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Asymptomatic *Plasmodium* parasitaemia in pregnant Nigerian women: almost a decade after Roll Back Malaria

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Summary Malaria during pregnancy is a major cause of fetal and maternal morbidity and mortality. In malaria-endemic areas, the condition may remain asymptomatic but is still associated with complications. The objective of this study was to determine the prevalence of asymptomatic malaria parasitaemia and its relationship with various sociodemographic characteristics. The study was performed at three hospitals in Enugu, the centre of southeast Nigeria, during the rainy season between March 2006 and October 2007. Pregnant women attending the antenatal clinic at the index pregnancy were randomly selected and counseled, and peripheral blood samples were collected for malaria parasite and packed cell volume estimation. Age, parity, gestational age at booking, degree of anaemia and parasite density were recorded. Of 125 pregnant women tested, 73 had microscopic *Plasmodium* parasitaemia, giving a prevalence of 58.4%. Asymptomatic malaria parasitaemia was more common in primigravidae, in the second trimester and in the younger age group. Anaemia in pregnancy was prevalent (55.2%) and there was no significant difference in the density of parasitaemia in those with mild, moderate and severe anaemia. The prevalence of *Plasmodium* parasitaemia in pregnant Nigerian women is still very high nearly a decade after Roll Back Malaria. It is therefore pertinent to reappraise Roll Back Malaria strategies or to design a more effective programme for the prevention and treatment of malaria in pregnancy.

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1. Introduction

Malaria in pregnancy is a major public health problem in tropical and subtropical regions of the world. In Africa, millions of women living in malaria-endemic areas become pregnant each year and most live in areas of relatively stable malaria transmission. Other susceptible groups include children below the age of 5 years, sickle cell anaemia patients, non-immune visitors to endemic areas and immunocompromised persons. Poverty, ignorance and malnutrition also contribute to the enormous burden of malaria in pregnancy. Maternal and perinatal morbidity and mortality are high, especially in neglected situations.[1,2] The main burden results from infection with *Plasmodium falciparum*. The impact of the other three human species (*P. vivax*, *P. malariae* and *P. ovale*) is less well understood.

The susceptibility of pregnant women to malaria parasites is well established.[3,4] It was previously thought that this is related to depression of the cell-mediated immune response to *P. falciparum* antigens.[5] Recently susceptibility has been linked to the level of antibodies to placental sequestrated parasites.[6] This is more marked in the first 24 weeks than during the third trimester.[7] Some researchers have also suggested that susceptibility to parasite sequestration may be linked to the vascular nature of the placenta.[8] Indeed, it has been shown that a subpopulation of *P. falciparum*-infected erythrocytes that are functionally distinct adhere to chondroitin sulphate-A (CSA) receptors expressed by syncytiotrophoblasts in the placenta.[9] Primigravidae and women at their second pregnancy are more susceptible, as anti-adhesion antibodies against CSA-binding parasites associated with protection only develop after successive pregnancies.[10]

The presence of parasites in peripheral blood without symptoms is common in hyperendemic areas and is associated with chronic anaemia and placental sequestration. Furthermore, the absence of parasites in peripheral blood does not exclude complications since it has been shown that up to 50% of women with placental parasitisation do not have peripheral parasitaemia.[11] It has also been shown that peripheral parasites in an immune individual express the same variant-specific antigen as placental parasites, an important finding that will assist in malaria vaccine research.[12]

In Nigeria, malaria is still a leading cause of anaemia in pregnancy despite the global partnership initiative aimed at prevention and treatment. It is thus doubtful whether these measures have been appropriately applied. Very few studies have actually evaluated the magnitude of problems faced by these asymptomatic cases. This underscores the need to evaluate the prevalence and some sociodemographic characteristics as a prelude to an in-depth research into intervention strategies.

2. Patients and methods

2.1. Study population

The study took place in Enugu, the centre of southeast Nigeria, during the rainy season between March 2006 and October 2007. The town is located in the hilly tropical rainforest approximately 230 m above sea level. The average annual temperature is between 23.1 °C and 31 °C with a rainfall of 1520—2030 mm. There are two major seasons, the rainy season (April—October) and the dry season (November—March). The area has a mixed rural and urban population with the majority being Igbos, with a projected population of 3.2 million of which 52.1% are female. The area is hyperendemic and malaria is predominantly caused by *P. falciparum*. Most of the drainage system has been blocked by indiscriminate refuse disposal. The study was carried out in three hospitals: two private hospitals in the heavily populated outskirts of Enugu (Abakpa and Emene), which provide antenatal services, delivery, postnatal care, emergency obstetrics and family planning services; and the University of Nigeria Teaching Hospital (UNTH), which has the largest medical facility in southeast Nigeria.

2.2. Study design

This was a multicentre, cross-sectional study. After obtaining ethical clearance from the relevant body, apparently healthy pregnant women attending antenatal care (ANC) who were on at least one form of malaria prophylaxis were counselled, oral informed consent was obtained and they were consecutively recruited. All the women were using insecticide-treated nets (ITN) and were given intermittent preventive treatment (IPT). We tried to find out from the women whether and how they treated the nets and also how often and for how long, as well as what type of bed they used and the time and hours spent inside the nets.

Patients with symptoms indicating malaria, for example fever, headache, weakness, anorexia and joint/muscle pains, were excluded from the study. Subjects who had not taken any form of antimalarial drugs in the index pregnancy and who did not sleep under ITNs were also excluded. Other exclusions included HIV-positive patients and patients with sickle cell disease, diabetes mellitus, hypertension and any other diagnosed medical condition in pregnancy in order to exclude the possible effect of these variables on the body's response to the presence of malaria parasites. The socioeconomic status of the women was either low or middle class.

Personal history, history of present pregnancy, past obstetric history, past medical history, family and social history, and review of systems was obtained. Gestational age was assessed from the last normal menstrual period. The expected date of delivery was calculated using Naegele's Rule, which is a pregnancy due date calculator named after a German obstetrician who devised the rule. It calculates approximately the expected date of delivery from the first day of the woman's last menstrual period and is done by totalling a year, deducting 3 months from that year and adding 7 days. Trimester was defined as first trimester (<14 weeks), second trimester (14—27 weeks) and third trimester (>27 weeks). Five subjects were recruited in the first trimester, 70 in the second trimester and 50 in the third trimester. Young age was defined as $\leq$30 years and old age as >30 years.

Medical and obstetric examinations were performed. The axillary temperature was taken to exclude fever, and temperature <37.5 °C was considered normal. Antenatal

laboratory investigations as performed at UNTH (including genotype, screening for syphilis, hepatitis B and HIV) were normal. HIV screening is performed for all pregnant women at UNTH.

2.3. Laboratory methods

Peripheral blood samples were collected using sterile needles and syringes after cleaning the volar surface of the arm with cotton wool moistened with methylated spirit. Thin and thick blood films were made from each of these samples, stained with Giemsa and then examined under a microscope using a ×100 objective lens in each case. Identification of species was done using the thin blood film. Parasite density was estimated on the thick film under oil immersion and viewed using the ×100 objective lens. Determination was done by counting the number of asexual forms of *P. falciparum* parasites against at least 100 leukocytes, or 200 leukocytes for the definitive count. The number of asexual parasites was calculated using the following formula:

$$\text{Parasites}/\mu\text{l} = \frac{\text{no. of asexual parasites} \times 8000 \text{ leukocytes}}{200 \text{ leukocytes}}$$

The degree of parasitaemia was graded thus: 1–999/μl, mild; 1000–9999/μl, moderate; and >10 000/μl, severe.

A negative result was recorded after thorough examination of 100 fields without any parasite.

Quality control was ensured by using freshly reconstituted and filtered Giemsa stains. The microscopist was very experienced and spent an average of 15 min to 1 h on each thick and thin film, respectively. Comparison was made both with known positive and negative thin films.

Samples were also estimated for packed cell volume (PCV) using the microhaematocrit centrifugation method. Mild anaemia was taken as PCV of 27–30%, moderate anaemia as PCV of 19–26% and severe anaemia as PCV of <19%. This classification is based on the definition of anaemia in pregnancy in the study environment, which is a PCV of <30%.[13] All patients with malaria parasites and anaemia were promptly and adequately treated free of charge.

2.4. Statistical analysis

Statistical analysis was done using SPSS version 11 (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to compare proportions within groups. *P*-values of ≤0.05 were considered significant.

3. Results

Of the 125 pregnant women examined, 73 had microscopic *Plasmodium* parasitaemia, giving a prevalence of 58.4%; 69 women had anaemia, giving a prevalence of 55.2%. Among the anaemic women, 43 (62.3%) were mildly anaemic, 24 (34.8%) were moderately anaemic and 2 (2.9%) were severely anaemic. It was also noted that the majority of the women who use an ITN purchased it from the open market and did not use it properly. All antimalarial prophylaxis was taken at home unsupervised. The commonest occurrence of parasitaemia was in the second trimester, where 61 (87.1%) of the 70 women tested positive. In the third trimester 11 (22%) of the 50 women tested positive, whilst the lowest level was in the first trimester, with 1 (20%) of the 5 women testing positive. These differences were statistically significant (χ^2 84.932; $P < 0.001$). *Plasmodium* parasitaemia was commonest in primigravidae, where 32 (65.3%) of 49 women were positive, followed by multigravidae with 39 (55.7%) of 70 women. The lowest occurrence was in grand multigravidae with 2 (33.3%) of 6 women testing positive. These relationships were statistically significant (χ^2 31.753; $P < 0.001$).

The prevalence of malaria parasites was higher in younger (65.7%; 46/70) than older pregnant women (49.1%; 27/55). This relationship was also statistically significant (χ^2 4.945; $P = 0.026$).

Of the 69 anaemic women, 41 (59.4%) had parasitaemia [22 (31.9%) mild, 17 (24.6%) moderate and 2 (2.9%) severe]. Moderately anaemic pregnant women had the highest prevalence of malaria parasitaemia (79.2%), followed by severely anaemic (50%) and then mildly anaemic (48.8%). However, these values were not statistically significant (χ^2 1.275, Spearman's correlation 0.044, likelihood ratio 1.655 at 95% CI; $P = 0.866$). This is illustrated in Table 1.

4. Discussion

Roll Back Malaria, launched in 1998, aimed to halve the world malaria burden by the year 2010. Africa contributes over 90%[14] of the world malaria burden with an estimated 1 million infant deaths annually[15], thus the African heads of state endorsed the Roll Back Malaria initiative at a summit in Abuja, Nigeria, in 2000.[16]

This study examined the prevalence of *Plasmodium* parasitaemia among pregnant Nigerian women nearly a decade after launching this global partnership initiative with less than 4 years to the targeted reference point of 2010. The study showed that the prevalence of *Plasmodium*

Table 1 Relationship between grade of anaemia and degree of parasitaemia

Grade of anaemia	No. (%)					Subtotal
	Anaemic cases	Mild parasitaemia	Moderate parasitaemia	Severe parasitaemia	No parasitaemia	
Mild	43 (62)	12 (28)	8 (19)	1 (2)	22 (51)	43
Moderate	24 (35)	9 (38)	9 (38)	1 (4)	5 (21)	24
Severe	2 (3)	1 (50)	0 (0)	0 (0)	1 (50)	2
Total	69 (100)	22 (32)	17 (25)	2 (3)	28 (41)	69

parasitaemia among pregnant Nigerian women was 58.4%, similar to the findings in Libreville (Gabon) and Awka (Nigeria) in 2003 where prevalences of 57% and 63.5%, respectively, were recorded.[17,18] However, an earlier study in Lagos, Nigeria, revealed a lower prevalence of 42.2%.[19]

This study also demonstrated that the prevalence of *Plasmodium* parasitaemia was higher in primigravidae than in multigravidae and grand multigravidae, with prevalences of 65.3%, 55.7% and 33.3%, respectively. This agrees with a study performed in Lagos where prevalences of 42.2% and 33.6% were recorded in primigravidae and multigravidae, respectively.[19] It also agrees with findings from other African countries.[20–22]

It was also observed that the prevalence was significantly higher in young women than in older women, and this correlated with previous work undertaken in Gabon where parasitaemia was more common in teenagers and young women than older women.[17]

The prevalence of anaemia in pregnant Nigerian women was found to be 55.2% using 30% as the reference cut-off for PCV.[13] This was similar to previous studies done in some African countries where prevalences of >50% were recorded.[17,23] Malaria is a significant risk factor in the determination of anaemia. Women with moderate anaemia had the highest prevalence of parasitaemia, and parasite density was also highest in this group of anaemic pregnant women. However, this is different from findings in Gabon and Malawi where women with mild anaemia had the highest prevalence of parasitaemia.[17,22]

We examined women who used some form of malaria prevention and observed similar results to studies done on women attending ANC for the first time who were probably not on any prophylaxis.[17,18] Although we could not verify the authenticity of the information obtained on the patient's use of the claimed antimalarials and/or ITNs, we had no reason to doubt them as they were properly counselled. However, we discovered that most of the women purchased the ITN on the open market, were ignorant of the basic methods of utilisation and had unsupervised ingestion of IPT. Furthermore, ITNs only give protection while sleeping inside the net, with increased vulnerability to mosquito bites while in the toilet, kitchen and balcony. This cast some doubt as to the effectiveness of the implementation strategies for reduction of the adverse effects of malaria in pregnancy despite enormous resources that have been invested in the project.

Another interesting discovery is that ITNs meant for free distribution are sold on the open market. This act is facilitated by some business men and politicians who parade as non-governmental organisations (NGO), thus hijacking this life-saving initiative. The government should therefore reassess the sincerity of some of these NGOs and redirect partnership if we are to achieve our goals. Further studies should aim at comparing women who properly use ITNs (supplied in the clinic) and IPT (taken in the clinic) with new ANC cases with no proven malaria prophylaxis.

Although the findings from this study correspond very well with earlier findings, the impact of the Roll Back Malaria initiative in the study environment has been poor despite the investment of huge human and material resources. This situation should be of serious concern to policy-makers. A reappraisal of Roll Back Malaria strategies or design of a more effective programme to suit our peculiar sociocultural environment is highly recommended. ITNs should be extended to doors and windows. Other antimalarial drugs apart from sulfadoxine/pyrimethamine combination should be tried to exclude the possibility of resistance.

Authors' contributions: UIN conceived the study; all authors were involved in the design of the study; UIN was involved in clinical examination of the subjects; TUN performed the microscopy to identify malaria parasites; all authors were involved in analysis and interpretation of the data; UIN drafted the manuscript. All authors revised the article for intellectual content and read and approved the final version. UIN and VOU are guarantors of the paper.

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