

Full Length Research Paper

Clinical benefits to pregnant women on the use of rapid diagnostic test to microscopy in malarial diagnosis in Jigawa State, Nigeria

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The study was aimed at comparing rapid diagnostic test kits (RDTs) and microscopy in detecting sequestered placental malaria or deep tissue malaria from pregnant women and the clinical benefits that can be derived. About 300 pregnant women were enrolled in the study. Five (5) ml of venous and placental blood was collected into an ethylenediaminetetraacetic acid (EDTA) tube, respectively. The blood samples were tested for malaria using microscopy and parascreen (RDTs). The hemoglobin (Hb) concentration was estimated by Hb color scale method. Out of the 300 enrolled, a total of 250 (82.5%) were positive with microscopy while the RDTs detected 300 (100%). Comparing the sensitivity, RDTs had 100% while microscopy had 88.3% and both had 100% specificity. Comparing the age group with frequency of infection, the 21 to 25 years age groups were the most vulnerable with 134 (45.54%). With parity, secundgravidae (1+1) had the highest with 104 (34.32%) and ≥ 4 parity had the least with 50 (16.50%). Those with Hb values ≤ 9 g/dl had the highest incidence with 245 (80.85%), 10 to 11.4 g/dl had 51 (16.86%) while ≥ 11.5 g/dl had the least with 4 (1.32%). About 16.5% were RDTs positive which might have been lost if only microscopy was done.

Key words: Rapid diagnostic tests (RDTs), microscopy, pregnancy, anaemia, parity, sequestration.

INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium species*. They are transmitted from person to person through the bite of an infected female anopheles mosquito (Fernandez, 2006). Malaria generally is a disease of major public health concern in African region, with 562 million people at high risk (World Health Organization (WHO), 2013). It was estimated that there were 166 million clinical cases of malaria in 2012 and up

to 90% malaria deaths of world total was from sub Saharan Africa. 77% of the deaths in the African region was among children < 5 years (WHO, 2013). Each year, 25 to 30 million women become pregnant in malaria-endemic areas of Africa, and similar numbers are exposed to malaria in Asia, Oceania, and South America. Malaria is an important cause of severe anemia in pregnant African women, and by this mechanism malaria

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causes an estimated 10,000 maternal deaths each year. Moreover, malaria infections result in 75,000 to 200,000 low birth weight babies each year, due to combinations of preterm delivery and fetal growth restriction (Guyatt and Snow, 2004). The yearly exposure of at least 50 million pregnancies to malaria infection makes it the most common and recurrent parasitic infection directly affecting placenta (Federal Ministry of Health (FMOH), 2006).

In Africa, perinatal mortality due to malaria is at about 1500/day. In areas where malaria is endemic, 20 to 40% of all babies born may have a low birth weight, hence making malaria in pregnancy one of the priority areas of Roll Back Malaria strategy. It affects more than 3 million pregnant women per year in developing countries, where it commonly causes poor birth outcome and maternal anemia (WHO, 2004). To revert malaria in Africa, there have to be tremendous efforts from all angles to curtail it. Concurrently, there has to be a shift away from the concept of eradication of malaria using indoor house spraying to integrated vector control approaches (WHO, 2006). Efforts to control the disease are as well hampered by the resistance to drugs shown by the *Plasmodia*, to the insecticides by the vectors and the lack of an effective vaccine (Elizabeth et al., 2005).

Malaria in pregnancy is an obstetric, social and medical problem requiring multidisciplinary and multidimensional solution. It is a debilitating, infectious disease characterized by chill, shaking and periodic bouts of intense fever. Pregnant women constitute the main adult risk group for malaria and 80% of deaths due to malaria in Africa occur in pregnant women and children < 5 years (Worts et al., 2006a).

Parasitaemia level and number of peripherally-detected malaria infections, but not the presence of fever, are associated with adverse birth outcomes. Hence, prompt malaria detection and treatment should be offered to pregnant women regardless of symptoms or other preventive measures used during pregnancy, and with increased focus on mothers living in remote areas. The physiological changes of pregnancy and the pathological changes due to malaria has a synergistic effect on the course of each other, thus making the life difficult to the mother and the child (Kakkilayer, 2006; Reyburn et al., 2007). In Africa, malaria in pregnancy is responsible for 400,000 cases of severe maternal anaemia and 200,000 newborn deaths each year. Placental infection, premature birth and low birth weight (a significant factor in infant mortality) are also caused by maternal malaria. In addition, severe maternal anemia increases the risk of perinatal complications.

Plasmodium falciparum causes three specific changes in the placenta. Infected erythrocytes (IE) containing mature trophozoite and schizont parasite stages accumulate in the intervillous spaces (the lake-like structures through which maternal blood circulates), sometimes to high densities. High placental parasitemia

has been associated with preterm delivery (PTD). Placental malaria may be accompanied by intervillous infiltrates of monocytes and macrophages, some containing malaria pigment (hemozoin). High-density monocyte infiltrates are especially common in first pregnancy, and are associated with low birth weight (LBW) and anemia (Brabin et al., 2004; Rogerson et al., 2003).

The problems in the new born include low birth weight, prematurity, intrauterine growth retardation (IUGR), malaria illness and mortality. The pathogenesis of placental malaria is only partially understood, but it is clear that it leads to distinct epidemiological pattern of malaria during pregnancy (Worts et al., 2006b). An integrated understanding of the epidemiological, immunological and pathological processes must be achieved in order to understand how to control malaria in pregnancy. In pregnant women, parasitological and both hematological and biochemical changes should be promptly investigated as part of good clinical practice to improve the differential diagnosis of fever and any possible derangements. This may also reduce the unnecessary prescription and use of anti malaria drugs, many of which are of questionable safety. During pregnancy, *P. falciparum* is sequestered in placenta, often without being detected in the peripheral blood (Moody, 2002).

Rapid diagnostic tests have considerable potential as a tool to improve the diagnosis of malaria. Several commercially available tests are sensitive, specific, and stable under operational conditions. Although microscopy remains the gold standard for diagnosis of malaria, its accuracy under operational condition in Africa is often low. Result of RDTs are rapidly available, less liable to the theoretical risk of being falsely negative due to parasite sequestration, and accessible to both prescriber and patient and can restore confidence in the laboratory (Reyburn et al., 2007). Although RDTs are significantly more costly than the traditional routine microscopy in hospital settings, they are potentially cost effective (Reyburn et al., 2007).

Diagnosis of malaria involves identification of the malaria parasite or its antigens/products in the blood of the patient. Although this seems simple, the efficacy of the diagnosis is subject to many factors. The different forms of the four species; the different stages of erythrocytic schizogony; the endemicity of different species; the population movements; the inter relation between the level of transmission, immunity, parasitemia, and the symptoms; the problem of recurrent malaria, drug resistance, persisting viable or non-viable parasitemia, and sequestration of the parasites in the deeper tissues; and the use of chemoprophylaxis or even presumptive treatment with the basis of clinical diagnosis can all have an impact on the identification and interpretation of malaria parasitemia on a diagnostic test (Bates et al., 2006b). The Jigawa State Ministry of Health in collaboration

Table 1. Baseline characteristics of patients for slides (microscopy) and RDTs.

Characteristic	Microscopy/RDTs
Mean age (years)	30±15
Fever in last 48 h	280
Low hemoglobin level (≤ 9 g/dl)	235
Previous use of antimalarial in current illness	215
Parity stages	4±3
Previously diagnosed positive	300
HIV status	Negative

Table 2. Statistical analysis depicting sensitivity, specificity and predictive values of RDTs and microscopy (n=300).

Methods	Positive S/C	Negative S/C	Sensitivity	Specificity	PPV	NPV
RDTs	300	0	100	100	100	100
Microscopy	250	50	83	100	100	83
P-Value	<0.05	<0.05	<0.05	>0.05	>0.05	<0.05

RDTs = rapid diagnostic tests, PPV = Positive predictive Value, NPV = Negative Predictive Value, S/C = Slide/Cartridge.

with PATHS deployed RDTs to formal health care system of rural areas as part of intensifying the need to avoid missed diagnosis especially to pregnant women and children < 5 years.

MATERIALS AND METHODS

Sample collection

A total of 300 pregnant women were recruited, all attending antenatal clinic at General Hospital, Ringim of Jigawa State. Informed consent was sorted from each participant for the study. At delivery, 5 ml of maternal and placental blood were collected into separate EDTA tubes for thick films, RDTs and hematological assessment.

Making and staining of thick films

Thick films of 2 cm in diameter were made from well mixed blood on a clean grease free microscope slide. The films were stained using Field's stain rapidly (Bates et al., 2006a). All films were later reviewed by the State Malaria Microscopy Quality Control Officer. Parascreen RDT kit was used according to manufacturer's procedures to test for the presence of malarial antigen in the blood samples.

Hemoglobin concentration estimation

Hemoglobin (Hb) color scale technique was used to estimate the Hb concentration of the blood samples in g/dl (Bates et al., 2006a).

Statistics

The results were analyzed using SPSS 15.0 statistical package.

RESULTS

Table 1 shows the baseline characteristics of the participants at commencement of the study, with mean age of 30 ± 15 years. Those with fever within the 48 h were 280, those detected with low haemoglobin level (≤ 9 g/dl) were 235 and those on drugs were 215. Parity ranged between 1 to 4, they were all previously diagnosed malaria positive and non reactive to human immunodeficiency virus (HIV). Table 2 shows the result of sensitivity, specificity and predictive values using both Microscopy and RDTs. Microscopy and RDTs both had 250 vs 300 of positive S/C, Negative S/C of 50 vs 0, Sensitivity 83 vs 100, Specificity 100 vs 100, PPV 100 vs 100, and NPV 83 vs 100, respectively. A significant value ($p < 0.05$) was observed in all but specificity and PPV. Table 3 shows the distribution of malarial infection among the different age groups ranging between 15 to 45 years of age. The age group of 21 to 25 years has the highest infection rate, followed by 15 to 20 age group. From 26 to 30 age group, the infection rate decreases down to 41 to 45 age group in both the diagnostic methods. Table 4 shows the relative malarial infection in association with the number of parity by the mother. Secundigravidae has the highest infection rate followed by those with first time pregnancy. Third with the high rate were those with third time pregnancy while those with the least infection rate were those with four and above parity status. Table 5 shows the hemoglobin distribution among the malaria infected pregnant women. Those with hemoglobin level of 9 g/dl and below have the highest populations followed by those with 10 to 11.4 g/dl while those with hemoglobin 11.5 g/dl and above have the least number of

Table 3. Age groups compared to the rate of malaria infection.

Age groups (years)	Microscopy n (%)		RDTs n (%)	
	Positive	Negative	Positive	Negative
15-20	78 (26.20)	13 (4.29)	91 (30.03)	0 (0)
21-25	104(34.67)	34 (11.33)	138(45.54)	0 (0)
26-30	28 (9.33)	4 (1.33)	32 (10.56)	0 (0)
31-35	24 (7.92)	1 (0.33)	25 (8.25)	0 (0)
36-40	6 (1.98)	4 (1.33)	10 (3.30)	0 (0)
41-45	3 (0.99)	1 (0.33)	4 (1.32)	0 (0)

Table 4. Showing association of parity status and malaria infection.

Parity	Total examined (n)	Microscopy n (%)	RDTs n (%)
		Positive	Positive
1+0	83	72 (24.00)	83 (27.67)
1+1	104	78 (25.74)	104 (34.32)
1+2	63	55 (18.15)	63 (20.79)
≥4	50	45 (14.85)	50 (16.5)

n = sample size, 1+0 = First pregnancy, 1+1 = Second pregnancy, 1+2 = Third pregnancy, ≥4 = Fourth pregnancy and above.

Table 5. Depicting association of hemoglobin content and malaria infection.

Hemoglobin (g/dl)	Total examined (n)	Microscopy n (%)	RDTs n (%)
		Positive	Positive
≥ 11.5	4	4 (01.33)	4 (01.33)
10-11.4	49	46 (15.33)	49 (16.33)
≤ 9.00	247	200 (66.67)	247 (82.33)

infected population.

DISCUSSION

In Jigawa state, the natural event of pregnancy puts women at greater risk of death at a higher rate than expected. An average of 1500 to 2000 pregnancies out of 100,000 live birth will end in the death of the mother, child or both. In some part of the world in developed countries, the number of pregnancies is fewer than 100 per 100,000 live birth (Department for International Development (DFID), 2006).

The challenges for diagnostic laboratory in Jigawa and most of the African regions which include defective microscope, intermittent power, poor supply of consumables, and time limit to examine slides are well known both to the laboratory managers and to their consumers. To improve these to the standard and comparable sensitivity and specificity of RDTs is not simple or easy to sustain. RDTs if embarked upon will supplement as a tool to offer improvement in accurate and precise diagnosis of malaria in our local setting were competent and other

basic requirements are lacking. In most of the request made to the laboratories in syndrome manner, the findings in most cases with respect to malaria parasites request in most cases turnout negative even in severe infections. This may be explained by sequestration of parasites into deep vascular beds. Other possibilities that may affect sensitivity of microscopy in our settings may include work overload, shortage of staff and substandard Romanowsky's stain that flooded our chemical stores throughout the nation. From the study it was observed that the routine may fail to indicate the presence of malaria parasites as a result of tissue sequestration in the placenta. Therefore, recognizing the increasing importance of accurate diagnosis in an era of negative clinical benefits experiencing by pregnant women, government should be encouraged by experts to place substantial orders for RDTs as guide to treatments of febrile illness (Reyburn et al., 2007).

Prompt detection and treatment with effective anti-malaria should be offered, irrespective of symptoms and use of other preventive measures in pregnancy. While frequent screening was associated with improved birth outcome, reaching mothers living in remote areas to

prevent late attendance and low number of visits at antenatal care is essential. What this study has added is that, the parasites in some patient might be sequestered or missed diagnosis in the placenta in about 16.6% (50) cases in the pregnant women attending this comprehensive hospital of the locality. This may contribute significantly in preventing the pregnancy complication due to plasmodiasis among this great population. In Cameroon, 20.1% of pregnant women in a similar study were detected by HRP-2 based RDT and therefore rescued from missed diagnosis using microscopy (WHO, 2004).

CONCLUSION AND RECOMMENDATION

Public enlightenment through the local media radio stations and traditional town criers will ultimately help in reducing the risk by attending clinic in the early stage of the pregnancy. Public/community sanitation should be enforced so as to clear away the harboring areas that proliferates the mosquitoes. Those attending antenatal clinic should be told on the risk of abandoning their routine drugs in relation to their health and the fetus. They should also be given a free set insecticide treated nets (ITN) as part of the Federal government effort on Roll Back Malaria program. The RDTs test kits should also be supplied free or at well reduced price to the reach of less privileged.

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Conflict of interest

The authors have no conflict of interest

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