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Full Length Research

Comparison of a rapid diagnostic (SD bioline malaria Ag p.f/pan) test kit with microscopy for detection of malaria parasites among blood donors in Kaduna state, Nigeria

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The screening of pre-transfusion blood donors for malarial parasitaemia to reduce the risk of transmission transmitted malaria is of paramount importance in efforts to enhance the safety of donor blood and control of morbidity and mortality caused by malaria in malaria endemic areas. This study evaluated the diagnostic performance of a rapid diagnostic test (RDT) for detection of histidine-rich protein II (HRP-II) of *Plasmodium falciparum* and *Plasmodium* lactate dehydrogenase forthe other *Plasmodium* species in the peripheral blood of 360 asymptomatic blood donors reporting at the blood transfusion in three secondary health facilities of Kaduna State, Nigeria for blood donation. Microscopic examination of Giemsa stained blood smears was used as the gold standard. Results showed that 27(7.5%) of the donors were parasitaemic by microscopy whereas 31(8.6%) were positive by RDT. Ten (10) of the donors were negative by microscopy but positive by RDT (false positive) while 6 were negative by RDT but positive by microscopy (false negative). Thorough microscopic examination by two experienced microscopists revealed that *P. falciparum* accounted for all (100%) the positive cases. There were no mixed infections and no other blood parasites were encountered. Further analysis of the results showed a sensitivity of 77.8%, specificity of 97%, positive predictive value of 67.7% and negative predictive value of 98.2%. RDT was substantially correlated (κ = 0.700) with the reference method. The RDT is a valuable tool for screening of blood donors and epidemiological studies. The cost of most of these RDTs remains a major obstacle to widespread use in areas of malaria endemicity.

Key words: Comparison,diagnostic,microscopy, malaria parasite, blood donors.

INTRODUCTION

Malaria remains an acute public health problem in many regions of the world despite tremendous progress in its control. According to the recent estimates, there were 214 million new cases of malaria and approximately 438,000 deaths in 2015 (WHO/UNICEF, 2015).More than two-thirds of malaria deaths globally were in children under 5 years of age. Fifteen countries mainly in sub-Saharan Africa, accounted for 80% of cases and 78% of deaths globally in 2015 (WHO/UNICEF, 2015). The report also indicates that about 3.2 billion people (almost half of the world's population) are at risk of malaria (WHO/UNICEF, 2015). Prevalence rates of malarial parasitaemia of between 4.1% and 77.4% have been reported in Nigeria (Akinboye and Ogunrinade, 1987; Ihanesebhor *et al*., 1996; Chikwem *et al*., 1997; Mbanugo and Emenalo, 2004; Ikeh and Okeke, 2005; Chigozie *et al*., 2006; Garba *et al*., 2009). This prevalence rate coupled with the annual requirement of about 1- 2 million units of blood in the country makes malaria a major transfusion transmissible infections (TTI) that is essential

to reckon with. Unfortunately, there are few strategies in place to prevent or reduce its impact in transfusion medicine in the country while tremendous efforts continue to go towards preventing the transmission of TTIs such as HIV and hepatitis B and C. Malaria case management remains a vital component of the malaria control strategies. This entails early diagnosis and prompt treatment with effective antimalarial medicines recommended for use in the country (FMOH, 2015). Because of The potential risks associated with transfusion related malaria (TRM), screening of donors is being strongly advocated in Africa(Mungai *et al*., 2001; KindeGazard *et al*., 2000; Mbanugo and Emenalo, 2004; Okocha *et al*., 2005; lkeh and Okeke, 2005; Umeanaeto*et al*., 2006; Mbanya and Tayou, 2007).Ithas also been noted that it was cheaper to screen blood donors for malaria parasitaemia than administrating antimalaria or prophylactic treatment especially when artesunate-containing drugs were used (Rajah *et al*., 2005).

Current methods of malaria diagnosis include light microscopy, fluorescent microscopy and Quantitative Buffy Coat (QBC), flow cytometry, automated blood cell analyzers, serological methods, molecular methods, laser adsorption mass spectrometry and Microarrays (Murray *et al*., 2003; Bronzan *et al*., 2008; Tangpukdee, 2009; Chiodini, 2014). Although significant advances have been achieved for the diagnosis of malaria, these technologies are still far from ideal, being time consuming, complex and poorly sensitive as well as requiring separate assays for sample processing and detection. Therefore, the development of a fast and sensitive method that can integrate sample processing with detection of malarial infection is desirable (Liu *et al*., 2016). Consequently, various modifications of the molecular tests are continually being developed and proposed. Recently, a two-stage sample-to-answer system based on nucleic acid amplification approach for timely (results within 1 hour) and highly sensitive (<1parasite/µl of blood) detection of malaria parasites have been developed (Liu *et al*., 2016). Also, a high-throughput malaria parasite separation using a viscoelastic fluid for an ultrasensitive PCR has been proposed (Nam *et al*., 2016). However, the affordability of these techniques in low resource settings like ours is doubtful for reasons ranging from the poverty level of these malaria endemic countries to political issues.

Microscopic detection of parasites on Giemsastained blood smears has been the mainstay for malaria diagnosis in laboratories for more than a century. This method is relatively simple and required minimal training of the microscopist; with an average sensitivity of about 50 to 100 parasites per micro liter (Wongsrichanalai *et al*., 2007; Iglesias *et al*., 2014).However, the quality of the results varies considerably from one laboratory to another, mainly due to the level of expertise of microscopists, quality of reagent and equipment, procedures, workload capacity and inefficient quality control procedures (Moura *et al*., 2014; WHO, 2009). Consequently, its perfectness as a goldstandard has been questioned (Wongsrichanalai *et al*., 2007; Iglesias *et al*., 2014). Nevertheless, it has continued to demonstrate a superior sensitivity over the RDT especially with increased *P*. *falciparum* parasitaemia (Ojurongbe *et al*., 2013; Oyeyemi *et al*., 2015).

Rapid diagnostic tests (RDTs) are immunochromatographic tests designed to detect parasite products in human blood. These methods are increasingly being used for malaria diagnosis because they are rapid and easier to use especially in resource limited settings and do not require trained personnel or special equipment (McMorrow *et al*., 2011). The need for a new, simple, reliable and cost effective diagnostic technique to overcome the deficiencies of light microscopy has also been recognized. Several RDTs for malarial parasitaemia have been developed. This study was undertaken to evaluate the diagnostic performance of the SD BIOLINE Malaria Ag P.f/Pan malaria combo test kit for the detection of malaria parasitaemia in blood donors in Kaduna state using microscopy as the reference standard.

METHODOLOGY

The study design and area

The study was a cross-sectional facility based study conducted in Kaduna State. The state is in North-western geo-political zone of Nigeria with a population of 6,066.562 (Encyclopedia Britannica, 2015) and 23 local government areas (LGAs) which are further grouped into 3 senatorial districts (north, central and south). It is a metropolitan as well as a cosmopolitan industrialized state with over 80 commercial and manufacturing industries. It is one of the education centers in Nigeria with many colleges and most recognized university in Nigeria. Agriculture is the mainstay of the state with about 80% of the people actively engaged in farming. It is defined by longitude 10°20' N and latitude 9° 03' E. The vegetation characteristic is that of the guinea savanna with scattered trees and shrubs. There are two distinct seasons the wet (rainy) which lasts from April to October and dry season that occurs from November to March. It experiences a rainfall of 1530mm in Kafanchan-Kagoro in the southeast and 10 15mm in Ikara/Makarfi districts in the northeast (Encyclopedia Britannica, 2015).

Ethical approval

Approval for the research was obtained from the ethical committee of the Kaduna State Ministry of Health. Patients' anonymity and good laboratory practice were

maintained. The findings were treated with utmost confidentiality and for the purpose of this research only.

Study population

A total of 360 apparently healthy individuals who reported for blood donation in three secondary health facilities of Kaduna state from January to December 2013 were recruited for the study. One hundred and twenty samples each were collected from General Hospital Kafanchan, MammanTsoho Memorial Hospital, Tudun Wada, Kaduna and HajiyaGamboSawaba Memorial Hospital, Zaria representing the southern, central and northern senatorial districts of the state respectively. These Hospitalsare secondary Health facilities offering specialized services for Kaduna State and neighboring states. Approval for the research was obtained from the Kaduna state Ministry of Health Ethics Committee. Blood specimens were collected from 360 consecutive, consenting, apparently healthy individuals who presented at the blood transfusion service centre of these health facilities for blood donation. They were HBV, HCV and HIV negative, aged between 18 and 65 years, weighed ≤50kg and had haematocrits of ≤36%.

Collection of samples

About 2 ml of blood was obtained from the 5ml of venous, blood collected from each of the donors for routine preliminary screening to determine the fitness of the donor into ethylene-diamine tetracetic acid (EDTA) bottles. The RDT was performed immediately or within one hour of specimen collection. In case of any delay, the samples were stored at 4^{o}C.

Detection of malaria parasites by microscopy

Thick and thin blood smears were made on-site at the time of collection and allowed to air dry. The dried blood smears were stained, examined and the malaria parasites identified as per standard methods (Cheesbrough, 2010).

Estimation of parasites density

The malaria parasite numbers/µl of blood was estimated by counting the number of malaria parasites seen against 200 white blood cells (WBC) in a Giemsa stained thick film and result expressed as described by Cheesbrough (2010).

$$\text{Number of parasites/µl of blood} = \frac{8000 \times \text{Number of parasites counted against 200 WBC}}{200}$$

Detection of malaria parasites usingRDT

The RDT was carried out using the SD BIOLINE Malaria Ag P.f/Pan malaria combo test kit manufactured by ATANDARD DIAGNOSTICS, INC. 156-68 Hagal-dong, Gieung-gu, Yongin-si, Korea 446-930, according to the manufacturer's instructions. The test detects the histidine rich protein 2 (HRP-2) antigens of *P. falciparum* and *Plasmodium* lactate dehydrogenase for the other *Plasmodium* species. Hence, can differentiate *P. falciparum* from the other three (*P. vivax*, *P.ovale* and *P.malariae* infections).

Data analysis

Results were analyzed using 2 × 2 contingency tables and McNemartest (Pembele *et al*., 2015). The kappa coefficient (Cohen's kappa coefficient as a measure of agreement for qualitative items) was determined to confirm the consistency of the results among the diagnostic tools. The kappa values were used to categorize the strength of agreement between the microscopic examination and RDT. Values were interpreted with the Landis and Koch classification (Pembele *et al*., 2015): poorly correlated (<0), slightly correlated (0- 0.20), fairly correlated (0.21-0.40), moderately correlated (0.41-0.60), substantially correlated (0.61-0.80), and perfectly correlated (0.81-1.0) (WHO, 1993; Mbarakurwa, 1996; Agomo *et al*., Tjitra *et al*., 1999; 1998; Garba *et al*., 2011 and Pembele *et al*., 2015). The key variables considered were;

Sensitivity (%)

$$= \frac{\text{number of True Positives (TP)}}{\text{number of True Positives (TP)} + \text{Number of false negatives (FN)}} \times 100$$

Specificity (%)

$$= \frac{\text{number of True Negatives (TN)}}{\text{number ofTrue Negatives (TN)} + \text{Number of false positives (FP)}} \times 100$$

$$\text{Positive Predicted Value (PPV, \%)} = \frac{TP}{TP+FP} X\ 100$$

$$\text{Negative Predicted Value (NPV, \%)} = \frac{TN}{TN+FN} X\ 100$$

Table 1. Distribution of Malarial parasites among blood donors in Kaduna state based on the malaria diagnostic methods used.

Test method	Number examined	Number positive (%)
RDT	360	31 (8.6)
Microscopy	360	27 (7.5)

Table 2. Contingency table of Comparison of the RDT with microscopy

RDT	MICROSCOPY		Total
	Positive	Negative	
Positive	21	10	31
Negative	6	323	329
Total	27	333	360

Table 3. Diagnostic performance of the RDT

Indices	Value
True positives	21
True negatives	323
False positives	10
False negatives	6
Sensitivity (%)	77.8
Specificity (%)	97
Positive predictive value (PPV) (%)	67.7
Negative predictive value (NPV) (%)	98.2
False negative rate (FNR) (%)	1.8
False positive rate (FPR) (%)	3
Efficacy (%)	95.6
Reliability (%)	74.7
Kappa (κ) value	0.7

$$\text{False Positive Rate (FPR, \%)} = \frac{FP}{FP+TN} \times 100$$

$$\text{False Negative Rate (FNR, \%)} = \frac{FN}{FN+TN} \times 100$$

$$\text{Efficacy/Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \times 100$$

$$\text{Test Reliability/J index} = \frac{(TP \times TN - FP \times FN)}{(TP + FN)(TN + FP)}.$$

RESULTS

Out of the 360 blood donors examined, 27 (7.5%) were parasitaemic by microscopywith an average parasite density of 126/µl of blood. The RDT detected antigenaemia in 31 (8.6%) blood donors (Table 1). A total of 21 (5.8%) donors were positive (True positives) and 323 (89.7%) were negative (True negatives) by both techniques. The result also showed that 10 donors were negative by microscopy but positive by RDT (false positives) whereas 6 were negative by RDT but positive by microscopy (False negatives). This gives rise to the results presented in Table 2. Analysis of the results using the standard format showed that RDT had sensitivity of 77.8, specificity of 97%, PPV of 67.7%, a NPV of 98.2% a FPR of 3%, a FNR of 1.8%and a substantially correlated κ value of 0.700 (Table 3).Thorough microscopic examination by two microscopists revealed only *P falciparum* accounting for all (100%) of the infections. No cases of mixed infections were encountered.

DISCUSSION

The result of this study showed that the SD BIOLINE Malaria Ag P.f/Pan malaria combo test kit detected more antigenaemia (8.6%) than microscopic parasitaemia (7.5%). This might be attributable to cross-reactivity with rheumatoid factors in the blood, but replacement of IgG with IgM in recent products reduces this problem (Laferl *et al*., 1997; Grobusch *et al*., 1999;Mishra *et al*., 1999),cross reactivity with the heterophile antibodies (Moody and Chiodini, 2002) and persistence of residual antigens for weeks after treatment (Heutmekers *et al*., 2012).The same observation was reported by Pembele *et al*. (2015).

The low proportion of false positive and false negative tests is characteristic of a good diagnostic test. False positive tests are attributed to cross-reactivity with the rheumatoid factor in the blood (Laferi *et al*., 1997; Grobusch *et al*., 1999) and cross-reactivity with heterophile antibodies (Moody and Chiodini, 2002). The false negative RDT may be due to low parasitaemia as in the majority of cases or by interpreting the RDT before the test line has fully developed (Heutmekers *et al*., 2012). This false negative result may also be caused by mutation of the HRP-2 gene (Wellems *et al*., 1991). The presence of anti-HRP-2 antibodies in humans has also been suggested as the reason why some tests were negative despite high parasite density (Biswas *et al*., 2005).

The sensitivity of 77.8% reported in this study is lower than the recommended value of >95% (WHO, 2000 and Whonsrichanalai *et al*., 2015). It is also lower than the80-100% previously reported in Nigeria (Ajumobi *et al*., 2015;) and other parts of the world(Buchachat *et al*., 2004; Hopkins *et al*., 2008;Msellem *et al*., 2009; Nicastri *et al*., 2009; Pemble *et al*., 2015).However, this sensitivity is higher than 42.5% reported elsewhere in

Nigeria(Oyeyemi *et al*., 2015).The high specificity of 97%, efficiency 95.6% and substantial correlation (κ = 0.700) with the reference method makes it a potential tool for the detection of asymptomatic malarial parasitaemia since the RDT is unlikely to miss-out the non-infected individuals. The type of samples evaluated and the regions from which they were collected have also been found to influence the performance of RDTs (Dzakah *et al*., 2014). The accuracy (sensitivity and specificity) of RDT is mostly dependent on the parasite species, transmission intensity, parasite density, and amount of circulating antigens, local polymorphisms of target antigen and persistence of antigens after treatment (Murray *et al*., 2008; Ishengoma *et al*., 2011).RDT sensitivity has been found to decline at parasite densities <500/µl of blood for *P. falciparum* (Whonsrichanalai *et al*., 2015). Extremely low sensitivity had been reported earlier for both HRP-2 and Pldh tests and batch specific problems were suspected (Rubio *et al*., 2001;Mason *et al*., 2002;Coleman *et al*., 2002; Huong *et al*., 2002; Iqbal *et al*., 2002). The storage temperature the RDT product was subjected to by the marketers could also cause a 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 result of this study further demonstrated the advantages of RDT technique over the microscopic technique as outlined by some previous researchers (Asianya *et al*., 1999; Peyron *et al*., 1994; Singh *et al*., 1997). They are commercially available in cassette form with all the necessary reagents. Reproducibility of the RDT was 100% and the test was easy to perform not requiring extensive training or equipment to perform or interpret the results. It was also noted that it was cheaper to screen blood donors for malaria parasitaemia than administrating antimalaria or prophylactic treatment especially the newly introduced ACTs. Similar observation was made by Rajab *et al*., (2005). The low parasite density recorded is not unexpected since the subjects were all apparently healthy.

Conclusion

In conclusion, we demonstrated that the SD BIOLINE Malaria Ag P.f/Pan malaria combo test kit is reliable, rapid, easy to use and simple to interpret. The RDT is a potential alternative to microscopy in places where the facilities for microscopy are poor. Therefore, we concur to earlier recommendations for the use of the RDT kit for the rapid screening of blood donors for malarial parasitaemia and epidemiological studies. This study contributes to the limited literature regarding the evaluation of RDTs for possible use in screening blood donors for malariaparasites in Kaduna state.

Comparative studies will be required to assess the relative utility of the available combined antigen detection tests in areas of malaria endemicity. However, current prices of most antigen detection tests including SD BIOLINE Malaria Ag P.f/Pan malaria combo test kit are too high to enable widespread utility in developing countries. Despite their advantages over microscopy and clinical diagnosis, their affordability in most malaria endemic areas can only be achieved if the cost of these rapid antigen detection tests is reduced.

Consent

Written informed consent was obtained from the blood donors.

Ethical approval

All experiments were examined and approved by theethical committee of the Kaduna State Ministry of Health.

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Competing interests

Authors have declared that no competing interests exist.

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