Assessment of malaria in pregnancy using rapid diagnostic tests and its association with HIV infection and hematologic parameters in South-Eastern Nigeria

P. falciparum **malaria in pregnancy was evaluated using histidine-rich proteins-2 RDT and related to HIV infection and hematologic parameters. Prevalence of malaria, HIV and anemia were 19.7%, 3.1% and 17.2% respectively.** ***Primigravidae*** **were significantly more infected with malaria. Malaria was not significantly associated with anemia, blood group, genotype and HIV infection.**

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The enormous significance of malaria in pregnancy for public health makes early and accurate diagnosis of malaria absolutely imperative. Obtaining results quickly from the examination of blood samples from pregnant women with suspected malaria is now made possible by the use of rapid malaria diagnostic tests (RDTs).[1] Although their use in developing countries has been limited by their high cost, RDTs have advantages over microscopy in their diagnostic precision and potential to help reduce drug costs due to over-prescription. In this study, we evaluated malaria in pregnancy using a *P. falciparum* RDT that detects histidine-rich proteins-2 (HRP-2) and its association with maternal HIV infection and hematologic parameters. This study was conducted in Abakaliki, south-eastern Nigeria, from June 2006 to December 2006 at the Ebonyi State University Teaching Hospital (EBSUTH). Approval was obtained from the Ethical Committee of the EBSUTH. Pregnant women at full term who were admitted at EBSUTH for childbirth and who fulfilled the following study inclusion criteria were enrolled: (i) attended the antenatal clinic at EBSUTH, (ii) had an uncomplicated singleton pregnancy ≥32 weeks' gestation (based on fundal height estimation), (iii) resident in Abakaliki or neighbouring local government areas, (iv) had no obvious clinical evidence of malaria (asymptomatic), and (v) had no known underlying chronic illness. After receiving informed consent, information about participants' age and parity were obtained from the case files of each individual and by interview. Shortly before child birth, about 5 mL of the maternal peripheral blood was obtained from each participant by venepuncture technique and placed in a sterile EDTA container for laboratory analysis.

A rapid diagnostic test kit, the Smart Check Malaria *P.f* cassette (Globalemed, 1101 King St. Suite 370, Alexandria, VA 22314 USA) was used to assess malaria infection. The hemoglobin concentration (HbC) was determined using the cyanmethemoglobin method.[2] The WHO definition of anemia in pregnancy, i.e., Hb<11g/dL,[3] was adopted. The hemoglobin genotype was determined by the Hemoglobin electrophoresis technique at alkaline pH using cellulose acetate membrane (CAM).[4] The ABO blood grouping test was performed using the slide method[2] with commercially available reagents (Murex Diagnostics, Inc. Dartford, UK). The HIV Tri Line Test kits (Biosystem INC, Vienna, Austria) were first used to screen maternal serum samples and to detect antibodies to HIV-1 and HIV-2. The HIV-seropositive samples were confirmed by immunoblot analysis using the BIORAD New Lav Blot kits (Bio-Rad Novapath Diagnostic Group US, Oxnard, CA, USA). Differences between proportions were evaluated using the χ^2 tests. Statistical significance was achieved at $p<0.05$. Further data from each of the laboratory measurements could not be included for logistic reasons.

Table 1. Prevalence of malaria infection, in relation to demography, HIV infection and hematologic parameters, among pregnant women at childbirth in Abakaliki, Nigeria.

Parameter	*Number examined*	*Number (%) with malaria Infection*	*95% Confidence interval*
Age			
24	79	19 (24.1)	14.7-33.5
25-29	106	2 0(18.7)	14.0-23.4
30–35	85	16 (18.8)	14.3-23.3
≥36	40	4 (10.0)	0.7-19.3
Total	300	59 (19.7)	15.2-24.2
Parity			
Primigravidae	72	21(29.2)	23.9-34.5
Multigravidae	228	38 (16.7)	15.2-24.2
Total	300	59 (19.7)	15.4-18.3
Hemoglobin concentration HbC (g/dL)			
<11	20	6 (30.0)	9.9-50.1
≥11	150	42 (28.0)	20.8-35.2
Total	170	48 (28.2)	19.6-36.8
Blood Group			
A	41	11 (25.0)	22.5-35.5
B	34	11 (32.4)	24.8-40.0
AB	5	1 (20.0)	13.9-26.1
0	90	16 (17.8)	13.5-22.1
Total	170	39 (22.9)	22.7-35.3
Genotype			
AS	50	19 (38.0)	30.0-46.0
AA	83	33 (39.8)	31.6-48.0
Total	133	52 (39.1)	30.9-47.3
HIV Status			
Positive	8	3 (37.5)	4.0-71.0
Negative	216	56 (25.9)	20.1-31.7
Total	224	59 (26.3)	17.9-29.3

Of the 300 women screened, 59 (19.7%, 95%CI., 15.2-24.2%) were positive for malaria infection with the primigravidae significantly more infected than the multigravidae (χ^2=4.63, df =1, $p<0.05$) (Table 1). The prevalence of malaria infection was highest among women 24 years old and under (24.1%,95%CI., 14.7-33.5%). The prevalence of HIV infection was 3.1% and that of anemia (Hb<11g/dL) was 17.2%. Malaria was not significantly associated with anemia, blood group, hemoglobin genotype and HIV infection.

This study showed a malaria prevalence of 19.7%. Higher prevalence rates ranging from 34% to 52.6% had been obtained in previous studies using RDTs.[5,6] Variations in transmission intensity, access to treatment, coverage and quality of antenatal services, and drug resistance may have accounted for the differences in the prevalence of maternal malaria.

Younger women appeared to be more susceptible, but age was not significantly associated with malaria. A number of reports have indicated a significant association between malaria during pregnancy and maternal age[7,8] while others did not.[9,10] The reason for these age-related differences in malaria prevalence is probably

related to host or environmental factors and requires further investigation. *Primigravidae* were significantly more likey to be infected with malaria than *multigravidae*. This was in general agreement with findings from studies conducted in other malarious areas indicating that gravidity and premunition influence susceptibility to malaria infection with the parasite are significantly higher in *primigravidae* than the *multigravidae*.[7,8]

Interestingly, malaria was not significantly associated with anemia and HIV infection. Although malaria is a major contributor of anemia among pregnant women in malarious areas, the etiology of maternal anemia is complex and multi-factorial,[3] and other factors could also be responsible for anemia in pregnancy. Our result showed that the prevalence of malaria was higher among the HIV-positive than the HIV-negative women in agreement with an earlier report.[10] However, the difference is not significant and since the numbers are very small, one less malaria infected HIV positive women would have removed any apparent difference between the groups. Furthermore, the mechanisms of the association between malaria and maternal HIV infection are potentially numerous and many aspects are still not fully understood. Further studies using molecular tools should be carried out.

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Key words: malaria, pregnancy, RDT, HIV, hematologic parameters.

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