ORIGINAL ARTICLE

Asymptomatic malaria parasitaemia using rapid diagnostic test in unbooked pregnant women in rural Ondo-south district, Nigeria

D.U. NWANERI, O.A. ADELEYE[*], A.B. ANDE[**]
Institute of Child Health, [*] Department of Community Health, [**] Department of Obstetrics and Gynaecology, University of Benin, Benin City, Edo State, Nigeria

Key words

Booking • Insecticide-treated • Malaria • Net • Pregnancy

Summary

Background. *Malaria is a major contributor of maternal and peri-natal morbidity and mortality. The disease may be asymptomatic despite sequestration of parasitized red blood cells in the placental micro-circulation with antecedent complications. In such condition, it may also be difficult to identify the malaria parasite by the peripheral blood film microscopy, thus the need for use of simple but reliable tool for malaria parasite diagnosis.*
Objective and method. *To determine the prevalence of asymptomatic malaria parasitaemia using the Rapid Diagnostic Test in pregnant unbooked women seen in a primary health centre during a malaria control campaign programme in rural Ondo-south, District Nigeria.*
Results. *Prevalence of asymptomatic malaria parasitaemia was 25.9%. Only 3 (3.5%) of the 85 women had the long lasting insecticide-treated nets. There was no significant association between malaria parasitaemia, and the age group, parity and gestation age.*
Conclusion. *Given the high prevalence of asymptomatic malaria in pregnancy, routine screening for malaria at booking and scaling-up of other malaria control strategies such as the use of long lasting insecticidal-treated nets and intermittent preventive therapy for pregnant women are recommended.*

Introduction

Malaria poses enormous public health burden worldwide [1]. Pregnant women and children under the age of 5 years are at risk of severe disease and mortality. The burden of malaria in pregnancy in sub-Saharan Africa is caused mainly by *Plasmodium falciparum* [1]. In malaria holo-endemic areas, malaria in pregnancy may remain asymptomatic despite sequestration of parasitized red blood cells in the placental micro-circulation with antecedent complications [2]. These complications include chronic anaemia, post-partum haemorrhage and death. The impact on the foetus is usually from maternal infection of the placenta and maternal anaemia resulting to low birth weight, prematurity, still birth, abortions, intra-uterine growth restriction, as well as congenital malaria [1, 2].
Prevalence rates for malaria parasitaemia in pregnancy (using microscopy) in hospital-based surveys in different parts of Nigeria included 3.1% in Sokoto in the north-west [3], 9.0% in Jos in the north-central [4] 27.4% in Lagos in the south-west [5] and 39.1% in Awka in the south-east [6].
Most of the available data were from urban areas, whereas most of Nigeria is rural [7]. This anomaly partly derives from the difficulty in conducting microscopy in rural areas because microscopists are few and power supply is erratic. However, Rapid Diagnostic Test (RDT), now recommended by the World Health Organization (WHO) for diagnostic purposes where microscopy is unavailable [8, 9] is not yet widely used in surveys in Nigeria.
The objective of this study therefore was to determine the prevalence of asymptomatic malaria parasitaemia in pregnant unbooked women in a rural area using the RDT. The findings from this study will add to the body of knowledge of asymptomatic malaria parasitaemia in pregnant women living in rural areas as well as give information on the usefulness of RDT for malaria diagnosis.

Materials and methods

This was a cross sectional descriptive study and was carried out in June 2011. The study site was Primary Health Care (PHC), Odo-Aiye; a rural community in Okitipupa Local Government Area (LGA) located in Ondo-south District, south-west Nigeria. The LGA lies within the equatorial evergreen rain forest of Nigeria where malaria is stable with no seasonal variation [1]. The major occupations of the inhabitants are farming, timber felling and lumbering [7].
Ethical clearance was obtained from the Ethics and Research Committee, University of Benin Teaching Hospital, Benin City, Nigeria. Verbal permission to conduct the study in the PHC was obtained from the Director

of the PHC. The written informed consent was read, interpreted and explained in the local dialect by trained research assistance to the participants; following which each participant then gave a verbal consent before being recruited in the study.

Recruitment of subjects was during the campaign for malaria control at the PHC, Odo-Aiye. Participants for this study included all pregnant women who were in attendance during the malaria campaign program. Pregnant women who though were present during the program but had already booked at the PHC or in any other organized health facility were excluded from this study. Pregnant women who had fever or who received antimalaria drugs in the preceeding two weeks to the study and those who were on intermittent preventive treatment (IPT) were also excluded from the final analysis of the study. Fever was defined as axillary body temperature ≥ 37.5°C or history of fever in preceeding 7 days to this study [1].

A total of 116 pregnant women attended the campaign program. In line with aforementioned criteria, 31 were excluded from the study because they had booked in the PHC; of whom 4 had received IPT for malaria and another 4 had fever during the period of recruitment for this study.

A validated questionnaire was administered to the participants by one of the authors and assisted by a trained research assistance who verbally translated the required information in the questionnaire to respondents where necessary. The questionnaire contained questions about the participants' demography, whether or not she had long lasting insecticide-treated net (LLIN), and whether she slept under the net. Family social class was obtained by the method proposed by Olusanya, Okpere and Ezimokhai [10], using level of education of the participant and the occupation of her husband. In this method of classification of social class, specific scores (0, 1, and 2 for the woman's level of education; and 1, 2 and 3 for her husband's occupation) was allotted to each participant and the sum of these scores was used to describe social class as I, II, III, IV, and V. Participants with scores I and II were classified as upper class, III-middle class; IV and V as lower class. The gestational age of the participants was calculated from the first day of the last menstrual period (LMP).

Each subject was then tested for malaria using the RDT kit for malaria parasite- *Plasmodium falciparum* (pf). The RDT was a one step invitro diagnostic test for qualitative detection of pf antigen known as the histidine-rich protein II (HRP-II) in capillary blood [11]. The RDT kit used for this study was the Standard Diagnostic (SD) Bioline malaria antigen pf test (Kyonggi-do, Korea). Each pack of the RDT kit used in this study was from lot number 082048 (reference number 05FK50) and contained 25 cartridge kits or test devices, 25 un-opened alcohol swabs, 25 appropriately calibrated pipette loop and 25 sterile lancets. One pack was meant for testing malaria parasite for 25 people and a new test cartridge or test device was used for each participant. Internal evaluation of the RDT kits which was certified by WHO showed that the SD Bioline RDT kit has 100.0% sensitivity for blood with > 50 parasite/μL and 98.0% specific for *P. falciparum*. The kits had storage temperature range of one to 40°C which was within the average environmental temperature in Ondo and the other southwestern region of Nigeria.

Malaria parasite test was performed by one of the authors who had received certified training on the use of RDT for malaria diagnosis. Procedure for the test was as follows: the pulp of the fourth finger of the subject was cleaned with an alcohol swab and when it was air dried, the cleaned area was pierced with the sterile lancet. A capillary pipette loop was used to collect 5.0 μL of blood (a black line calibrated on the pipette loop provided in the test kit). The blood was then transferred to the sample window on the test kit cartridge. Four drops of provided buffer was then added to the buffer well window on the cartridge. Each test kit was read between 15-30 minutes. Malaria positive was by the presence of two colour bands ('T' Test line and 'C' Control line) within the result window, no matter which band appeared first and no matter the intensity of the colour band. Negative result was by presence of only one colour band 'C' (Control line) within the result window. If the control band failed to appear within the result window or no colour band at both the 'T' and 'C' regions, the result was considered invalid and the test was repeated for that particular subject using a new test device.

Women with positive malaria parasite received the recommended anti-malarials in line with the national guideline for treatment of malaria in pregnancy.

Data analysis

The data obtained for this research was entered into the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc Chicago, Illinois, USA) spread sheet where analysis was also done. The major outcome variable was the presence of malaria parasitemia. Associations with this variable were tested using chi-square and student t tests as appropriate. The level of significance of each test was set at $p < 0.05$.

Tab. I. Demographic data of the subjects.

Demographic data	n = 85 (%)
Age	
18-24 years	30 (35.3)
25-31 years	46 (54.1)
32-38 years	8 (9.4)
39-45 years	1 (1.2)
Educational status	
No formal education	7 (8.2)
Primary	22 (25.9)
Secondary	54 (63.5)
Tertiary	2 (2.4)
Social class	
Upper	1 (1.2)
Middle	9 (10.6)
Lower	75 (88.2)

Results

Mean (± SD) age of the 85 pregnant women recruited for this study was 26.1 ± 4.7 years (age range 18-40 years); mean gestational age was 27.1 ± 8.1 weeks (range 6-40 weeks). Forty-five (52.9%) were in the third trimester, 35 (41.2%) were in second trimester and 5 (5.9%) were in the first trimester. Forty-eight (56.5%) were multipara, 20 (23.5%) were primigravidae and 17 (20.0%) were grand multiparous women. Fifty-four (63.5%) of the women had secondary education, 22 (25.9%) had primary, 2 (2.4%) had tertiary education and 7 (8.2%) had no formal education. Table I shows the age group and social class of the respondents. Majority (88.2%) of the women were from the lower social class and most were 31 years and below.

The prevalence of malaria parasitaemia in this study was 22 (25.9%). There was no significant difference in mean age of infected (26.5 ± 5.1 years) and non-infected subjects (25.9 ± 4.6 years); (t = 0.46, p = 0.65, 95%CI = -1.78, 2.85).

Only 3 (3.5%) out of the 85 women owned LLIN; and only one of the three slept under a LLIN. Reason given by the other two who did not sleep under the net was that the net was difficult to mount. Malaria parasitaemia was not significantly associated with age, parity, gestational age and social class (Tab. II).

Discussion

The high prevalence of asymptomatic malaria in pregnancy in this study supports similar findings in other stable malarial transmission areas [1, 3-6]. Susceptibility to *Plasmodium* parasitaemia has been linked to the level of antibodies to placental sequestrated parasites [2]. The most preferentially adherence is to the chondroitin sulphate-A (CSA) receptors expressed by the syncytiotrophoblasts in the placenata [12]. This adherence is common in primigravidae and women in second pregnancy as the anti-adhesion antibodies against CSA binding parasites usually develop in women after successive pregnancies [2]. The consequence of untreated asymptomatic malaria parasitaemia in pregnancy is enormous [3-6], therefore adequate and prompt implementation of malaria control policy for pregnant women should be intensified to protect these women and their un-born babies from the antecedent complications of malaria disease.

In most African countries, pregnant women usually book for antenatal care by the 2nd trimester [13], there is the need for malaria parasite screening as a routine test at booking to identify and treat asymptomatic malaria parasitaemia with the recommended anti-malarial drug before the commencement of IPT. Some authors have also suggested that in areas with high prevalence of asymptomatic malaria in pregnancy, it may be more appropriate to institute intermittent screening for malaria parasite using simple tools such as the RDT as well as treat the individual with presence of asymptomatic malaria parasitaemia as alternative to IPT especially in areas where sulphadoxine-pyrimethamine (SP) resistance is very high [14].

Malaria detection using RDT is a major break-through in malaria control [8]. Though this study did not compare the prevalence of malaria parasitaemia by RDT and microscopy, the prevalence found (25.9%) was comparable to 27.4% observed in a study among pregnant women using microscopy in Lagos, in the same south-west Nigeria [5]. Malaria screening and diagnosis in pregnancy using RDT especially at booking may also be a cost effective malaria control strategy in pregnancy and should be an integral component of malaria control in pregnancy in holo-endemic regions.

Only 3.5% of the pregnant women had the LLIN. The use of the LLIN is now one of the most important preventive tools against malaria. LLIN has been shown to reduce the number of infective mosquito bites by 70.0 – 90.0% in a variety of ecologic settings as well as reduce the prevalence of malaria in children and adults [15, 16]. Unfortunately, most women of reproductive age group in Nigeria do not possess the LLIN and the few who had the LLIN barely sleep under the net [15].

Tab. II. Malaria parasitaemia and age group, gestation, parity and social class of the subjects.

	Malaria Parasitaemia			
	Positive (%)	Negative (%)	χ^2	p-value
Age group				
18-24 years (n = 30)	5 (16.7)	25 (83.3)		
25-31yeras (n = 46)	14 (30.4)	32 (69.6)	4.69	0.20
32-38 years (n = 8)	2 (25.0)	6 (75.0)		
39-45 years (n = 1)	1 (100.0)	0 (0.0)		
Trimester of gestation				
First (n = 5)	2 (40.0)	3 (60.0)		
Second (n = 35)	12 (34.3)	23 (65.7)	3.35	0.19
Third (n = 45)	8 (17.8)	37 (82.2)		
Parity				
Primigravida (n = 20)	7 (35.0)	13 (65.0)		
Multipara (n = 48)	10 (20.8)	38 (79.2)	1.62	0.45
Grand multipara (n = 17)	5 (29.4)	12 (70.6)		
Social class				
Upper (n = 1)	0 (0.0)	1 (100.0)		
Middle (n = 9)	3 (33.3)	6 (66.7)	0.62	0.73
Lower (n = 75)	19(25.3)	56 (74.7)		

Limitations of the study

The observations were not sufficient to permit statistical significance in the observed differences. In addition, microscopy on the specimens could have offered an opportunity to assess the validity of the RDT results in the community.

Conclusions

This study showed that one-quarter of the pregnant women had asymptomatic malaria parasitaemia and that RDT can be an invaluable instrument for malaria diagnosis in rural areas. The authors therefore recommended routine malaria parasite screening for all pregnant women at booking as well as scaling-up the availability of LLIN for vector control and the RDT kits for malaria diagnosis in rural areas. Health education on the use of LLIN as important tool for control of malaria should be intensified in malaria control National Advocacy, Communication, and Social Mobilization Strategic Framework and Implementation Plan in Nigeria.

ACKNOWLEDGMENT

The authors wish to thank staff of Primary Health Centre Odo-Aiye, Okitipupa LGA for their assistance in recruiting the women for this study and Nwaneri Adaeze for typing and editing the manuscript.

References

[1] *WHO world malaria report 2008*. Available in http://malaria.who.int/wmr 2008/malaria2008. pdf. Accessed on 02/06/2011.

[2] Elliot SR, Brennan AK, Beeson JG, et al. *Placental malaria induces variant antibody of the cytophylic subtype immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity*. Infect Immun 2005;73:5903-7.

[3] Isah AY, Amanabo MA, Ekele BA. *Prevalence of malaria parasitaemia amongst asymptomatic pregnant women attending a Nigerian Teaching Hospital*. Ann Afr Med 2011;10:171-4.

[4] Ikeh EI, Akudo SN, Uguru VE. *Prevalence of malaria parasitaemia in pregnant women attending antenatal clinic at Jos University Teaching Hospital, Nigeria*. Afri J Clinical & Experimental Micrbio 2005;6:91-4.

[5] Iriemenam NC, Dosunmu AO, Oyibo WA, et al. *Knowledge, attitude, perception of malaria and evaluation of malaria parasitaemia among pregnant women attending antenatal care clinic in metropolitan Lagos, Nigeria*. J Vector Borne Dis 2011;48:12-7.

[6] Mbanefo EC, Umeh JM, Oguoma VM, et al. *Antenatal Malaria Parasitaemia and haemoglobin profile of pregnant mothers in Awka, Anambra State, Southeast Nigeria*. Amer-Eurasian J Sci Res 2009;4:235-9.

[7] Federal Republic of Nigeria. 2006 population and housing census of Nigeria. Federal Republic of Nigeria official gazette, Lagos 2007.

[8] *Malaria diagnostic tool. WHO 2010*. Available in www.wpro.who.int>malariarapid diagnostictests>RDTevaluationprogramme. Accessed on 24/09/2012.

[9] Durrheim DN, Govere J, la Grange JJ, et al. *Rapid immunochromatographic diagnosis and Rolling Back Malaria-experiences from an African control program*. Afr J Med Sci 2001;30suppl:21-4.

[10] Olusanya O, Okpere E, Ezimokhai M. *The importance of social class in voluntary fertility control in a developing country*. W Afr J Med 1985;4:205-12.

[11] Howard RJ, Uni S, Aikawa M, et al. *Secretion of a malarial histidine-rich protein (pf HRP II) from Plasmodium falciparum-infected erythrocytes*. J Cell Bio 1986;103:1269-77.

[12] Duffy PE, Fried M. *Malaria during pregnancy; parasites, antibodies and chondroitin sulphate-A*. Biochem Soc Trans 1999;27:478-82.

[13] Okonofua F, Adediran M, Adetugbo-Davies A, et al. *Prevalence of malaria parasitaemia in pregnant women*. Medicare 1991;4:16-8.

[14] Tagbor H, Bruce J, Browne E, et al. *Malaria in pregnancy in an area of stable and intense transmission: is it asymptomatic?* Trop Med Intl Hlth 2008;13:1016-21.

[15] Igwe PC, Inem V, Ebuehi OM, et al. *The effect of insecticide treated bed net use on malaria episodes, parasitaemia and haemoglobin concentration among primigravidae in a peri-urban settlement in southeast Nigeria*. J Rural & Trop Publ 2007;6:24-32.

[16] D'Alessandro U, Olaleye BO, Mc Guire W, et al. *Reduction in morbidity and mortality from malaria in Gambian children following the introduction of a National Insecticide Impregnated Bed Net Programme*. Lancet 1995;345:479-83.

■ Received on October 24, 2012. Accepted on December 6, 2012.

■ Correspondence: D.U. Nwaneri, Institute of Child Health, University of Benin, PMB 1154, Benin City, Edo State, Nigeria - Tel. +2348056321577 +23480139172309 - E-mail: damiannwaneri@yahoo.com