Comparative Analysis of Rapid Diagnostic Kits and Microscopy in Plasmodium falciparum infected population in Abeokuta, Nigeria

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ABSTRACT

Malaria is an endemic disease in many parts of the tropical countries characterized with high rate of mobidity and frequent mortality particularly among children and immunocompromised individuals. phenotypic detection of most prevalence species; *Plasmodium falciparum* (*pfal*) is a major factor indices towards malaria prevention and management in southwest Nigeria. Objective: The study evaluates comparative analysis of commonly used diagnostic tools in detection of *pfal* among infected population in Abeokuta, Nigeria. Blood samples of 320 febrile patients attending the out-patient departments of the major health facilities in Abeokuta, Nigeria were examined for *pfal* antigenemia using Rapid Diagnostic Test kit and further phenotypically characterized with microscopy and their demographic factors (age and sex) as risk factor for *pfal* infectivity were evaluated. Comparative prevalence and detection rate of RDT Kits and Microscopy techniques were determined. Also correlation of parasiteamia with the degree of RDT positivity was evaluated while the Cut off point for RDT positivity based on parasite count (Parasitaemia) was determined. Results: Among the subjects examined, detection rate of *pfal* is significantly low with use of RDT (male; 7.2% and female; 15.9%) compare to microscopy (male; 20.3% and female 32.8%) ($p<0.05$). Generally, a high prevalence rate of 16.3% and 19.7% were found in the age groups <1-10 years and 31years and above respectively followed by age group 21-30 years which recorded 11.3%, while the least prevalence rate of 5.9% was recorded by age group 11-20 years using Gold standard. *Pfal* parasite density and RDT positivity significantly correlate ($r=0.713$, $p=0.001$) with subject parasitaemia level cut off mean value of 2.6%. Conclusion: *Pfal* infection remains prevalent, irrespective of sex and age. The use of both microscopy and RDT would facilitate accuracy in malaria diagnosis mostly in endemic area with primary diagnostic resources. However, RDT method should only be employed as adjunct to Microscopy for effective parasite detection, prevention and management

Keywords: *P. falciparum*, Diagnosis, Microscopy, RDT, Antigenemia, Parasitemia

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1. INTRODUCTION

Malaria caused by *Plasmodium falciparum* (*Pfal*), is a protozoan infection of red blood cells, transmitted through bites during blood-meal of female *Anopheles* mosquitoes, a definitive host, into man who serves as an intermediate host (Arora and Arora, 2008; Cheesbrough, 2006; Ross, 1999). Malaria has been declared as one of the most dreaded diseases of mankind, as an estimated 400 thousand people die of malaria each year (Ilesanmi *et al.,* 2017). Its high mortality in children and pregnant women and immunocompromised individuals, pose a major challenge in Africa especially in Nigeria (WHO, 2013; WHO, 2005).

Pfal infection still remains a major public health problem in about 104 countries (Abdoulaye *et al.,* 2016; WHO, 2013). It is estimated that 3.3 billion people worldwide are at risk of malaria, with 90% of cases occurring in Africa (WHO, 2011) while about 40% of malaria deaths occurred in just two countries – Nigeria and the Democratic Republic of Congo (WHO, 2013). Records showed that Nigeria, with a population density of over 134 million has more than 100 million cases of malaria and a mortality of 300,000 annually (WHO, 2015). To improve case management, the World Health Organization (WHO) recommends that parasitological confirmation by microscopy or malaria rapid diagnostic tests (RDTs) is conducted in all patients with a suspected prognosis of *Pfal* infection prior to commencing treatment (WHO, 2010).

Microscopic detection and identification of *Plasmodium species* in Giemsa-stained thick blood films (for screening the presence of malaria parasite), and thin blood films (for species' confirmation) remains the gold standard for laboratory diagnosis (Bharti *et al.,* 2007). Microscopy is laborious and ill-suited for high-throughput use, and species determination at low parasite density as it requires skill personnel and electricity supply. Therefore, in remote rural settings, e.g. peripheral medical clinics with no electricity and no health-facility resources, microscopy is often unavailable (Erdman *et al.,* 2008). Therefore, administration of RDTs, (lateral-flow immunochromatographic tests) to detect specific antigens produced by malaria parasites and are rapid and simple to carry out. It does not require electricity or specific equipment or skills in malaria management. This indeed is a paradigm shift in case management based on the World Health Organization (WHO) recommendation. However, the value of a "test before you treat" policy depends on accurate diagnosis since false-negative tests may result into serious consequences for individual patients and population at risk (WHO, 2013; Moody *et al.,* 2002).

2. METHODS

Sampling: Venous bloods were randomly collected from 320 febrile patients who were attending out-patient clinics at the major health facilities in Abeokuta, Nigeria. The blood samples were transported and stored at 4°C in cold chain before analysis.

Ethical consideration: The protocol and blood collection for the study was approved by Research Ethics Committee of Sacred Heart Hospital, Abeokuta which serves as a referral centre for Internal medicine. Ethical approval was given for the study and each participant was assigned with unique study number and bio-data of each subject remain confidential.

Microscopic examination: Both thick and thin blood films of each subject recruited for the study were made from the blood samples in triplicate, fixed and stained with Giemsa and examined using oil immersion objective (100x objective lens) and parasitemia was recorded as described by Cheesbrough (2010).

Rapid diagnostic tests (RDT) preparation: The blood samples that were collected into the EDTA bottles from febrile patients were tested using SD Bioline Rapid Test kits based on Immunochromatographic method. Briefly, 2 drops from each of the blood sample were placed in an inner ring on well labelled RDT kits using different sterile applicator sticks, 4 drops of Assay diluent was added into the outer well to form a blood buffer mixture. After 15 minutes according to the manufacturer's instruction, immunochromatographic Assay results were observed and recorded in which the presence of two bands (test line and the control line) indicated a positive result, while one band (only the control line) showed a negative result (Kim *et al.,* 2008; Lee *et al.,* 2008; Park *et al.,* 2006).

Data analysis: Prevalence of *Pfal* infection rate was determined according to gender and age groups of the subjects using descriptive statistical analysis and significance difference of comparative detection rate of microscopy (Gold standard) and RDT method was also determined with chi square at p value <0.05. Correlations between parasitaemia level between microscopy and RDT among the subjects and the cut off level were determined taking significant at p<0.05 using SPSS version 23.

3. RESULTS

Prevalence of malaria according to sex using microscopy method showed higher prevalence rate of 32.8% among female when compared to 20.3%% rate found in male. Similarly, the prevalence rate of malaria according to sex using RDT method revealed higher rate of 15.9% among female, when compared with 7.2 % recorded in male (Figure 1).Microscopy and RDT methods are compared based on their prevalence rate. It was observed that microscopy method had a higher prevalence rate of 32.8% for female and 20.3% for male than the results obtained for the RDT with 15.9% and 7.2% for female and male respectively (Figure 1).

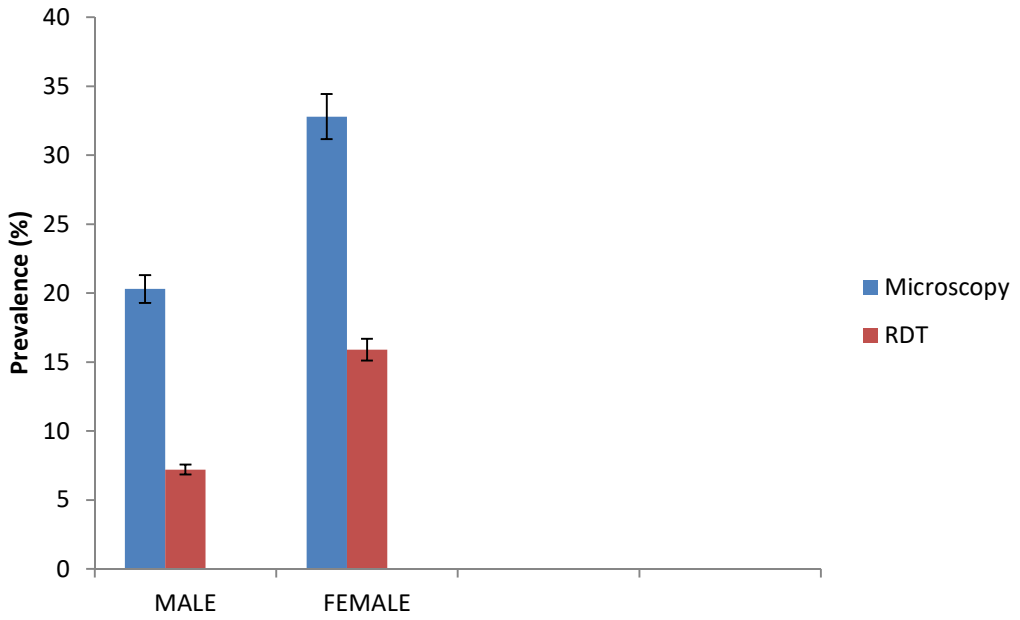

Figure 1: Comparison Prevalence rate of malaria parasite using microscopy and RDT methods among Gender

Table 1 shows the comparison of detection rate between Microscopy and RDT methods according to age. With microscopy method, a high malaria detection rate of 16.3% and 19.7% were found in the age groups <1-10 years and 31years and above respectively followed by age group 21-30 years which recorded 11.3%, while the least detection rate of 5.9% was recorded by age group 11-20 years whereas with RDT method, the highest detection rate of 10.0% was found in the age group <1-10 years, followed by 6.6% recorded by age group 21-30 years and 5.0% by age group 31years and above, while the least rate of 1.6% was recorded by age group 11-20 years.

Comparatively, the detection rate among different age groups was found to be higher with the microscopy method than that obtained from the RDT method which is also significant (P< 0.05).

Table 1: Comparison of Detection Rate between Microscopy and RDT by Age

Age	N	Microscopy	RDT	X^2	P-value
		Positive n_1(%)	Positive n_2(%)		
<1 - 10 years	84	52(16.3)	32(10.0)	34.813	0.001
11 - 20 years	24	19(5.9)	5(1.6)	5.195	0.047
21 - 30 years	57	36(11.3)	21(6.6)	37.333	0.001
31 years and above	79	63(19.7)	16(5.0)	14.519	0.001
Total	244	170(53.1)	74(23.1)		

Key: N-Total number of sample examined, n_1-number positive for Microscopy, n_2-number

Table 2 shows the comparison of detection rate between Microscopy and RDT methods according to Sex. Male recorded higher detection rate of 20.3% for Microscopy when compared to 7.2% recorded by RDT. Similarly, 32.8% and 15.9% were recorded for female in Microscopy and RDT respectively (Table 2). Detection rate of malaria parasite by Microscopy and RDT methods significantly differ in both Male and Female subjects (P< 0.001).

Table 2: Comparison of Detection rate between microscopy and RDT according to sex

Sex	n/N	Microscopy	RDT	X^2	P-value
		Positive n_1(%)	Positive n_2(%)		
Male	88/123	65(20.3)	23(7.2)	25.243	0.001
Female	156/197	105(32.8)	51(15.9)	60.295	0.001
Total	244/320	170(53.1)	74(23.1)		

Key: n-total number of positive subjects; N-Total number of sample examined for each sex; n_1-number positive for Microscopy, n_2-number positive for RDT

A significant strong correlation was estimated between malaria parasite density and RDT positivity using Pearson's 2-tailed correlation analysis (r=0.713, p=0.001) (Figure 4).

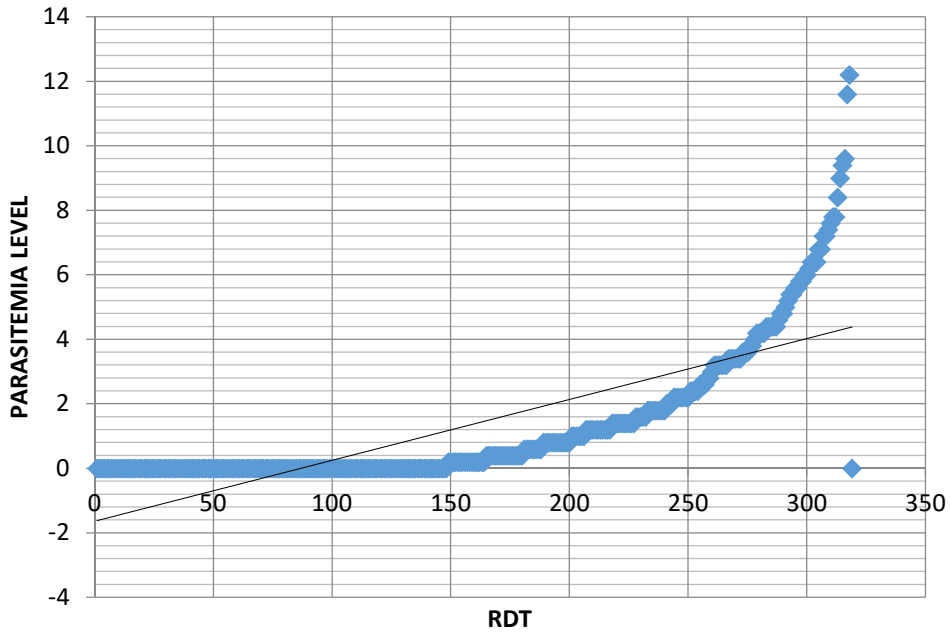

Figure 2: Correlations between Parasitaemia Level and RDT Positivity among the Subjects (r = 0.713, p = 0.001)

The Cut off point for RDT positivity based on parasite count (Parasitaemia) for all the subjects recruited for the study was shown in Table 3. Among the subjects tested positive for malaria parasite, 0.20% and 12.2% fall between the minimum and maximum range of positivity for RDT parasitemia detection and the cut off mean value estimated for parasitaemia is 2.6%.

Table 3: Cut off point for RDT Positivity based on Parasite count (Parasitaemia)

Parasitemia level	Minimum (%)	Maximum (%)	Cut-off value (Mean±SD) (%)
Total sample tested positive (N=170)	0.20	12.20	2.60±2.47

Key: N- Total Number positive, %-rate of positive samples.

4. DISCUSSION

In the present study, endemicity of malaria in the Southwest Nigeria is now a threat to livelihood and socio-economic well being of many residents. Inaccurate diagnosis of *Pfal* infection among various age group and gender is a serious challenge. It was observed the prevalence of malaria according to sex using both microscopy and RDT methods showed higher prevalence rate among female when compared to the male. Similar finding of high malaria prevalence among female than male was reported by Olasunkanmi *et al.,* (2013) in Abeokuta; Kalu *et al.,* (2012) at Aba and Umuhia in Abia State and at Oyi Local Government in Anambra State, Nigeria (Onyido *et al.,* 2011).

These recorded higher malaria parasite prevalence rates in females could be due to low immune status of as a result of monthly ovulation, pregnancy, child birth and breast feeding of the nursing mothers which tend to lower the female immune response to infection, thereby, predisposing them to malaria infection and other diseases. Comparison of detection rate between Microscopy and RDT methods by age was found to be higher with microscopy method than that RDT method with significance difference ($P< 0.05$). This may be due to the presence of high number of malaria parasites in the blood samples but with relatively low antigenaemia. This therefore shows that the microscopy method is better than the RDT method for diagnosis of *Pfal* infection. The high rate of detection of *pfal* infection in age group below 10 years suggests congenital infection from mother to child and undeveloped T lymphocyte cells needed for the antibody production against the malaria parasite, thus made this age group vulnerable to malaria (Ruqayyat *et al.,* 2017).

However, the higher percentage *pfal* infection detection rate observed among age group 31years and above is however, in contrast to recent similar study where it was generally observed that the more the ages, the more the body's immune system can suppress the density of parasites, thus affecting its clinical manifestation (Siahaan *et al.,* 2018). It may be suggested that negligence and carefree attitude among age group 31years and above towards *pfal* infection preventive measures and strategies cum sample distribution. A significant strong correlation was estimated between *pfal* parasite density and RDT positivity using Pearson's 2-tailed correlation analysis ($r=0.713$, $p=0.001$) (Figure 4). This shows that the higher the level of parasitaemia recorded in microscopy, the higher the RDT positivity rate. This implies that *pfal* infection still remains a public health problem as its diagnosis with both microscopy and RDT could detect the infection. Also, this evidence suggests that symptom-based diagnosis is not affirmative for parasite detection but *pfal* infection. Therefore, the use of microscopy or RDT for *pfal* parasite detection is significant and relevant for proper diagnosis. The Cut off point for RDT positivity based on parasite count (Parasitaemia) for all the subjects recruited for the study tested positive for *pfal* parasite is 2.6% while the parasitemia level ranged between 0.20% and 12.2%. This further indicates the efficacy of RDT for detection of *pfal* parasitemia and can be attributed to the RDT accuracy in detecting clinical episodes of malaria in symptomatic and asymptomatic individuals.

5. CONCLUSION

From this study, malaria still poses a public health challenge to the populace with high prevalence irrespective of sex and age. Therefore, prompt and accurate diagnosis of malaria remains the effective management and could provide early preventive intervention. Also, use of both microscopy and RDT would facilitate accuracy in malaria diagnosis mostly in endemic area with low primary diagnostic resources.

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Conflict of Interest:
None

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