COMPARISON OF RAPID DIAGNOSTIC TEST (RDT), POLYMERASE CHAIN REACTION (PCR) AND MICROSCOPY METHODS IN THE DIAGNOSIS OF MALARIA AMONG AIRPORT WORKERS IN LAGOS

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ABSTRACT
Malaria is one of the leading causes of death in Africa, and it is highly endemic in Nigeria. It is responsible for about 70% of out-patient attendance at secondary health care facilities in Lagos State. This study assessed the diagnostic performance of three malaria detection methods among Nigerian airport workers. 268 blood samples were collected from febrile and non-febrile Airport workers in Lagos, and processed using three diagnostic methods: thick and thin Giemsa blood stained microscopy method for parasite species identification and count; SD-Bioline PfLDH/panLDH rapid diagnostic test (RDT) device; and Polymerase Chain Reaction (PCR) amplification as a two step procedure using dried blood spot (DBS). A total prevalence of 2.6% was detected by microscopy, 4.1% by RDT and 4.9% by PCR. Microscopy parasite density is 22 to 5.837/µl at prevalence rate of 4.9%. The species identified were 69% *Plasmodium falciparum* and 31% *Plasmodium malariae,* while *P. falciparum* and *P. malariae* co-infections were observed. The performance evaluation shows PCR having 100% sensitivity while microscopy and RDT had 53.8 and 84.6%, respectively. The negative predictive values are 97.8, 99.3 and 100% for microscopy, RDT and PCR respectively. The result of the PCR and RDT methods indicated that the gold standard method (microscopy) is less sensitive because additional 2.3% positive cases were detected by the PCR method.

Key words: Malaria diagnostic, RDT, PCR, microscopy, Lagos.

INTRODUCTION

Malaria affects mostly poor populations in tropical and sub-tropical areas of the world, including Sub-Saharan Africa and Southeast Asia (Brian et al., 2008; Isah et al., 2013). This has been attributed to the presence of temperature and rainfall conditions that are suitable for the development of the malaria-causing Plasmodium parasites in Anopheles mosquitoes (Brain et al., 2008).

Malaria is ranked second after human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS) in Africa and third after pneumonia and diarrheal diseases, as the leading cause of death in the general population and among children below 5 years with more than 90% of the global malaria deaths in Africa (WHO, 2013). The most vulnerable groups are young children and pregnant women (CDC, 2014). Other vulnerable groups are visitors from non-endemic regions of the world, those with sickle cell anemia and people living with HIV/AIDS (Lagos State Ministry of Health, 2015).

Although there has been malaria mortality reduction by 49% in the general population and by 54% in children below 5 years in Africa, an estimate of 209 million cases and 627,000 malaria deaths occurred in 2012. Although malaria burden disproportionately affects sub-Saharan Africa, both Nigeria and the Democratic Republic of Congo recorded 40% of the global malaria deaths in 2013 (WHO, 2013).

Malaria is highly endemic in Nigeria with 97% of the population at risk of infection (FMoH, 2005). Every year, 100 million estimated cases of

malaria and 300,000 deaths occur in Nigeria (Nigeria Malaria fact sheet 2011). Malaria is responsible for 29% of childhood deaths, 25% of infant mortality and 11% of maternal mortality in the country yearly (Nigeria Malaria Indicator Survey, 2010). It accounts for about 63% of all out-patient hospital attendance and 30% of all hospital admissions among children below five years (FMoH, 2005). In Lagos State, malaria has been reported to be the cause of consultation by 70% of outpatients attending secondary health care facilities (LSMoH, 2013).

Malaria impedes human development and accounts for more than N132 billion yearly losses due to the cost of treatment and absenteeism from work, schools and farms (FMoH, 2005). Malaria parasite is transmitted by the female anopheles mosquitoes. The species commonly responsible for malaria transmission in Lagos and other parts of the south West Nigeria are *Anopheles gambiense* and *Anopheles funestus* (LSMoH, 2015). These mosquito species have also been reported to breed in clear stagnant water in broken pots, unused discarded tyres and other areas where water can collect (LSMoH, 2015).

The clinical diagnosis where malaria is suspected based on the history, symptoms and clinical findings must always be confirmed by a Laboratory diagnosis which involves identification of malaria parasite or its antigen/products in the blood of the patient (Summiya, 2009). Giemsa stain of peripheral blood smear for the demonstration of malaria parasites is the gold standard for diagnosis of malaria (Nandwani et al., 2003).

However, malaria microscopy has been documented to have some limitations. They include reduced sensitivity at low parasitaemia, longer turn around time of 45 to 60 min and requirement for quality assurance of reagents. With low parasitaemia, using Giemsa stained blood smear method requires long periods of observation and experienced microscopists (Jamshaid et al., 1999). Malaria Rapid Diagnostic Tests (RDTs) detects specific parasite-associated antigens or proteins from infected individuals. It is fast, simple and less laborious in terms of quality assurance. However, sensitivity of this method also decreases with low parasitaemia (Georges, 1996).

Parasites nucleic acids detection using PCR is another technique though more accurate than microscopy but expensive and requires a specialized Laboratory (Georges, 1996). The analysis of DNA by the PCR can be useful when the results of conventional techniques for diagnosis of malaria are negative and it presents an accurate species identification and can detect low level Parasitaemia with sensitivity and specificity rate of 100% (Anthony, 2002).

The present study is hereby designed to evaluate the methods for the accurate diagnosis of malaria parasites among the participants.

MATERIALS AND METHODS

Study site

This study was carried out in the Aviation Medical Clinic, Lagos State. The patients are aviation workers and were taken to represent Lagos population. The clinic is located within the Murtala Mohammed Airport area. It is the medical department of the Federal Airports Authority of Nigeria (FAAN). It provides medical facilities to staff of FAAN, Nigerian Airspace Management Agency (NAMA). and Accident Investigation and Protection Bureau (AIB). It also responds to emergencies involving passengers and other airport users.

Ethical approval

This was sought and obtained from the Institutional Review Board of the Nigerian Institute of Medical Research (IRB-NIMR).

Sample collection and processing

268 blood samples were collected into Ethylene diamine tetra acetic acid (EDTA) containers and taken to the laboratory for examination. Dried blood spot was done created using sterile micropipette tips; 50 µl of blood sample was dropped unto Whatman's filter paper No.3. This was allowed to air-dry and kept in a desiccator until the DNA extraction was done as described by Berecsky et al. (2005).

Thick and blood thin films

Thick films were made onto a clean grease free microscope slides, air dried and stained with 3% Giemsa for 45 min, and were examined under

the microscope using 100× objective lens. Parasite density was determined by World Health Organization (WHO) method as detailed in Arsene et al. (2012).

Rapid diagnostic test

Rapid malaria test was performed using SD Bioline malaria Ag Pf/pan test kits according to the manufacturer's instruction. The results obtained was recorded in comparison with the control line as positive if a unique *Pf-p*LDH line appears indicating *P. falciparum* infection and /or pan-*p*LDH line indicating either an infection with *P. falciparum* or a mixed infection with *P. falciparum* and one or more of the non-falciparum species.

Parasite DNA extraction

Extraction of plasmodium DNA from dried blood spots is done by heating samples in a suspension of 5% Chelex 100 at a temperature of 100°C in an alkaline suspension, breaking down proteins including heat-labile enzymes. Chelex 100, an ion chelator, inactivates nucleases thereby limiting the destruction of the DNA and chelating heavy metals that may damage parasite DNA (WWARN SOP-03).

Polymerase chain reaction (PCR)

PCR amplifications was performed as a two-step procedure: Plasmodium genus-specific primers and Species-specific primers using dried blood spot (DBS) samples made on Whatman filter paper No.3 as detailed by Mekonnen et al. (2014).

RESULTS

A total of 268 blood films made from participants' blood samples were examined. 140 (52.2%) were males and 128 (47.8%) females. Table 1 shows the Malaria parasite species as stratified by Gender among participants, a total of 255 (95.1%) study participants were negative for all investigated species, while 7(5%) males and 3(2%) among females were positive. 7(2.6%) were positive by microscopy while 11(4.1%) and 13(4.9%) were positive by RDT and PCR respectively (Table 2). The parasite density range among the infected participants was 22 to 5,837 parasites/µl of blood. The relationship between age and prevalence of malaria parasite among the participants show that infected individuals were more among age group 35 to 44years as shown in Table 3.

Table 4 shows the performance evaluation where PCR had 100% sensitivity, while microscopy while microscopy and RDT had 53.8 and 84.6%, respectively. All the methods had the same specificity and positive predictive value of 100%. The prevalence obtained using microscopy is 4.7% while RDT and PCR were 4.9 %. Microscopy method had a negative predictive value of 97.8% while RDT and PCR had 99.3 and 100%, respectively.

Table 1. Malaria parasite species stratified by gender among participants airport workers in Lagos.

Sex	*P. falciparum* (%)	*P. falciparum* + *P. malariae* (%)	Negative (%)	Total (%)	χ^2
Male	7 (5.0)	3 (2.1)	130 (92.9)	140 (52.2)	1.88
Female	2 (1.6)	1 (0.8)	125 (97.7)	128 (47.8)	-
Total	9 (3.4)	4 (1.5)	255 (95.1)	268 (100)	-

Pf = *P.falciparum*, Pf + Pm = *P. falciparum* and *P. Malariae.*

Table 2. Relationship between microscopy, RDT and PCR.

Variable	Positive (%)	Negative (%)	Total (%)	95% confidence interval	χ^2
Microscopy	7 (2.6)	261 (97.4)	268 (100)	-1479.68-1747.69	
RDT	11 (4.1)	257 (95.9)	268 (100)	-1390.74 -1658.76	2.641
PCR	13 (4.9)	255 (95.1)	268 (100)	-1403.45-1671.48	

Table 3. Age related prevalence of malaria parasite among airport workers in Lagos.

Age group (Years)	No. examined	No. infected	Infected (%)	χ^2
15-24	9	0	0	4.25
25-34	78	5	6.4	
35-44	53	7	13.2	
45-54	107	1	0.9	
55-64	17	0	0	
65 and above	4	0	0	

Statistically significant Chi-square test P< 0.05 (i.e. P=0.002).

Table 4. Performance evaluation**.**

Variable	Prevalence (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Microscopy	4.7	53.8	100	100	97.8
RDT	4.9	84.6	100	100	99.3
PCR	4.9	100	100	100	100

PPV%: positive predictive value; NPV%: Negative predictive value.

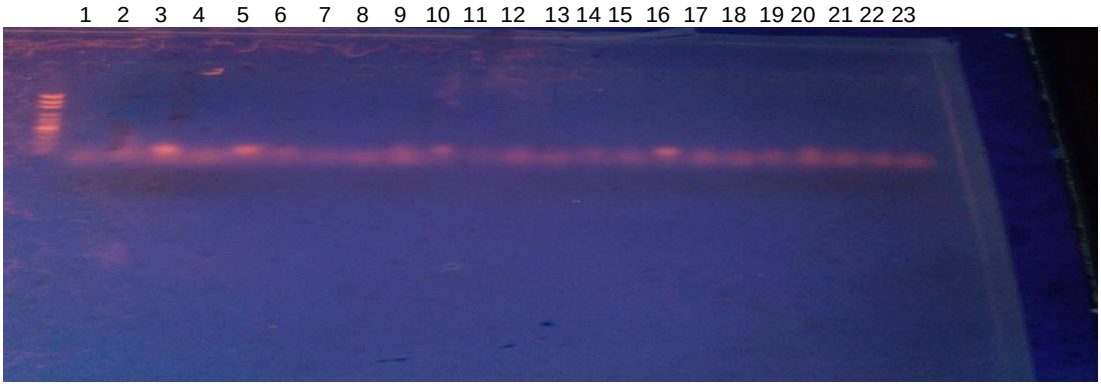

Figure 1. PCR result. Lane 3, 5, 7, 8, 10, 13, 18, 19, 20 - *P. falciparum*, Lane 4, 6, 9, 17 - *P. falciparum* + *P. malariae* and Lane 11 - Negative control.

Figure 1 shows the species differentiation of the 13 PCR-confirmed cases. 9 (69%) were positive for *P. falciparum* and 4 (31%) were positive for *P. falciparum* and *P. malariae* double infections. This shows that the malaria species stratified by gender among airport workers χ^2 is not significant at 5% level of significance. There is a significant association in gender related to malaria parasite among the airport workers sampled.

The table shows that 0.26 > 0.05. There are significant differences between (Microscopy, RDT, and PCR) the three tests 5% level.

DISCUSSION AND CONCLUSION

This study was carried out between May and September, 2014 among airport workers in Lagos to detect the malaria parasite prevalence using microscopy, SD bioline malaria antigen Pf-pLDH/pan-pLDH and Polymerase chain reaction. These methods were evaluated to determine their efficacy. There has been no previous report on the prevalence of malaria parasite among airport workers in Nigeria. The work done by Dougnon et al. (2015) was carried out just among travelers who also attend Aviation Medical Clinics.

The results obtained in this study show a prevalence of 4.9% which is contrary to the 22% reported by Dougnon et al. (2015). The difference in this result can be attributed to the sample size of the previous study and the type of participants recruited compared to the recent report. It can also be due to the fact that the group used in the previous work was symptomatic.

Airport staff are part of the citizens, so the results obtained correlates with the 7.7% prevalence rate reported by Agomo et al. (2009) among pregnant women attending ante-natal clinics in Lagos, south-west Nigeria; and 6.8% reported by Uko et al. (1998) in the study of malaria infection of the placenta and neonates in Calabar. On the other hand, higher prevalences of 28, 32.5 and 35.7% had been obtained in other parts of Nigeria by Agboola et al. (2010) among donors in Lagos; Isah et al. (2013) among donors in Sokoto; and Umaru et al. (2015) among those attending the General hospital Markafi Kaduna state respectively.

Of the 268 participants tested, 140 (52%) were males while 128 (48%) were females. Among the males, 10 (3.7%) were positive while 3 (1.1%) females were positive for Plasmodium species. The gender distribution of the prevalence shows that male participants are more infected with malaria parasites than females which correlate with the report of the study conducted by Dougnou et al. (2015) among travelers in Lagos Nigeria in the same facility and the findings of Umaru et al. (2015) among patients attending General hospital Makarfi, Kaduna. This suggests that males are more prone to malaria than females, since women tend to sleep under mosquito treated nets than males (Dougnou et al., 2015). Also males are often less compliant with chemo-prophylaxis than women (Helena et al., 2005). The specie of Plasmodium prevalent among the group was *P. falciparum* with 69%.

There is a significant difference (p.0.05) between the three methods used. In this study, 7 (2.6%) participants were positive for malaria parasite by microscopy, 11 (4. 1%) were positive by RDT and 13 (4.9%) by PCR. Out of the total 13 (4.9%) confirmed positives by PCR, 6 (2.2%) was missed by microscopy which may be due to low parasitaemia while only 2 (0.75%) was missed by RDT. This is so because Plasmodium infected asymptomatic individuals have low parasite densities which are often missed by microscopy according to Miguel et al. (2013). All the *P. malariae* positives were detected by PCR which may be due to the level of *P. malariae* parasitaemia in their peripheral blood. The performance of the methods used was greatly influenced by the level of parasitaemia in the peripheral blood.

This study shows that *P. falciparum* and *P. malariae* were the only species of *Plasmodium* associated with the study population. Igbeneghu and Odaibo (2012) reported *P. falciparum* and *P. malariae* in Iwo community, Southwestern Nigeria. Also Ademowo et al. (1995) reported *P. falciparum* and P. malariae in a rural community in southwestern Nigeria. May et al. (1999) and Molineaux et al. (1980) reported *P. falciparum*, *P. malariae* and *P. ovale* in Ibadan, southwestern Nigeria and Garki Northern Nigeria respectively,

In this study, the sensitivity of microscopy was 53.8% which may be due to very low parasitaemia in the participants, nevertheless, the specificity was very high (100%). In addition, the sensitivity of RDT was 84.6 which correlate with the 85 and 87%. Hopkins et al. (2007) in the comparison of HRP2- and pLDH- based rapid diagnostic tests for malaria in Kampala, Uganda; and Iqbal et al. (2001) in the diagnosis of imported malaria by pLDH and PfHRP2-based immunocapture assays respectively.

The RDT also had a specificity of 100% which may be attributable to the fact that the enzyme activity in pLDH is no longer detectable after parasite clearance. The positive predictive value (PPV) was 100% while the negative predictive value (NPV) was 99.3% which means that an individual with positive RDT has a high probability of having malaria. The evaluation of PCR reveals a sensitivity and specificity of 100% each with 100% of PPV and NPV. This means that any individual with a positive PCR has malaria. This is because PCR method has the ability to detect parasite macromolecules (Kassberger et al., 2002)

The age distribution of participants in this study revealed that malaria is more prevalent among participants in group 35 to 44 age years (13.2%). This come to an agreement with the reports of Agomo et al. (2009) that among pregnant women in Lagos, Nigeria malaria prevalence increase in age group above 34 years and that of Marielle et al. (2003) which states that pregnant women in Gabon reported a high prevalence within age group 36 to 39 years. Prevalence of 6.4 and 0.9% was recorded amongst the age groups 25 to 34 years and 45 to 54 years, respectively.

Conflicts of interest

The authors have none to declare.

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