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Diagnosis of malaria in pregnancy: A comparison of microscopy with rapid diagnostic tests

Sarah I. Umeh[1]*, Chika Paulinus Enwuru[2] and Richard C. Egbuobi[3]

[1]Department of Microbiology, Federal University of Technology, Owerri, Nigeria.
[2]Department of Medical Laboratory Technology, Imo State College of Nursing and Health Sciences, Amaigbo, Nigeria.
[3]Department of Medical Laboratory Sciences, Imo State University, Owerri, Nigeria.

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ABSTRACT

Malaria in pregnancy in Nigeria is a serious life threatening infection to the pregnant mother and the fetus. A study was conducted to test the effectiveness of rapid diagnostic tests in the parasitological diagnosis of malaria in pregnant women in the absence of reliable microscopy facilities. Blood samples of 500 pregnant women were tested for malaria parasite by Giemsa stained smear and Malaria Pf$^{(R)}$ rapid diagnostic test (RDT). Thirty percent of the samples were positive by microscopy while 27% were positive by the RDT. The younger pregnant women (20 to 25 years) and women in the second trimester of pregnancy (13 to 24 weeks) have more malaria prevalence than other age groups. The sensitivity of the Malaria Pf test was 86.7%, the specificity was 98.6%. The positive predictive value was 96.3% and the negative predictive value was 94.5%. In the absence of good quality microscopy facilities, and experienced laboratory personnel, the Malaria Pf test should be employed as a viable alternative to ensure a reliable parasite based diagnosis of malaria in pregnant women for prompt and accurate treatment.

Keywords: Malaria, pregnancy, *Plasmodium falciparum*, rapid diagnostic tests.

*Corresponding author. E-mail: elisaraumehsam@yahoo.co.uk. Tel: +2348063365313.

INTRODUCTION

The year 2015, for the attainment of the millennium Development Goals (MDGs) is fast approaching. Malaria is one of the diseases listed for control in the MDG 6 (UN, 2000) and is still a public health challenge in the sub-Saharan African, particularly in Nigeria. Malaria is a potentially fatal disease especially when caused by *Plasmodium falciparum* (Sidney, 2008). Literature indicates that malaria in pregnancy poses a serious risk for both the mother and the unborn child. The consequences of malaria in pregnancy range from maternal anaemia, low birth weight, spontaneous abortion, stillbirth, among others (Steketee et al., 1996; Tagbor et al., 2008; Luxemburger et al., 1997).

Diagnosis and treatment of malaria is an integral part of the World Health Organisation malaria control strategy (Sidney, 2008; WHO, 2006). Administration of antimalarial drugs to vulnerable population groups such as pregnant women has also formed part of the global malaria control measures (Sidney, 2008). However, the risk of exposing the pregnant mother and unborn baby to antimalarials unnecessarily necessitates parasitological confirmation of infection before treatment. Thus the WHO recommends that anyone suspected of having malaria should receive diagnosis and treatment with an effective drug within 24 h of the onset of symptoms (WHO, 2006). However, WHO indicates that there is a major setback to malaria control programmes. The unavailability of facilities for laboratory diagnosis of malaria at the periphery of the health services or at community levels where most cases of malaria are managed is a major hurdle (WHO, 2006). In the light of this, intermittent preventive therapy in pregnancy was also recommended by WHO (Murray and Benneth, 2009), but there is need to intensify effort at parasitological confirmation of malaria diagnosis before treatment. This is especially so in *P. falciparum* infection which has perceived resistance to many antimalarials including the artesunates (Alkker et al., 2007). This trend has led to the emergence of more

expensive and more complex drugs for the treatment of malaria (Zaman and Beg, 2004) with increased risk of drug toxicity (WHO, 2006). Mis-diagnosis of malaria results in increased morbidity and mortality, prolonged severe illness and missed opportunities. On the other hand, WHO states that timely, accurate and accessible detection of malaria parasites has an important role in reducing the malaria burden and promoting a more rational use of increasingly expensive antimalarials (WHO, 2006). The use of parasite based diagnosis supports the exclusion of malaria in the differential diagnosis of febrile illnesses.

Several methods to diagnose malaria exist, each with a certain degree of accuracy. They include:
Light microscopy using stains such as Giemsa, Leishman and rapid Fields stain. These are relatively cheap, readily available stains used on thick and thin blood films and remain the gold standard in the diagnosis of malaria. Acridine orange stain is expensive and unsuitable for routine diagnosis of malaria.

Molecular method using Polymerase Chain Reaction (PCR) is highly sensitive but expensive. Immunological tests such as indirect Fluorescent Antibody tests (IFAT), Indirect Haemaglutination tests (IHA) and Enzyme Linked Immunsorbent Assay (ELISA) are antibody detection methods that are less sensitive and therefore not suitable for routine diagnosis of malaria. Antigen detecting immunological methods are less expensive, more sensitive and rapid in application. This has been variously studied for its effectiveness in the diagnosis of malaria in different settings (Murray and Benneth, 2009; Zaman and Beg, 2004).

In this study, the Malaria Pf antigen detecting rapid diagnostic test was used to compare microscopy in the parasite based diagnosis of malaria in pregnant women in a rural setting in Imo State, Nigeria.

METHODOLOGY

Study area

The cross sectional study was conducted on all pregnant women attending ante-natal clinics at three hospitals:

1. Agaz Hospital, Akokwa, Ideato North
2. Chika Medical Centre, Osina, Ideato North
3. Osina Community Hospital, Osina, Ideato North

Ideato North Local Government Area is located in Imo West Senatorial Zone of Imo State in the South-east Nigeria. The area has a tropical rain forest and as such has a stable Plasmodium transmission.

Study sample

A total of 500 pregnant women were tested from January to December, 2009. The mean age of the women was 29.9 years with a range of 20 to 39 years. The mean gestation period was 23.6 weeks with a gestational range of 8 to 37 weeks. One hundred and seventy pregnant women out of the 500 studied were in the first trimester (1 to 12 weeks) of their pregnancy, another 170 were in the second trimester (13 to 24 weeks) and 160 in the third trimester (25 to 40 weeks).

Methods of testing

An informed consent from the pregnant woman was obtained and her venous blood obtained by Giemsa staining technique (Cheesbrough, 1987).

Ethical approval was obtained from the Medical Directors of the three Hospitals respectively.

Microscopy

Thick and thin blood smears were made on clean grease-free glass slides. The thick blood smear was stained (without fixing) with 10% Giemsa solution for 30 min. The thin smear was fixed in absolute methanol for about 2 min and then stained with 10% Giemsa solution for 30 min (Cheesbrough, 1987). The stains were washed in running tap water, left to air-dry in a slanting position. The film was examined on the microscope under oil immersion for malaria parasites by trained medical laboratory personnel. Both the thick and thin blood films were examined under a minimum of 200 high power fields before a patient was declared as either being negative or positive for malaria parasite.

Rapid diagnostic test

The rapid diagnostic test used in this study is the Malaria Pf antigen detection kit (ANTEC DIAGNOSTICS LIMITED, UK). The kit consists of:

(i) A test cassette sealed in an aluminium pouch
(ii) Assay diluent (buffer)
(iii) Disposable specimen dropper (pipette)

The cassette was removed from the pouch and placed on a level surface, and with the dropper held vertically, the whole blood was drawn to the marked line (about 10 µl) and transferred to the sample well on the cassette. Three drops of the assay diluent (about 120 µl) were added and allowed to flow to the result window on the cassette. The cassette was then examined for the appearance of coloured lines on the result window after 15 min.

Interpretation of the test

The test was positive if 2 coloured lines appeared on the result window, 1 on the control (C) region and the other on the test (T) region. A negative test was indicated by the appearance of only 1 coloured line on the control (C) region and none on the test region. An invalid test was indicated by the non-appearance of coloured line on the control region with or without a coloured line on the test region.

RESULTS

A total of 500 women were tested. The results were statistically analysed by Medcalc software version 12.7.5, which showed that the prevalence of malaria among pregnant women in this study area was 30%. One

Table 1. Statistical values of malaria PF test.

SENS (%)	SPEC (%)	PPV (%)	NPV (%)	FPR (%)	FNR (%)	LR+	LR-	F-MEASURE (%)
86.7	98.6	96.3	94.5	1.4	13.3	6.2	0.13	92.3

SENS = Sensitivity, SPEC = Specificity, PPV = Positive predictive value, NPV = Negative predictive value, FPR = False positive rate, FNR = False negative rate, LR+ = Likelihood ratio for positive test, LR- = Likelihood ratio for negative test, F-Measure = Harmonic mean of Precision (positive predictive value) and Recall (Sensitivity).

Table 2. Age range of pregnant women that tested positive for malaria parasite by both methods.

Age (years)	20-25	26-30	31-35	36-40	Total
No tested	140	120	123	117	500
Microscopy	50 (35.7%)	34 (28.3%)	31 (25.2%)	35 (29.9%)	150 (30.0%)
RDT*	41 (29.3%)	30 (25.0%)	29 (23.6%)	35 (29.9%)	135 (27.0%)

* = Rapid Diagnostic Test

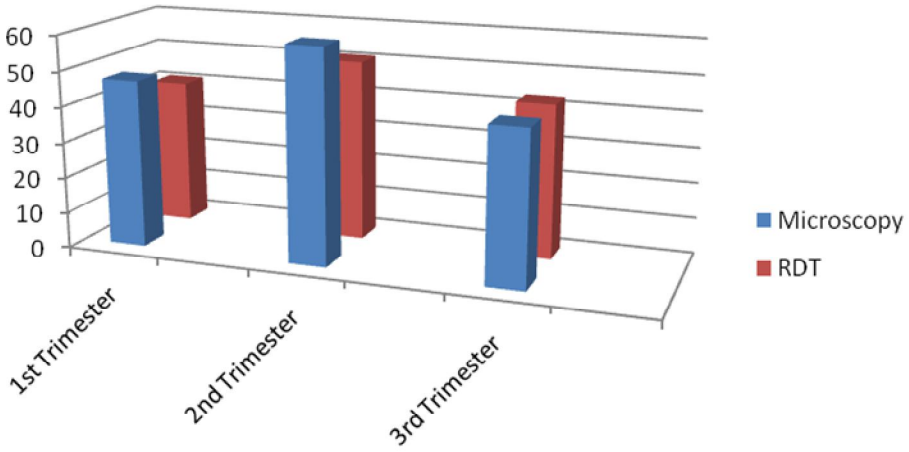

Figure 1. Number of positive microscopy tests compared to positive RDT in the three gestation stages. RDT = Rapid Diagnostic Test.

hundred and fifty pregnant women were positive by microscopy while 135 (27%) were positive by the Malaria Pf test. About 13.3% of those positive by microscopy were negative by Malaria Pf. On the other hand, 3.3% of those positive by Malaria Pf test were negative by microscopy. Compared with microscopy, the sensitivity (Altman and Bland, 1994a) of the Malaria Pf was 86.7% and the specificity was 98.6%. The positive predictive value (Altman and Bland, 1994b) was 96.3% and negative predictive value was 94.5% (Table 1). The younger pregnant women (20 to 25 years) were more infected than the older ones (Table 2). In addition, pregnant women in the second trimester (13 to 24 weeks) were more infected than other age groups (Figure 1).

DISCUSSION

This study revealed a malaria prevalence of 30% among pregnant women in the two communities of Akokwa and Osina in Ideato North Local Government Area of Imo State. Women in the second trimester were more infected (60/170; 35.3%) than the rest. In addition, the younger age group (20 to 25 years) were more infected (50/140; 35.7%) than the older age groups. This is similar to the findings of Tay et al. (2013). It has also revealed that the Malaria Pf Rapid Diagnostic Test has a sensitivity of 86.7%, specificity of 98.6%, a positive predictive value of 96.3% and negative predictive value of 94.5% when compared with the gold standard that is microscopy for the diagnosis of malaria. This sensitivity is lower than the World Health Organization prescribed sensitivity of over 90% for an acceptable rapid diagnostic test for malaria (WHO, 2006).

The false negative rate of 13.3% is worrisome. It means that up to 13.3% of the pregnant women who have malaria could be left untreated if patients are treated solely on the basis of this test. It is interesting to

point out that majority of the false negative specimens in this study had low parasitaemia. This agrees with similar studies with other rapid diagnostics tests where false negative results were reported in patients with parasitaemia of less than 50 parasites/µl of blood (Guthman et al., 2002; Shiff et al., 1994). In contrast, Tagbor et al. (2008) and Murray and Benneth (2009) have at different times suggested the possibility of more sensitive rapid diagnostic tests than microscopy at low parasite densities. Furthermore, some studies have reported that false negative results with rapid diagnostic tests could be as a result of mutant parasites that escape the antigenic determinant of the RDT (Lee et al., 2006; Baker et al., 2005).

The false positive rate in this study is 1.4%. This could possibly be explained by the fact that patients who were possibly already on treatment and whose parasitaemia has cleared could still have tested positive by the rapid diagnostic test picking up remnant antigens. Studies have shown antigens to be present several days after clearance of parasitaemia from the blood stream, making the RDT unsuitable for monitoring treatment and level of parasitaemia. The sequestration of parasitized erythrocytes in tissue capillaries and placental malaria in the absence of peripheral blood parasitaemia may have also yielded positive RDT and negative microscopy for malaria parasites (Tjitra et al., 2001; Murray and Benneth, 2009; Moody and Chiodini, 2000).

There is also a reported cross reactivity between RDTs that detect histidine-rich protein 2 and rheumatoid factor, which gives a false positive result for malaria parasites (Murray and Benneth, 2009; Laferi et al., 1997; Igbal et al., 2000). Certain challenge exists in providing the facilities for accurate microscopic diagnosis of malaria at the rural communities. This is the setting where the malaria menace threatens the life of the pregnant woman and the unborn baby. There is risk involved in exposing every pregnant woman to random choice of antimalarial drugs. It can strongly be asserted that the findings of this study were good to suggest the adoption of the Malaria Pf Rapid Diagnostic Test in the parasitological confirmation of malaria diagnosis (Enwuru et al., 2011). As stated by WHO, this will, with some degree of certainty, help to rule out malaria in pregnant women (WHO, 2006; Murray and Benneth, 2009). It is further suggested that RDT will curb malaria miss-diagnosis and help identify true antimalarial drug resistance (which seems to have been exaggerated), in the face of counterfeit antimalarial drugs. Some of these fake antimalarial drugs also receive false reports of efficacy when taken during the course of self-limiting febrile conditions. In addition, money will be saved by only treating those who are infected.

CONCLUSION AND RECOMMENDATION

The few shortcomings of the RDT observed in the study notwithstanding, the Malaria Pf Rapid Diagnostic Test is a veritable tool for parasite based diagnosis of malaria in pregnant women. This is so especially in the rural communities where facilities for microscopic diagnosis of malaria are not readily available and where experienced laboratory personnel may also not be available.

REFERENCES

Alkker AP, Lim P, Sem R, Shaw NK, Yi P, Mey-Bouth D, Tsuyuoka R, Maguire JD, Fandeur T, Ariey F, Wongsrichanalai C, Meshnick SR, 2007. Pfmdr1 and in-vivo resistance to artesunate-mefloquin in *falciparum malaria* on the Cambodian-Thai border. Am J Trop Med Hyg, 76(4):641-647.

Altman DG, Bland JM, 1994. Diagnostic Tests 1: Sensitivity and Specificity. BMJ, 308(6943):1552.

Altman DG, Bland JM, 1994. Diagnostic Tests 2: Predictive Values. BMJ, 309(6947):102.

Baker J, McCarthy J, Gatton M, Klye D.E, Belizario V, Luchavez J, Bell D, Cheng Q, 2005. Genetic Diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. J Infect Dis, 192(5):870-877.

Cheesbrough M, 1987. Medical Laboratory Manual for Tropical Countries. Vol 1, 2nd ed. Tropical Health Technology/Butterworths-Heinemann Limited, UK. pp: 221-251.

Enwuru CP, Umeh SI, Agbasi UM, Egbuobi RC, 2011. Laboratory diagnosis of malaria in children under 5 years in a rural community: microscopy versus malaria pf test. Afr J Clin Exper Microbiol, 12(2):54-57.

Guthman JP, Ruiz A, Priotto G, Kiguli J, Bonte L, Legros D, 2002. Validity, reliability and ease of use in the field of five rapid tests for the diagnosis of *Plasmodium falciparum* malaria in Uganda. Trans R Soc Trop Med Hyg, 96:254-257.

Igbal J, Sher A, Rab A, 2000. *Plasmodium falciparum* Histidine-Rich Protein 2-based Immunocapture diagnostic Assay for Malaria: Cross reactivity with Rheumatoid Factor. J Clin Microbiol, 38:1184-1186.

Laferi H, Handel K, Pichler H, 1997. False positive dipstick test for malaria. N Eng J Med, 337:1635-1636.

Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, McCarthy J, 2006. Effect of sequence variation in *Plasmodium falciparum* Histidine-Rich Protein 2 on binding of specific monoclonal antibodies: Implications for Rapid Diagnostic Tests for malaria. J Clin Microbiol, 44(8):2773-2778.

Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, White NJ, 1997. The epidemiology of severe malaria in an area of low transmission in Thailand. Trans R Soc Trop Med Hyg, 91(3):256-262.

Moody AH, Chiodini PL, 2000. Methods for the detection of blood parasites. Clin Lab Heamatol, 22:189-201.

Murray CK, Benneth JW, 2009. Rapid Diagnosis of Malaria. Interdiscip Persp Infect Dis. doi: 10. 1155/2009/415953.

Shiff CJ, Minjas J, Premji Z, 1994. The ParaSight®-F test, a simple rapid manual dipstick test to detect *Plasmodium falciparum* infection. Parasitol Today, 10: 494-495.

Sidney D, 2008. Malaria In: *Encyclopaedia of Earth*, Culter J, Cleveland (Ed.) Center for Disease Control and Prevention. (Washington DC. Environmental Information Coalition, National Council for Science and Environment). www.coearth.org/article/malaria

Steketee RW, Wirima JJ, Campbell CC, 1996. Developing effective strategies for malaria prevention programs for pregnant African women. Am J Trop Med Hyg, 55:95-100.

Tagbor H, Bruce J, Browne E, Greenwood B, Chandramohan D, 2008. Performance of the OptMal® dipstick in the diagnosis of malaria infection in pregnancy. Ther Clin Risk Manag, 4(3):631-636.

Tay SCK, Agboli E, Abruquah HH, Walana W, 2013. Malaria and anaemia in pregnant and non-pregnant women of child-bearing age at the University Hospital, Kumasi, Ghana. Open J Med Microbiol, 3:193-200.

Tjitra E, Suprianto S, McBroom J, Curie BJ, Anstey NM, 2001. Persistent ICT malaria P.f/P.v panmalarial and HRP2 antigen

reactivity after treatment of *Plasmodium falciparum* malaria is associated with gametocytemia and results in false positive diagnosis of *Plasmodium vivax* in convalescence. J Clin Microbiol, 39:1025-1031.

UN, 2000. United Nations Millennium Declaration. Document A/RES/55/2. New York: United Nations. www.un.org/millennium/declaration/ares552e.pdf (accessed 28/10/2013).

WHO, 2006. The role of Laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission. Report of a WHO technical consultation. (WHO/HTM/MAL/2006.1111).

Zaman V, Beg MA, 2004. Laboratory diagnosis of malaria. Infect Dis J, 13(2):47-48.