with the usage of prophylactic measures. These available control measures should be re-evaluated. Aggressive campaign for malaria preventive measures is crucial and early antenatal booking will ultimately lower malaria prevalence.

## INTRODUCTION

Malaria during pregnancy is a very serious public health challenge and an important risk to the mother, her unborn child and her newborn. [1] Malaria is caused by protozoan parasite of the genus Plasmodium inoculated into human red blood cells by female anopheles mosquitoes. These five human plasmodium species include Plasmodium falciparium, Plasmodium vivax, P. ovale, P. malariae, and P. knowlesi. [2,3] In 2017, in WHO regions of Africa and South-East Asia, P. falciparum accounted for 99.7% and 62.8% respectively. In WHO regions of America, P vivax

accounted for 74.1% of malaria cases. [3] According to the latest world malaria report, in 2017, there were 219 million cases of malaria, differing from 217 million cases in 2016. In 2017, there were 435,000 estimated malaria deaths which were almost comparable to the previous year. High share of the global malaria burden has continued to be borne by WHO African Region. In 2017 universally, five countries were reported to have carried almost half of malaria cases. Nigeria was highest with 25%.  Pregnant women are at risk due to reduced immunity during pregnancy [4].

Malaria during pregnancy, especially in regions of stable

malaria transmission is asymptomatic. There is the growth of an efficacious anti-disease immunity to malaria as a consequence of regular exposure to the bite of female anopheles mosquitoes. This halts the life-threatening parasite burdens thereby subduing the pro-inflammatory response that cause illness. This semi-immunity acquired is capable of keeping the infection at an asymptomatic level during pregnancy in the majority of cases. [2] These asymptomatic cases constitute an important percentage of infection reservoirs. [3] It is reported that 1-50% of pregnant women may transmit malaria parasites predominantly in the placenta unnoticed, depending on the endemicity of the malaria in the area. [2] The unpleasant outcome of plasmodiasis in pregnancy includes maternal anemia, deaths, premature delivery, low birth weight and pregnancy loss. [3] Aftermath of malaria in pregnancy on the infant include, attitudinal behaviors, stunted growth and neurological deficiency. [2,5]

Malaria control has continued to face a serious challenge in Africa. [4] Use of antimalaria chemophylaxis and the use of insecticide treated nets (ITNs) have been the main bedrock of malaria prevention. [2] Pregnant women on antimalaria chemoprophylaxis are at reduced risk of adverse effect of plasmodiasis. Use of insecticide treated nets reduce human-mosquitoes contact and thereby decrease the incidence of malaria with its consequences. [6,7] The World Health Organization (WHO) put forward a whole package of interventions to prevent malaria infections in pregnancy. These include use of long-lasting insecticides nets (LLINs) and administration of intermittent preventive treatment at each routine antenatal visit beginning from second trimester of pregnancy. [8] In 2005, Nigeria adopted the WHO-IPTp-SP strategy (intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine = IPTp-SP) of providing IPTp with SP to replace weekly prophylaxis. [9] IPTp-sp is meant to be given at each routine antenatal care visit, beginning as early as possible in the second trimester. [10] Effort to deliver IPTp-SP services during antenatal visits has not been successful or impactful. [11]

The world Malaria report of 2017 outlined a lot of challenges being face in the control of malaria infection. These include reasonable and expected international and domestic funding, risks constituted by conflict in malaria endemic zones, abnormal climate changes, and the emergence of parasite resistance to antimalarial medicines and resistance of mosquito to insecticide sprays. World health Organization is presently rendering assistance to malaria emergency responses in Nigeria especially in zones where ongoing humanitarian crises constituted serious health challenge. [12] However, malaria infection is a risk for 97% of Nigerian of which less than 5year old children and pregnant women are most vulnerable. [13] There are different diagnostic methods that may be used to detect malaria parasites. These include microscopy, Rapid diagnostic Tests (RTD), and polymerase chain reaction (PCR). Microscopy remains the gold standard in the diagnosis of malaria; it is cheap, reliable and allows for quantification of malaria parasite. PCR is more sensitive especially when parasitaemia is low. At present, molecular diagnostic tools do not have any role in the clinical management of malaria. [3]. Malaria infection is therefore a preventable and treatable mosquito-borne illness. It has been advocated that all suspected cases of malaria be confirmed by microscopy or Rapid Diagnostic Test (RTD) before commencement of treatment. [14]

## MATERIALS AND METHODS

*Study Design and subjects*

This was set up as a cross-sectional study on 548 randomly selected pregnant attending antenatal in two big health institutions, University of Nigeria Teaching Hospital, Ituku Ozalla and Balm of Gilead Hospital, Mary land Enugu. A well structured questionnaire was used to generate information on socio-demographic data and knowledge and use of preventive measures against malarial infection.

*Sample Size*

The method used by Fana et al was adopted to calculate the sample size [2]. The malaria prevalence of 58.4% from previous study was used at a 95% confidence interval (CI) and a 5% margin of error. [15] A sample of 374 was required. A total of 548 were enrolled because of high turnout of participants.

*Inclusion Criteria*

Pregnant women at any gestational age who resided within the study area and who gave their consent were included in the study. Each person was recruited once.

*Exclusion Criteria*

Women with chronic debilitating disease such as human immunodeficiency virus (HIV), malnutrition were excluded from the study.

*Data Collection*

The purpose and objective of the study were explained to the participants and those who gave their consents were given forms to sign. Those that signed were given well structured questionnaire to fill. Well trained Scientists helped them to fill the questionnaires.

*Ethical Clearance*

This work was approved by Ethics Committee of University of Nigeria, NHREC/05/10/2008B-FWA000024-IRB00002323

*Sample Collection and Preparation*

Samples were collected by a well-trained phlebotomist. About 2ml of venous blood was collected aseptically into ethylenediamine tetra-acetic acid containers. Thick and thin films were made on clean, grease-free slides

*Laboratory Analysis*

Thick and thin films were used to identify the parasites using Giemsa techniques as recommended by WHO [16] while hemoglobin was determined using methemoglobin method as described by Baker et al., [17] Thick and thin films were made on the same slide. For the thick film, 3 drops of blood were placed at one end of a clean, grease-free microscopic slide and spread over a diameter of 15mm, while a drop of blood was used to make the thin film and were appropriately labeled. The thin film end was fixed with absolute methanol for 2 seconds. Both films were allowed to air-dry and subsequently flooded with 3% Giemsa stain for 45 minutes and then washed and allowed to dry. Microscopic examinations of the stained slides were done using oil immersion objective lens ($\times$ 100)

*Malaria Parasite Density*

Method approved by World Health Organization on Malaria Microscopy procedure was used. The malaria parasites and white blood cells were counted with two tally counters. The parasite density was calculated using estimated average white cell count of 800/µl.

## STATISTICAL ANALYSIS

All statistical analyses were done using GraphPad Prism version 7.0. Chi-square test (x2) and Fisher's exact test were used to test for significant association between malaria parasitaemia and various demographic characteristics of the sampled population at 95% confidence interval. P-value < 0.05 was considered statistically significant.

## RESULTS

The baseline demographic characteristics of the pregnant women were summarized in table 1. A total of 548 pregnant women who gave their consent participated in the study. The mean age of the participants was 29.3 years, (range 20-44) most of them had secondary education and above (88%), and 67.5% lived in urban areas. There were 59.5% pregnant women who used insecticide treated net, 70.1% used insecticide sprays. Of the 548 pregnant women, 28.8% were primigravidae, 37.2% secondigravidae, and 33.9% were multigravidae, 25.9% were in their first trimester, 45.6% and 28.5% were in their second and third trimester respectively. There were 52.9% that used IPT-SP while 47.1% did not use.

Plasmodium falciparum was the only type of Plasmodium species seen and identified. Of the 548 pregnant women sampled 294 yielded positive malaria parasitaemia giving a prevalence of 53.7%. Table 2 shows the prevalence of malaria parasitaemia and mean malaria density, the highest malaria prevalence was found in age group 20-24 (78.7%) and the least was 40-44 (28.6%) with mean parasite density of 635µl and 355µl respectively and age was found to be significantly associated with malaria prevalence (P<0.0001). level of education, place of residence, gravidity, gestational age, use of ITN, use of insecticide sprays, and use of IPT-SP were all significantly associated with prevalence of malaria parasitaemia ( P<0.0001).

Table 3 shows the odd ratio of malaria infection to demographic variables. Demographic variables that significantly associated with increased risk of malaria infections were age (OD= 1.61, 95% C.I. 1.5-2.5), level of education (OD=16.8, 95% C.I. 6.030-47.01), area of residence (OD= 5.6 95% C.I. 3.72-8.61), and gravidity (OD=8.0 95% C.I. 4.97-12.96)

Table 4 displays the odd ratio of malaria infection to malaria preventive measures. Control measures that greatly associated with reduced risk of malaria infections were uses of insecticide treated nets (OD= 0.006, 95% C.I. 0.0020-0.0155), use of insecticide spray (OD= 0.102, 95% C.I. 0.0620-0.1663), and the use of IPTp-SP (OD= 0.008 95% C.I. 0.004-0.154)

The mean hemoglobin (Hb) of infected and non-infected pregnant women was shown in table 5. Uninfected pregnant women recorded higher Hb, with mean value of 12.52g/dl while infected pregnant women recorded lower value, with mean value 10.25g/dl.

Statistically, unpaired T-test showed a significant difference in Hb of infected and non-infected pregnant women (P< 0.0001).

**Table 1**

Demographic characteristics of the study population

| Demographic characteristics | Number (%) |
|---|---|
| **Age group** | |
| 20 – 24 | 94 (17.15) |
| 25 – 29 | 204 (37.23) |
| 30 – 34 | 166 (30.29) |
| 35 – 39 | 70 (12.77) |
| 40 – 44 | 14 (2.55) |
| **Level of education** | |
| Primary | 66 (12.04) |
| Secondary | 298 (54.38) |
| Tertiary | 184 (33.58) |
| **Use of ITNs** | |
| Yes | 326 (59.49) |
| No | 222 ( 40.51) |
| **Area of Residence** | |
| Urban | 370 (67.52) |
| Rural | 178 (32.48) |
| **Use of insecticide sprays** | |
| Yes | 384 (70.07) |
| No | 164 (29.93) |
| **Gravidity** | |
| Primigravidae | 158 (28.83) |
| Secundigravidae | 204 (37.23) |
| Multigravidae | 186 (33.94) |
| **Gestational age** | |
| First trimester | 142 (25.91) |
| Second trimester | 250 (45.62) |
| Third trimester | 156 (28.47) |
| **Use of IPTp-SP** | |
| Yes | 290 (52.92) |
| No | 258 (47.08) |

**Table 2**

Prevalence of malaria parasitaemia and mean parasite density of pregnant women with respect to age, education level, use of ITNS, use of insecticide sprays, area of residence, gravidity, gestational age and use of IPT-SP

| | Number examined | Number infected | % Infected | Mean parasite density (MPD) | p-value | Chi-square ($X^2$) |
|---|---|---|---|---|---|---|
| **Age (years)** | | | | | | |
| 20 – 24 | 94 | 74 | 78.72% | 635 | <0.0001 | 32.3 |
| 25 – 29 | 204 | 102 | 50% | 491 | | |
| 30 – 34 | 166 | 84 | 50.60% | 590 | | |
| 35 – 39 | 70 | 30 | 42.86% | 458 | | |
| 40 – 44 | 14 | 4 | 28.57% | 355 | | |
| **Education Level** | | | | | | |
| Primary | 66 | 62 | 93.94% | 688 | <0.0001 | 154.2 |
| Secondary | 298 | 198 | 64.44% | 562 | | |
| Tertiary | 184 | 34 | 18.49%s | 492 | | |
| **Use of ITNs** | | | | | | |
| YES | 386 | 76 | 23.31% | 387 | <0.0001 | |
| NO | 222 | 218 | 98.20% | 585 | | |
| **Use of Insecticide sprays** | | | | | | |
| YES | 384 | 152 | 39.58% | 495 | <0.0001 | |
| NO | 164 | 142 | 86.59% | 644 | | |
| **Area of Residence** | | | | | | |
| Urban | 370 | 152 | 41.08% | 470 | <0.0001 | |
| Rural | 178 | 142 | 79.78% | 617 | | |
| **Gravidity** | | | | | | |
| Primigravidae | 158 | 134 | 84.81% | 612 | <0.0001 | 87.46 |
| Secundigravidae | 204 | 88 | 43.14% | 513 | | |
| Multigravidae | 186 | 72 | 38.71% | 486 | | |
| **Gestational age** | | | | | | |
| 1st trimester | 142 | 94 | 66.20% | 530 | <0.0001 | 21.36 |
| 2nd trimester | 250 | 138 | 55.20% | 492 | | |
| 3rd trimester | 156 | 62 | 39.74% | 310 | | |
| **Use of IPT-SP** | | | | | | |
| YES | 290 | 46 | 15.86% | 400 | <0.0001 | |
| NO | 258 | 248 | 96.12% | 517 | | |

Note – Mean parasite density (MPD) = Number of parasites/μl of blood

**Table 3**

Risk ratio of demographic variables and malaria parasitaemia in pregnant women attending antenatal clinics in Enugu, Nigeria

| Demographic Characteristics | Number examined | Number infected | Odd Ratio (95% C.I.) | P-value |
|---|---|---|---|---|
| **Age** | | | | |
| ≤ 29 | 298 | 176 | 1.61(1.15-2.27 | <0.0057 |
| > 29 | 250 | 118 | | |
| **Level of Education** Primary | | | | |
| > Primary | 66 | 62 | 16.8(6.030-47.01) | < 0.0001 |
| | 484 | 232 | | |
| **Residence** | | | | |
| Rural | 178 | 142 | 5.6(3.72-8.61) | < 0.0001 |
| Urban | 370 | 152 | | |
| **Gravidity** | | | | |
| Primigravidae | 158 | 134 | 8.0(4.97-12.96) | < 0.0001 |
| > Multigravidae | 390 | 160 | | |

**Table 4**

Risk ratio of methods of prevention and malaria parasitaemia in pregnant women attending antenatal in Enugu, Nigeria

| Preventive Measures | Number examined | Number infected | Odd ratio (95% C.I.) | P-value |
|---|---|---|---|---|
| **Use of ITNs** | | | | |
| Yes | 326 | 76 | 0.006(0.0020-0.0155) | <0.0001 |
| No | 222 | 218 | | |
| **Use of Insecticide Spray** | | | | |
| Yes | 384 | 152 | | |
| No | 164 | 142 | 0.102(0.0620-0.1663) | < 0.0001 |
| **Use o IPT-SP** | | | | |
| Yes | 290 | 46 | 0.0076(0.0038-0.154) | < 0.0001 |
| No | 258 | 248 | | |

**Table 5**

Mean hb of infected and non-infected pregnant women

| Malaria parasitaemia | Mean Hb ± S.D |
|---|---|
| Infected | 10.25 ± 0.8361 |
| Uninfected | 12.52 ± 1.1423 |
| P-value | <0.0001 |

## DISCUSSION

Pregnant women in malaria endemic areas, especially in sub-Saharan African are challenged by high cases of Plasmodium falciparum with increased rate of maternal morbidity. P. falciparum was the only species of plasmodium encountered in this study and this is in line with the work of many researchers. [18] It was likewise reported that P. falciparum is the most prevalent species on the African continent responsible for most malaria-related deaths globally. [14]

The prevalence of malaria parasitaemia in this study was 53.7% and this is in contrast to the low prevalence reported by in Lagos, Jos, Kaduna, in North Central Nigeria and in Malawi [6,18,19,20,21]. Previous work done in Enugu in 2009 reported a prevalence of 58.4%. [15] The prevalence here was also found to be lower than what was reported in Calabar 70% and Gombe 92%, all in Nigeria. [22, 23] The variations in prevalence may be due to differences in the study location. The high prevalence of 53.7% obtained may be due to the environmental condition in Enugu where there are a lot of stagnant water available thereby providing breeding places for mosquitoes. This therefore encourages increased contact between mosquito and human. [18] It has also been reported that climatic change may change transmission patterns and the malaria burden, giving rise conditions that favor vector breeding and thereby increasing the risk for malaria infection [3]

There was a major association between age and prevalence of malaria parasitaemia. Pregnant women of younger age were found to be at higher risk with highest parasite densities. These findings have also been reported by other researchers where malaria prevalence decreased with increasing age. [2, 22 ,24]. In this study, gravidity was significantly associated with malaria prevalence and this agrees with the work of many researchers. [2,18,24,25] but at variance with the study done in Lagos. [6] Pregnancy has always been accompanied by lowered immunity that may cause loss of acquired immunity to malaria especially in primagravida. [2, 10] The lower prevalence in multigravidas has been attributed to the possession of specific immunity to placental malaria as a result of previous exposure to malaria infection. This acquired specific immunity is built with previous infection and subsequent pregnancies. [2, 10, 26]

Results obtained from this study showed that area of residence significantly associated with malaria prevalence, the prevalence was higher in women that live in rural areas. This was in consonance with what was reported in Kaduna and can be attributed to poor environmental condition that encourages the breeding of mosquitoes in rural areas. [25]

Gestational ages were statistically significant as women in their first trimester had the highest prevalence. This agrees with previous studies were higher frequencies had reported in first trimester than second and third trimesters. [18, 26] This may be as a result of intermittent preventive treatment

IPTp-SP) given to pregnant women which usually starts at the second trimester. [10,18]

Education level showed a significant association with the prevalence of malaria. All women with primary education tested positive to malaria parasitaemia while lower prevalence was observed in women with secondary and tertiary education. Malaria parasitaemia therefore decreased with increasing education level. This agrees with work of many researchers in and outside Nigeria. [2, 24, 25, 26] However, previous work done in Lagos showed that education was not a significant risk factor [ 6]. The high prevalence rate in women with primary education is probably due to lack of basic knowledge in the dangers of malaria, their life style and poor environmental conditions. The importance of education in the control of malaria cannot be over-emphasized. [2]

The use of LLIN was found to be significantly associated with malaria parasitaemia in this study, as the rate increased with decrease or non use of LLINs. LLINs effectively reduce human-mosquito contact thus reducing malaria transmission among users. Similar results were recorded by many researchers. [6,25] As this has been found to be important risk factor associated with P. falciparum malaria, it is important to sustain the distribution of ITNs and ensure compliance. Use of insecticide sprays also accounted for a decrease in the prevalence of malaria infection in this study and this is in consonance with work carried out in Lagos. [6]. Non-use of ITNs and insecticide sprays increased the risk of malaria infection in pregnant women.

Our study also shows that the use of IPT-SP conferred a strong protection from malaria in pregnant women, with prevalence of 15.9% in IPT-SP users and 96.1% in non-users. Similar result was reported by some other researchers. [8, 25] Since the use of IPT-SP has been shown to play a major role in prevalence of malaria parasitaemia, women should be encouraged to consistently use this preventive measure once antenatal visit commences and ensure compliance. However, from the study, it was observed that a percentage of women who used the intermittent preventive therapy were still infected. This could be due to environmental factors or resistance to the drugs hence further research should be done to develop more potent therapy. Combined use of preventive measures such as insecticide sprays, LLINs and IPT-SP should proffer a stronger resistance to malaria infection during pregnancy. WHO strongly advocate a policy of test, treat and track to improve the quality of care and surveillance and this should

be sustained and enforced. [3]

## CONCLUSIONS

Malaria prevalence in this study was high. Malaria infection has continued to be a serious challenge to pregnant women in Enugu due to poor environmental conditions, low level of education, and non compliance to the use of preventive measures. It is therefore pertinent to monitor these measures and embark on aggressive campaign to enlighten the public especially the vulnerable group like the pregnant women to see the need to adhere to these measures. There is therefore need to strategize and scale up these preventive measures. Early attendance to antenatal is of paramount importance as this will help to reduce the risk of malaria in pregnancy. Seminars can be held to this effect to enlighten women on its importance and dangers associated with not taking the therapy.

## STUDY LIMITATIONS

Notwithstanding that the study has some crucial findings, it also has some limitations. The study relied on self reported measures which may have been inaccurate. The use of microscopy may have underestimated or over-estimated the prevalence of malaria. Hence, the use of molecular studies such as PCR would give a more sensitive and specific result. The study used only ITNs, IPTp-SP, Insecticide sprays and did not assess other measures such as indoor residual sprays, mosquito repellent coils and untreated nets. The study is a cross-sectional and you cannot draw any conclusion about causality between risk factors and the outcome because both are being measured at the same time and so cannot actually tell which one of them come first. They are also prone to selection bias because it was only those who gave their consent that participated.

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