Sumerianz Journal of Biotechnology, 2020, Vol. 3, No. 6, pp. 38-42
ISSN(e): 2617-3050, ISSN(p): 2617-3123
Website: https://www.sumerianz.com

Original Article Open Access

Comparative Assessment of Microscopy and Rapid Diagnostic Test (RDT) As Malaria Diagnostic Tools in Kano, Northern Nigeria

Farouk S. Nas
Department of Biological Science, Bayero University Kano, Nigeria

Abdullahi Yahaya
Department of Biological Science, Kano University of Science and Technology Wudil, Nigeria

Lurwan Mu'azu
Department of Biological Sciences, Federal University Gusau, Nigeria

Muhammad Ali*
Department of Microbiology, Federal University Gusau, Nigeria
Email: alimuhd4real@gmail.com

Shamsu I. Abdullahi
Department of Microbiology, Federal University Gusau, Nigeria

Article History
Received: May 20, 2020
Revised: June 12, 2020
Accepted: June 20, 2020
Published: June 23, 2020

Abstract

The impact of malaria globally has motivated interest in developing prompt and accurate diagnostic strategies to provide an effective management of the disease. The aim of the study was to compare microscopy and Rapid Diagnostic Test (RDT) as tools for diagnosis of malaria. A cross sectional study was conducted on 235 febrile patients, who were directed to Parasitology laboratory of Murtala Muhammad Specialist Hospital Kano State for blood screening for malaria parasites from February to July, 2017. A total of 235 samples of blood were collected from consented respondents and screened for malaria parasites microscopically using Blood film examination and by using Rapid Diagnostic Test (RDT) for detection of malaria parasites in the blood samples. A simple structured questionnaire was used to get demographics of the respondents. Of the 235 patients involved in the study, 158 (67.2%) tested positive for *Plasmodium falciparum* by RDT, whereas 173 (73.6%) tested positive by microscopy. There is no significant difference in the infection rates between microcopy and Rapid Diagnostic Test (RDT). From the result, the specificity, sensitivity and negative predictive values of Rapid Diagnostic Test (RDT) were low when compared to microscopy while the positive predictive value was high. The microscopy test methods showed superior sensitivity compared to Rapid Diagnostic Test (RDT). However, Rapid Diagnostic Test (RDT) could be useful for quick intervention in order to eliminate dangers associated with delayed malaria diagnosis.

Keywords: Kano; Malaria; Microscopy; Plasmodium falciparum; Rapid diagnosis test.

1. Introduction

Malaria is one of the febrile illnesses and the most common fatal disease worldwide and about half of the world population is at risk of malaria. It is caused by one or more species of protozoan parasite called *Plasmodium*. The species include *P. falciparum, P. ovale, P. vivax and P. malariae.* According to World malaria report in 2011, there were about 216 million cases of malaria and an estimated 655,000 death in the year 2010 [1]. Most of the malaria cases and death occur in sub-Saharan Africa and approximately 50% of Nigerian population experienced at least one or more episode of malaria per year. However, official estimate of the disease suggest as much as four rounds per person per year on the average [2]. The trend in malaria disease is rapidly increasing due to current resistance of the disease to first line anti-malarial drugs [3]. The magnitude of incidence and death due to malaria is a multiple of all other tropical diseases cumulatively. Malaria is responsible for over 81% of reported cases of tropical disease in Nigeria [4]. This demonstrated that the disease could be the largest contributor to disease burden and productivity losses resulting from major tropical disease in Nigeria. Report on malaria about Nigeria given by the Malaria report in 2005 shows that the incidence of the disease throughout the country has been on the increase over the years ranging from 1.12 million from 1990 and 2.25 million by the beginning of millennium (2000) and 2.61 million in the year 2003.

The main strategy for fighting malaria is rapid and accurate diagnosis method followed by effective treatment of the disease [4]. The quick and accurate diagnosis of malaria is essential for both malaria surveillance and effective treatment. In 2010, the WHO recommended that all patients with suspected malaria should have their diagnosis confirmed by rapid diagnostic test or microscopy before treatment [5]. In endemic countries such as Nigeria, microscopy is still considered as the 'gold standard' for malaria diagnosis. Microscopy has a sensitivity of 50 – 500 parasites/µl [6], it is inexpensive and has advantage of allowing the identification of species and parasite density [7]. During microscopy, it is necessary to observe many fields for parasite detection, which implies the requirement of at

*Corresponding Author

least two expert microscopists [8]. In many malaria-endemic regions such as Nigeria, diagnosis using microscopy has certain limitation such as shortage of skilled microscopists, inadequate quality control, and the possibility of misdiagnosis due to low parasitaemia or mixed infections [9, 10]. In addition to that microscopy has low sensitivity when performed by poorly trained personnel in endemic areas, especially in primary and secondary health facilities. This may cause over- or under- diagnosis of malaria, with excessive use of anti-malarial drugs or negligent treatment, which invariably contributes to malaria morbidity and development of resistance [11]. Therefore, in the absence of well prepared technician for microscopic diagnosis in many areas, the World Health Organization (WHO) recommend RDTs as a good alternative method for diagnosing malaria [11, 12]. In most parts of sub-Saharan Africa, Rapid Diagnostic Tests have become the primary tool for parasitological confirmation or diagnosis of malaria [13]. Unlike microscopy, Rapid Diagnostic Test detect malaria antigens not malaria parasite. This give them additional advantage in the ability to diagnose malaria in patients with low-grade parasitaemia below detection limit of microscopy [14, 15]. However, the specificity of the commonly used RDT that detects histidine rich protein-II (HRP-II) of *P. falciparum* is limited when the parasite is cleared and the antigen remain in circulation for about 28 days (false positive) [1]. Accurate and prompt diagnosis is a key to effective treatment and management of patients with malaria parasitemia which will eventually help in reducing malaria morbidity and mortality. The aim of the study was to compare microscopy and Rapid Diagnostic Test (RDT) in the diagnosis of malaria among febrile patients in Kano State, Northern Nigeria.

2. Materials and Methods

2.1. Ethical Approval

An approval (MOH/off/797/T.I/023) for the study was obtained from Research and Ethic committee Health Services Management Board (HSMB) under Kano State Ministry of Health. The study objective was clearly explained to the patient and informed consent was obtained from the patient before proceeding to the study

2.2. Study Area

Kano State is located in North-Western Nigeria at latitude 1103'N and longitude 80 3'E. It share borders with Kaduna State to South-West, Jigawa State to the East, Bauchi State to the South-East and Katsina state to the North. It has a total area of 20,131 km2 (7,777 sqm) and estimated population of 13.4 million [16]. Climate Kano has been described as 'AW' type as identified by Koppen's classification [17]. This type of vegetation is savanna type characterized by predominantly herbaceous vegetation with scattered or widely space trees Ali, *et al.* [17].

2.3. Determination of Sample Size

Sample size for the study was determined using a standard formula for calculation of minimum sample size [18]. Sample size for the study was given by the formula; N = (Z1-a)2 (p) (1-p) / d2 where; N = minimum sample size, Z1--a = value of standard normal deviate which at 95% confidence interval has found to be 1.96, P = the best estimate of prevalence obtained from literature review (81%) and d = difference between the true population rate and sample that can be tolerated, this is the absolute precision (in percentage) on either side of the population. N = (1.96)2 (0.81) (1-0.81)/ (0.05)2 = 3.84 x 0.81 x 0.19/0.0025= 236.39 as samples for the study. Therefore, a total of 235 subjects were used for convenience.

2.4. Study Population

In this study, blood samples from a total number of 235 patients (127 from males and 108 from females) were collected. All the subjects involved in this study were febrile patients diagnosed for malaria tests. The study was conducted for a period of 6 month from February 2017 – July, 2017at Murtala Muhammad Specialist Hospital Kano, Northern Nigeria.

Table-1. Demographic distribution of the Subjects with Percentage Frequency

Parameter	Frequency (n)	Percentage (%)
Age (Years)		
Less than 20	105	44.7
21 – 40	64	27.2
41 – 60	47	20.0
61 – Above	19	08.1
Sex		
Male	127	54
Female	108	46
Settlement		
Urban	132	56
Rural	103	44

2.5. Samples Collection

Five milliliter (5 ml) of blood samples were collected from 235subjects testing for malaria parasites from a period of from February to July, 2017. The blood samples were stored in test tubes and stored at 4°C prior to

laboratory analysis. A simple structured questionnaire was administered to obtain demographic data such residential area, age, education level and education level of the subjects.

2.6. Examination of Blood Samples

Examination of blood samples for *Plasmodium* in this study was conducted with thick blood film using Giemsa staining technique as described by Cheesbrough [19]. Giemsa stain was diluted 1 in 10 by adding 5 ml of strain of 45 ml buffered distilled water (pH 7.0). The blood films were flooded with freshly diluted Giemsa stain for 30 minutes. The stain was then washed off and the slides were allowed to air dry. The dried smear was examined microscopically using X100 objective.

2.7. Malaria RDT

Approximately 0.5 ml of blood was used to diagnosed malaria using Ag Pf/Pan malaria RDT kit (Standard Diagnostic Inc. South Korea), following the manufacturer's instructions. The Rapid Diagnostic Test is a qualitative immune-chromatographic test that detects *P. falciparum* HRP-II and Plasmodium lactate dehydrogenase, which is a glycolytic enzyme common to *Plasmodium* asexual stage parasites.

2.8. Statistical Analysis

Statistical analysis package for social sciences (SPSS) version 10.0 was used for statistical analysis of the data generated. Chi square was used to compare between two or more variables. Statistical significance was considered at p-value <0.05.

3. Results

3.1. Prevalence of Malaria According to Diagnostic Methods

The prevalence of malaria based on the diagnostic method used in this study is presented in Table 2. The result showed that out of the 235 patients involved in the study, 158 (67.2%) tested positive for Plasmodium falciparum by RDT, whereas 173 (73.6%) tested positive by microscopy.

Table-2. Prevalence of malaria based on the diagnostic methods used

Methods	No. of samples	No. of positive	No. of negative	P value
Microscopy	235	173 (73.6%)	62 (26.4%)	0.4497*
RDT	235	158 (67.2%)	77 (32.8%)	

Key: * Result not significant (p<0.05)

3.2. Comparison Between the Diagnostic Methods

The result of the performance of the microscopy and RDT is presented indicated in Table 3 below. A reference was generated and use as gold standard for the assessment of sensitivity of each method used in the analysis. This was explained as true positive if all the two methods tested positive and as true negative, if all the two methods tested negative. The true positive and negative values for both the methods stood as 147 and 53 respectively. The microscopy has more false (26) than RDT (11) while RDT recorded higher false negative value (24) than microscopy (09).

Table-3. Comparison between the diagnostic methods used

	Microscopy	RDT
True positive (TP)	147	147
True negative (TN)	53	53
False positive (FP)	26	11
False negative (FN)	09	24

3.3. Sensitivity and Specificity of Microscopy and RDT

The sensitivity and specificity of Microscopy and RDT is presented in Table 4. Sensitivity is the probability that a truly infected individual will test positive, while specificity is the probability that a truly uninfected individual will test negative. The microscopy was more sensitive than RDT (95.5%) although the RDT was more specific (82.8%) than microscopy (67.1%). The RDT has more positive predictive value (93.0%) than microscopy, although the microscopy showed more negative predictive value (85.5%).

Table-4. Sensitivity and specificity of Microscopy and RDT methods

Results	Microscopy	RDT
Sensitivity (%)	95.5	86.0
Specificity (%)	67.1	82.8
PPV (%)	85.0	93.0
NPV (%)	85.5	68.8

Key: PPV = Positive predictive value, NPV = Negative predictive value

4. Discussion

Accurate diagnosis of Plasmodium species is important not only for establishing correct treatment regiment, but also for application of effective malaria control strategies in highly endemic regions. In the present study, two

different diagnostic methods (Microscopy and Rapid Diagnostic Test) were employed for screening of febrile patients in Kano State, Nigeria. The prevalence of P. falciparum obtained from the sampled patients ranges from 73.6% and 67.27% for Microscopy and Rapid Diagnostic Test (RDT) respectively (the result is not significant (P= 0.4497)). This high prevalence was in conformity with the report of Obimakinde, *et al.* [20] who recorded 71.4% and 65% for Microscopy and RDT respectively, on the other hand, finding of this study was in contrast with the report of Pembele, *et al.* [21] where the microscopic analysis revealed 34.7% while RDT 44.44%. The finding of this study indicated that microscopic analysis of malaria parasite has higher prevalence than RDT. This result also agrees with the findings of Umeh, *et al.* [22] that of Mohammed, *et al.* [23] in Imo and Kano State respectively where microscopic analysis presented higher prevalence than RDT. However, this finding was in contrast to that of Mfuh, *et al.* [24] who found the prevalence of malaria by microscopy and RDT as 31% and 45% respectively.

Based on the finding of this study, higher percentage of prevalence by microscopy led to higher sensitivity and lower specificity than RDT. Similarly, RDT has more positive predictive value than microscopy, although the microscopy showed more negative predictive value. This finding correlates with that of Oyeniran, *et al.* [25]. The higher percentage prevalence malaria parasite detected by microscopy than RDT in this study may be attributed to the fact that the microscopy has an average sensitivity of about 50 – 100 parasites per microliter of blood while RDT are not sensitive to detect the intensity parasitemia of less than 100 parasites per microliter of blood. The RDT revealed the lowest prevalence of the infection in the present study which could be probably as a result of its inability to detect parasites when counts are relatively low. According to Berhane, *et al.* [26], the major constraint of RDT includes production of high false negatives due to gene deletions. The specificity of the commonly used RDT that detects histidine rich protein-II (HRP-II) of *P. falciparum* is limited when the parasite is cleared and the antigen remain in circulation for about 28 days (false positive) [1].

5. Conclusion

In this study, two different diagnostic methods (Microscopy and Rapid Diagnostic Test) were employed for the screening of febrile patients for detection of malaria parasite. The microscopy revealed the highest prevalence of the infection than RDT. The RDT produced least prevalence for the infection which could be probably as result of inability o the method to detect the presence of the parasite when parasitic counts are very low. However, RDT is quick and convenient method to use. It is recommended that the use of microscopy for detection of malaria parasite should be encouraged due to its sensitivity.

Acknowledgement

The authors wish to acknowledge the staff of General Outpatient Department (GODP) of Murtala Muhammad Specialist Hospital Kano State for samples provision. Thanks to the Technical staff of Parasitology laboratory of the hospital for use of Laboratory facilities.

Conflict of interest

The authors declare no conflict of interest exist

References

[1] World Health Organization WHO, 2003. *Universal access to malaria diagnostic testing. An operational manual*. Geneva: World Health Organization.

[2] World Health Organization WHO, 2002. *Universal access to malaria diagnostic testing. An operational manual*. Geneva: World Health Organization.

[3] World Health Organization WHO, 2000. *New perspectives: malaria diagnosis*. Geneva: World Health Organization.

[4] Alaba, A. O. and Alaba, O. B., 2003. "Malaria in rural Nigeria: Implication for the millennium development goals." *African Development Review*, vol. 21, pp. 73-85.

[5] World Health Organization WHO, 2010. *Guidelines for the treatment of malaria*. Geneva: World Health Organization.

[6] Feleke, D. G., Tarko, S., and Hadush, H., 2017. "Performance comparison of CareStartTM HRP"/pLDH combo rapid malaria test with light microscopy in North-Western Tigray, Ethiopia: A cross-sectional study." *BMC Infect Dis.*, vol. 17, p. 399.

[7] World Health Organization WHO, 2016. *World malaria report 2016*. Geneva: World Health Organization.

[8] World Health Organization WHO, 2009. *Parasitological confirmation of malaria diagnosis*. Geneva: World Health Organization.

[9] Hanscheid, T., 2003. "Current strategies to avoid misdiagnosis of malaria." *Clin Microbiol Infect*, vol. 9, pp. 497-504.

[10] Ohrt, C., Purnomo, M., Sutamihardja, M. A., Tang, D., and Kain, K. C., 2002. "Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials." *J. Infect. Dis.*, vol. 186, pp. 540–6.

[11] Ugah, U. I., Alo, M. N., Owolabi, J. O., Okata-Nwali, O. D., and Ekejindu, I. M., 2017. "Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area." *Malar. J.*, vol. 16, p. 189.

[12] Kim, S. H., Nam, M. H., Roh, K. H., Park, H. C., and Nam, D. H., 2008. "Evaluation of a rapid diagnostic test specific for Plasmodium vivax." *Trop Med Int Health*, vol. 13, pp. 1495–1500.

[13] Kozycki, C. T., Umulisa, N., Rulisa, S., Mwikarago, I., and Musabyimana, J. P., 2017. "False negative malaria rapid diagnostic tests in Rwanda: impact of Plasmodiumfalciparum isolates lacking hrp2 and declining malaria transmission." *Malar. J.*, vol. 16, p. 123.

[14] Azikiwe, C. C., Ifezulike, C. C., Siminialayi, I. M., Amazu, L. U., Enye, J. C., and Nwakwunite, O. E., 2012. "A comparative laboratory diagnosis of malaria: microscopy versus rapid diagnostic test kits." *Asian Pac. J. Trop. Biomed.*, vol. 2, pp. 307–10.

[15] Batwala, V., Magnussen, P., and Nuwaha, F., 2010. "Are rapid diagnostic tests moreaccurate in diagnosis of plasmodium falciparum malaria compared to microscopy at rural health centres?" *Malar. J.*, vol. 9, p. 349.

[16] National Population Commission NPC, 2014. *National population census result*. Abuja, Nigeria, pp. 1-565.

[17] Ali, M., Aisha, M., Ali, A., and Zage, U., 2019. "Effects of land uses on physicochemical properties of soil in Kano metropolis, Northern Nigeria." *Annals of Geographical Studies*, vol. 2, pp. 5-10.

[18] Nas, F. S., Yahaya, A., Muazu, L., Halliru, S. N., and Ali, M., 2020. "Prevalence of Trichomonas vaginalis among pregnant women attending ante-natal care in Kano, Nigeria." *Eur. J. Med. Health Sci.*, vol. 2, pp. 39-45. Available: https://doi.org/10.34104/ejmhs.020.39045

[19] Cheesbrough, M., 2006. *District laboratory practice in tropical countries*. 2nd ed. Cambridge University Press, pp. 80-85.

[20] Obimakinde, E. T., Simon-Oke, I. A., and Osunyemi, O. S., 2018. "The effectiveness of microscopy: Rapid diagnostic test and molecular assay in diagnosing malaria." *J. Parasit. Dis. Diagn. Ther.*, vol. 3, pp. 10-14.

[21] Pembele, G. N., Lázara, R. R., and Jorge, F., 2015. "Detection and species identification of malaria parasites by Nested-PCR: Comparison with light microscopy and with SD BIOLINE malaria Ag Test in Luanda, Angola." *Int. J. Trop. Dis. Health*, vol. 10, pp. 1-13.

[22] Umeh, S. I., Enwuru, C. P., and Egbuobi, R. C., 2013. "Diagnosis of malaria in pregnancy: a comparison of microscopy with rapid diagnostic tests." *Microbiol. Res. Int.*, vol. 1, pp. 35-9.

[23] Mohammed, Y., Dabo, N. T., and Kawo, A. H., 2015. "Comparison of microscopic examination and rapid diagnostic tests used to diagnose malaria among pregnant women in Kano, North–Western, Nigeria." *Int. J. Sci. Technol.*, vol. 5, pp. 47-52.

[24] Mfuh, K. O., Olivia, A. A., Obase, N. B., Livo, F. E., Calixt, D. M., Krupa, G., Rose, G. F. L., Diane, W. T., and Vivek, R. N. A., 2019. "Comparison of thick‐film microscopy, rapid diagnostic test, and polymerase chain reaction for accurate diagnosis of plasmodium falciparum malaria." *Malar. J.*, vol. 18, p. 73. Available: https://doi.org/10.1186/s12936-019-2711-4

[25] Oyeniran, O. A., Ajayi, O. O., Afolabi, A. Y., Oladipo, E. K., and Adepeju, A. A., 2017. "Comparison of rapid diagnostic tests and microscopy for Malaria." *Afr. J. Cln. Exper. Microbiol.*, vol. 15, pp. 158-164. Available: http://dx.doi.org/10.4314/ajcam.v15i3.7

[26] Berhane, A., Russom, M., Bahta, I., Hagos, F., Ghirmai, M., and Uqubay, S., 2017. "Rapid diagnostic tests to detect Plasmodium falciparum infections in Eritrea: an investigation of reported false negative RDT results." *Malar. J.*, vol. 16, p. 105.