Research Journal of
Parasitology

**Academic
Journals Inc.**

CrossMark
← click for updates

Comparative Assessment of Microscopy and Rapid Diagnostic Test (RDT) as Malaria Diagnostic Tools

Oyetunde T. Oyeyemi, Akinwale F. Ogunlade and Isaac O. Oyewole
Department of Biosciences and Biotechnology, Babcock University, Ilishan-Remo, Ogun State, Nigeria

Corresponding Author: Oyetunde T. Oyeyemi, Department of Biosciences and Biotechnology, Babcock University, Ilishan-Remo, Ogun State, Nigeria Tel: +2348163546787

ABSTRACT

Management of malaria requires prompt diagnosis of malaria by microscopy, Rapid Diagnostic Tests (RDTs), or other available tools. The aim of this study was to compare the effectiveness of RDT and microscopy in detection of malaria parasite in a malaria endemic area of Nigeria among different population groups. The cross sectional study was conducted on 251 febrile patients who were directed to the laboratory department for blood screening for malaria parasites at Ogunlade Hospital, Ijebu Ode, Ogun state. Blood samples were collected and screened for malaria parasites microscopically and by using First Response RDT. The prevalence of malaria obtained through microscopy (66.8%) was significantly higher than in RDT (36.8%) (p<0.05). Considering microscopy as the gold standard, RDT exhibited high specificity (87.1%) and low sensitivity (42.5%) with positive predictive and negative predictive values of 86.6 and 43.5%, respectively. The sensitivity of RDT increased significantly with increase in *P. falciparum* parasitaemia (p<0.0001). The routine microscopy test demonstrated a superior sensitivity compared to First Response RDT method of malaria diagnosis, however, RDT could be a useful tool in individuals suspected to show high degree of disease spectrum for quick intervention in order to avert danger associated with delayed diagnosis.

Key words: Malaria, rapid diagnosis, sensitivity, performance

INTRODUCTION

In areas where there is increasing population of malaria infected individuals, rapid and efficient diagnostic methods are needed for rational therapy. Rational therapy is necessary to avoid non-target effects, to save cost on alternative drugs and to delay the advent of parasite resistance (Wongsrichanalai *et al.*, 2007).

The use of rapid diagnostic tests suggested to have largest impact with microscopy has been widely advocated. Its implementation has been reported to be cost effective (Batwala *et al.*, 2011), cause significant reduction in referrals and in patient's length of hospital stay (Boyce *et al.*, 2015). Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5-15 µL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip (Wongsrichanalai *et al.*, 2007). The low capital involvement, non-requirement of electricity, easy operation and result interpretation are its advantages over microscopy. The adoption of RDT by community health workers could facilitate diagnosis in local malaria-endemic areas with limited health personnel and facilities (Harvey *et al.*, 2008). However, the accuracy of a clinical diagnosis is dependent on the disease endemicity level,

malaria season and the age group under consideration (Dicko *et al.,* 2005; Mwangi *et al.,* 2005), thus the discrepancies observed in RDT sensitivities in several observational studies (Grobusch *et al.,* 2003; Iqbal *et al.,* 2003; Fernando *et al.,* 2004).

Most commonly used RDTs are coated with monoclonal antibodies specific for malaria parasite with Histidine-Rich Protein 2 (HRP-2) and pan-pLDH or aldolase enzyme being the targeted antigens for *P. falciparum* and the four common *Plasmodium* species, respectively (Wongsrichanalai *et al.,* 2007). This study assessed the diagnostic performances of RDT in order to guide treatment decision in a low resourced Nigerian malaria endemic area.

MATERIALS AND METHODS

Study design and study area: The study was a nonrandomized descriptive and cross-sectional study. The study was carried out in Ogunlade Hospital, Ijebu Ode, Ogun state. Ijebu Ode is located in South-Western Nigeria. The city is inhabited by the Ijebus, a sub-group of the Yoruba ethnic group who speak the Ijebu dialect of Yoruba. It was conducted from July to September, 2012. During this period, vegetations were thick and canals/drainages were often seen with stagnant water thus providing good resting and breeding sites for malaria parasites mosquito vectors. However, the prevailing sociocultural practices and the poor socioeconomic status of the dwellers make malarial endemicity a year round event.

Sample size: Sample size was determined using the method of Naing *et al.* (2006). Briefly, using the prevalence data 50.0%, precision 0.05(5%) and statistical power 80%, the minimum sample size arrived at was 223 subjects. In all, a total of 251 participants were recruited during medical consultation after which they were directed to the hospital laboratory for RDT and malaria parasite tests.

Parasitological examination: All parasitological examinations were carried out in Ogunlade Hospital, Ijebu Ode, Ogun state. The study included febrile patients of both sexes attending outpatient clinics for parasitological examinations. Thick and thin blood smears were prepared from venous blood samples collected from the antecubital fossa. All samples were collected by trained laboratory staff on duty. The films were properly dried and stained with 10% Giemsa solution then washed after 10 min using clean water. A drop of immersion oil was applied on dried stained slide and examined microscopically for malaria parasites using 100X objective lens. Asexual stage parasite of any level was regarded as a positive smear; smears were considered negative if the examination of 100 high power fields did not reveal asexual parasites (Hopkins *et al.,* 2008).

Rapid Diagnostic Test (RDT): Blood samples collected from the study participants were also tested for malaria parasites using First Response® RDT (Premier Medical Corporation Limited, Kachigam, Daman, India). First Response® is an immunochromatographic test coated with monoclonal antibody that recognizes the specific Histidine Rich Protein-2 (HRP-2) associated with the presence of *P. falciparum*. The test uses approximately 5 µL of blood and is readable after 15 min following the manufacturer's instructions. Participants with positive RDT results were prescribed immediate treatment. A drop of anticoagulated blood (5 µL) obtained from the subjects was placed on the RDT cards and two drops of buffer was added to form a blood-buffer mixture. The mixtures were drawn up the card across the lines of bound antibody. Positive results indicate the

presence of three bands (test line 2, test line 1 and control). For negative results, only the control line appeared. Results were observed and recorded. All malaria positive smears were reviewed by another microscopist for confirmation.

Informed consent and ethical approval: Oral informed consent was obtained from the selected patients. Ethical approval with the reference number (OOUTH/DA.326/899) was obtained from Olabisi Onabanjo University Teaching Hospital, Ogun State, Nigeria.

Inclusion criteria: Inclusion criterion for this study was individual with fever and without focus (foci) of infection (Msellem *et al.*, 2009). The usual residence of the area and those without other visible signs of ailments were also included.

Data analysis: Data was carefully entered in Excel spread sheet (version 2007) and transferred to GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA 92037 USA) for analyses. Chi-square analysis was used to determine the significance differences in proportion of malaria parasite infection determined by microscopy and RDT. The same was used to assess significant variations in diagnostic parameters in different groups. Proportions were calculated and the diagnostic performance was determined by calculating the test sensitivity, specificity, predictive values, Diagnostic Likelihood Ratios (DLR) and Diagnostic Odd Ratios (DOR). The sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease while, specificity refers to the ability of the test to correctly identify those patients without the disease. The Positive Predictive Value (PPV) is the probability that an individual with a positive screening result has the disease while Negative Predictive Value (NPV) is the probability that an individual with a negative screening result is without the disease. Diagnostic likelihood ratio is the ratio of expected test result in subjects with the disease to the subjects without the disease. Diagnostic odd ratio is the ratio of the odds of positivity in subjects with disease relative to the odds in subjects without disease. Microscopy was used as the reference standard. The p-values less than 0.05 were considered statistical significance.

RESULTS

The subjects comprised 10 infants (1 month-4 years), 19 school-aged children (5-12 years), 25 teenagers (13-19 years) and 197 adults (18 years and above). The gender distribution of the participants included 75 males and 176 females. The overall prevalence of malaria parasites obtained through microscopy (66.8%) was significantly higher than in RDT (36.8%) (p<0.05). Species of malaria parasite identified in all study participants was *Plasmodium falciparum.* The parasite density ranged from 40-1080 μL^{-1} of blood. The mean parasite density ("SD) decreased with age of the subjects with 329.6"315.9, 224.4"283.2, 216.7"283.6 and 373.3"256.3 recorded in the adult, teenager, school-aged children and infants, respectively. However, there were no significant differences in intensity of infection across the various population groups (p<0.05).

The number of subjects with true/false negative and true/false positive RDT's results was presented in Table 1. A rather low sensitivity (42.5%) and high specificity (87.1%) were recorded in the general population. Sensitivity of RDT seemed to increase with ages of the participants with 33.3, 41.2 and 42.6% recorded in school children, teenagers and adult subjects, respectively (Table 2). However, there was a clear deviation from this general trend in infants with significantly higher sensitivity value (71.3%). The same followed for other diagnostic indicators. Generally, there were no significant differences in diagnostic parameters across the different groups (p<0.05). The positive diagnostic likelihood ratio of RDT was higher (4.1) in adult than in teenagers (1.1) and

Table 1: No. of subjects with true/false negative and true/false positive RDT's diagnostic test results

Categories	True positive	False positive	True negative	False negative	Total
General population	7 1 (28.3)	11 (4.4)	74 (29.5)	96 (38.2)	251
Adult	55 (27.9)	7 (3.6)	61 (31.0)	74 (37.6)	197
Teenagers	7 (28.0)	3 (12.0)	5 (20.0)	10 (40.0)	25
School children	4 (21.1)	1 (5.3)	6 (31.6)	8 (42.1)	19
Infant	5 (50.0)	0 (0.0)	3 (30.0)	2 (20.0)	10
χ^2					8.037
p value					0.443

Chi-square (χ^2) showing no association (p>0.05) between true/false diagnostic results in different categories of population

Table 2: Diagnostic performance of rapid diagnostic test in different groups

Diagnostic parameters	General population	Adult	Teenagers	School children	Infants	p-values
Sensitivity	42.5	42.6	41.2	33.3	71.3	0.712
Specificity	87.1	89.7	62.5	85.7	100	0.752
Positive predictive value	86.6	88.7	70.0	80.0	100	0.866
Negative predictive value	43.5	45.2	33.3	42.9	60	0.806
Positive diagnostic likelihood ratio	3.3	4.1	1.1	2.3	NC	
Negative diagnostic likelihood ratio	0.7	0.6	0.9	0.8	0.3	
Diagnostic odd ratio	5.0	6.5	1.2	3.0	NC	

NC: Not computed or infinite value since no value was recorded for false positive

Table 3: Differential sensitivity of rapid diagnostic test at varied parasitaemia

Parasite density (/µL of blood)	No. Examined	Sensitivity RDT (%)	Sensitivity (microscopy) (%)
<100	73	22.4	100
100-500	45	34.9	100
501-1000	46	81.3	100
1000 >	1	100.0	100
χ^2			40.27
p-value			0.0001

Chi-square (χ^2) showing association (p<0.05) between parasite density and RDT sensitivity

school children (2.3). The least negative diagnostic likelihood ratio (0.3) was recorded in infants. A similar pattern was observed in diagnostic odd ratios with very high DOR value and 6.5 in infants and the adult population, respectively. The sensitivity of RDT increased significantly with increase in malaria parasite density (Table 3) (p<0.05).

DISCUSSION

The low proportion of false positive test reported in this study is a characteristic of a good test. The rheumatoid factor cross-reacting in the blood could generate a false positive test line but replacement of IgG with IgM in recent products reduces this problem (Laferi *et al.*, 1997; Grobusch *et al.*, 1999). Also, cross-reactivity with heterophile antibodies may be another cause of false positive test (Moody and Chiodini, 2002). The high proportion of false negative test resulted in the rather low sensitivity recorded in the general population. This false negative result may be caused by mutation of the HRP-2 gene (Wellems *et al.*, 1991). The presence of anti-HRP-2 antibodies in humans may suggest why some tests were negative despite high parasite density (Biswas *et al.*, 2005). The storage temperature the RDT product was subjected to by the marketers could also explain the observed low sensitivity. Exposure of RDT kit to high temperatures has been implicated as a possible cause of poor performance in the tropics. Denaturation of antibodies in the test membrane can impair binding to the target antigen at high temperature. Heat can also cause damage to the nitrocellulose membrane forming the strip thus changing its flow characteristics or causing the antibody to detach from the membrane (Chiodini *et al.*, 2007).

The sensitivity of RDT reported in this study is lower than previous reports in Nigeria (Ajumobi *et al.*, 2015) and other parts of the world. Sensitivities of 96, 97 and 97.6% had been reported in Zambia, Zanzibar and Thailand, respectively (Hopkins *et al.*, 2008; Msellem *et al.*, 2009; Nicastri *et al.*, 2009). However, the specificity 87% is similar to 88% reported by Msellem *et al.* (2009) but lower than 93 and 100% recorded in Thailand (Buchachart *et al.*, 2004) and among Nigerian travellers (Dougnon *et al.*, 2015). The sensitivity reported in this study is yet to attain the 95% recommended World Health Organization value (WHO., 2000). This low sensitivity is disadvantageous as it will impair control intervention since a fraction of the infected population will be left untreated especially if RDT is the only available diagnostic tool. However, the high specificity will improve the cost effectiveness of malaria diagnosis since the RDT is unlikely to miss-out the non-infected individuals.

Diagnostic Likelihood Ratio (DLR) is more clinically useful than the sole usage of sensitivity in estimating the probability of disease in an individual (Akobeng, 2007). The very high positive diagnostic likelihood ratio in the infant category is a result of absence of false positive test in the group. The RDT presented a very good likelihood of presenting a positive test in infected infant and adult population compared to their uninfected counterparts. This is subject to the high positive diagnostic and low negative diagnostic likelihood ratios reported in our study. Malarial RDT can therefore serve as useful tool in early diagnosis of malaria in infants in order to avert the accompanied burdens associated with late diagnosis. However, the result is inconclusive as very few infants were representative of the study population. Another test indicator not commonly used in clinical research is Diagnostic Odd Ratio (DOR) which is also dependent on the spectrum of disease severity (Moons *et al.*, 1997). Expectedly, the high DOR values in the infant and adult populations are indicators of good test results in the groups. The value 1.2 recorded in the teenager's category shows that the RDT tool does not discriminate malarial infected patients from those without infection. This could be resulted from lower degree of parasitaemia reported in the group. Increase in sensitivity with *P. falciparum* parasitaemia is similar to what was reported elsewhere (Gasser *et al.*, 2005; Miller, 2006).

The use of microscopy in diagnosis of malarial infection in adult population that comprises pregnant women can pose some challenges due to placental sequestration of parasites thus reducing the sensitivity of microscopy. However, the detection of peripheral blood HRP-2 genes (the principle through which the RDT works) is possible with malaria parasites RDT therefore, making RDT very useful in such condition (Leke *et al.*, 1999). The ability to detect placental infection by antigen detection when microscopy does not identify parasitaemia could have a significant impact on material and fetal health care (Murray *et al.*, 2008). The RDT's utilization in community-base malaria care is difficult to sustain due to problems of low cost effectiveness and availability. One limitation of our study was that our data failed to compare malarial RDT diagnostic performance in the pregnant women with present data.

CONCLUSION

Malaria is still a serious problem in the study area. The RDT used in the diagnosis is parasite density dependent and may show low or poor performance in low infected population. This approach can be best adopted for infant and adult population of pregnant women being the most malarial susceptible groups. Further investigation however is needed to ascertain the cause of low sensitivity of the RDT recorded in this study.

REFERENCES

Ajumobi, O., K. Sabitu, P. Nguku, J. Kwaga and G. Ntadom *et al.*, 2015. Performance of an HRP-2 rapid diagnostic test in nigerian children less than 5 years of age. Am. J. Trop. Med. Hygiene, 92: 828-833.

Akobeng, A.K., 2007. Understanding diagnostic tests 2: Likelihood ratios, pre and post test probabilities and their use in clinical practice. Acta Paediatrica, 96: 487-491.

Batwala, V., P. Magnussen, K.S. Hansen and F. Nuwaha, 2011. Cost-effectiveness of malaria microscopy and rapid diagnostic tests versus presumptive diagnosis: Implications for malaria control in Uganda. Malaria J., Vol. 10.

Biswas, S., D. Tomar and D.N. Rao, 2005. Investigation of the kinetics of histidine-rich protein 2 and of the antibody responses to this antigen, in a group of malaria patients from India. Ann. Trop. Med. Parasitol., 99: 553-562.

Boyce, R.M., A. Muiru, R. Reyes, M. Ntaro, E. Mulogo, M. Matte and M.J. Siedner, 2015. Impact of rapid diagnostic tests for the diagnosis and treatment of malaria at a peripheral health facility in Western Uganda: An interrupted time series analysis. Malaria J., Vol. 14. 10.1186/s12936-015-0725-0

Buchachart, K., S. Krudsood, M. Nacher, D. Chindanond, P. Rungmatcha, S. Kano and S. Looareesuwan, 2004. Evaluation of the KAT-quick malaria rapid test for rapid diagnosis of falciparum malaria in Thailand. Southeast Asian J. Trop. Med. Public Health, 35: 35-37.

Chiodini, P.L., K. Bowers, P. Jorgensen, J.W. Barnwell and K.K. Grady *et al.*, 2007. The heat stability of *Plasmodium* lactate dehydrogenase-based and histidine-rich protein 2-based malaria rapid diagnostic tests. Trans. Royal Soc. Trop. Med. Hygiene, 101: 331-337.

Dicko, A., C. Mantel, B. Kouriba, I. Sagara and M.A. Thera *et al.*, 2005. Season, fever prevalence and pyrogenic threshold for malaria disease definition in an endemic area of Mali. Trop. Med. Int. Health, 10: 550-556.

Dougnon, T.V., H.S. Bankole, Y.M.G. Hounmanou, S. Echebiri, P. Atchade and J. Mohammed, 2015. Comparative study of malaria prevalence among travellers in Nigeria (West Africa) using slide microscopy and a rapid diagnosis test. J. Parasitol. Res., Vol. 2015. 10.1155/2015/108707

Fernando, S.D., N.D. Karunaweera and W.P. Fernando, 2004. Evaluation of a rapid whole blood immunochromatographic assay for the diagnosis of *Plasmodium falciparum* and *Plasmodium vivax* malaria. Ceylon Med. J., 49: 7-11.

Gasser, R.A., A.J. Magill, T. Ruebush, R.S. Miller and J. Sirichaisinthop *et al.*, 2005. Malaria diagnosis: Performance of NOW ICT Malaria in a large scale field trial. Proceedings of Abstract 2338 of the 54th Annual Meeting of the American Society of Tropical Medicine and Hygiene, December 11-15, 2005, Atlanta, Georgia, USA.

Grobusch, M.P., U. Alpermann, S. Schwenke, T. Jelinek and D.C. Warhurst, 1999. False-positive rapid tests for malaria in patients with rheumatoid factor. Lancet, 353: 297-297.

Grobusch, M.P., T. Hanscheid, K. Gobels, H. Slevogt, T. Zoller, G. Rogler and D. Teichmann, 2003. Comparison of three antigen detection tests for diagnosis and follow-up of falciparum malaria in travellers returning to Berlin, Germany. Parasitol. Res., 89: 354-357.

Harvey, S.A., L. Jennings, M. Chinyama, F. Masaninga, K. Mulholland and D.R. Bell, 2008. Improving community health worker use of malaria rapid diagnostic tests in Zambia: Package instructions, job aid and job aid-plus-training. Malaria J., Vol. 7. 10.1186/1475-2875-7-160

Hopkins, H., L. Bebell, W. Kambale, C. Dokomajilar, P.J. Rosenthal and G. Dorsey, 2008. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. J. Infect. Dis., 197: 510-518.

Iqbal, J., A. Muneer, N. Khalid and M.A. Ahmed, 2003. Performance of the OptiMAL test for malaria diagnosis among suspected malaria patients at the rural health centers. Am. J. Trop. Med. Hygiene, 68: 624-628.

Laferi, H., K. Kandel and H. Pichler, 1997. False positive dipstick test for malaria. New Engl. J. Med., 337: 1635-1636.

Leke, R.F.G., R.R. Djokam, R. Mbu, R.J. Leke and J. Fogako *et al.*, 1999. Detection of the *Plasmodium falciparum* antigen histidine-rich protein 2 in blood of pregnant women: Implications for diagnosing placental malaria. J. Clin. Microbiol., 37: 2992-2996.

Miller, R., 2006. Comparison of performance characteristics of the Binax NOW Malaria test using venous and fingerstick samples. Proceedings of Abstract 533 of the 55th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 12-16, 2006, Atlanta, Georgia, USA.

Moody, A.H. and P.L. Chiodini, 2002. Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection. Br. J. Biomed. Sci., 59: 228-231.

Moons, K.G.M., G.A. van Es, J.W. Deckers, J.D.F. Habbema and D.E. Grobbee, 1997. Limitations of sensitivity, specificity, likelihood ratio and baye's theorem in assessing diagnostic probabilities: A clinical example. Epidemiology, 8: 12-17.

Msellem, M.I., A. Martensson, G. Rodllant, A. Bhattarai and J. Stromberg *et al.*, 2009. Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar-A crossover validation study. PLoS Med., Vol. 6. 10.1371/journal.pmed.1000070

Murray, C.K., R.A. Gasser Jr., A.J. Magill and R.S. Miller, 2008. Update on rapid diagnostic testing for malaria. Clin. Microbiol. Rev., 21: 97-110.

Mwangi, T.W., M. Mohammed, H. Dayo, R.W. Snow and K. Marsh, 2005. Clinical algorithms for malaria diagnosis lack utility among people of different age groups. Trop. Med. Int. Health, 10: 530-536.

Naing, L., T. Winn and B.N. Rusli, 2006. Practical issues in calculating the sample size for prevalence studies. Ach. Orofacial Sci., 1: 9-14.

Nicastri, E., N. Bevilacqua, M.S. Schepisi, M.G. Paglia and S. Meschi *et al.*, 2009. Accuracy of malaria diagnosis by microscopy, rapid diagnostic test and PCR methods and evidence of antimalarial overprescription in non-severe febrile patients in two Tanzanian hospitals. Am. J. Trop. Med. Hygiene, 80: 712-717.

WHO., 2000. Malaria diagnosis new perspectives. Report of a Joint WHO/USAID Informal Consultation, October 25-27, 2000, Geneva.

Wellems, T.E., A. Walker-Jonah and L.J. Panton, 1991. Genetic mapping of the chloroquine-resistance locus on *Plasmodium falciparum* chromosome 7. Proc. Nat. Acad. Sci. USA., 88: 3382-3386.

Wongsrichanalai, C., M.J. Barcus, S. Muth, A. Sutamihardja and W.H. Wernsdorfer, 2007. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT).anthones inhibitors of α-glucosidase and glycation from *Garcinia nobilis*. Am. J. Trop. Med. Hygiene, 77: 119-127.