AGRICULTURAL AND BIOLOGICAL SCIENCES

COMPARATIVE DIAGNOSIS OF MALARIA USING ROUTINE MICROSCOPY AND RAPID DIAGNOSTIC TECHNIQUE WITH LACTAN DEHYDROGENASE.

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ABSTRACT
This study investigated the comparative analysis of rapid diagnostic techniques (RDT) and microcospy for malaria diagnosis using a population of clients attending National Institute for Pharmaceutical Research and Development (NIPRD), Research Clinic. Two hundred and fifty-one clients with a clinical suspicion of malaria based on fever from Idu and surrounding communities were tested with both methods. Diagnostic test outcomes were separated into different age groups to assess the impact of age on test outcome. The significance of age-groups was assessed with Chi-Squared test at 95% confidence interval. Of the 251 clients 110(43.82%) were positive by RDT, compared to 87(34.66%) by microscopy. The difference between the two methods was not statistically significant (Tab. t = 2.306 > Cal t = 0.3833; p > 0.05). Rapid diagnostic technique RDT test, detected malaria more in age group 11-20 years (17.3%) and 21-30 years old 38(15.14%), though lower in older ages 31 - ≥50 years (<7%). Using Chi-Squared ($\chi 2$), there was no significant difference in the detection of malaria in the age groups (Tab $\chi 2$=9.488 > Cal χ 2 df.4, 1 = 3.32, p > 0.05). In relation to sex, seroprevalence of malaria parasite was higher 41(35.96%) males compared to 46(35.58%) females with no significant difference (Tab $\chi 2$ = 9.488 > Cal $\chi 2$ =df.4, 1 = 4.58; p > 0.05). In all, RDT detected higher values in both sexes 51(44.74%) and 59(43.07%) for males and females respectively. The Pf (HRPII/PAN-LDH); HRP2-based RDT showed higher sensitivity compared to microscopy in detection of malaria and may perhaps be more appropriate for screening of malaria infection. Large scaleassessment of RDT for malaria screening is endorsed forPrimary Health Care (PHC) centres, and field research on malaria studies.

KEYWORDS: *Rapid diagnostic technique (RDT), Microscopy, Malaria*

INTRODUCTION

Malaria is one of the highest killer diseases affecting most tropical countries, especially, Africa. It affects over 500 million people worldwide and over one million children die annually from malaria parasite (Amazu *et al.,* 2009). Of the entire human malaria parasite, Plasmodium falciparum, is the most pathogenic and is frequently fatal if untreated in time (Nandwani *et al.*, 2005). Clinical diagnosis is imprecise but remains the basis of therapeutic care for the majority of febrile patient in malaria endemic areas, where laboratory is often out of reach. Rational therapy of malaria is essential to avoid non- target effects, to delay the advent of resistance and to save costs on alternative drugs. Accurate diagnosis is the only way of effecting rational therapy. Confirmatory diagnosis before treatment initiation recently regained attention, partly influenced by the spread of drug resistance and thus the requirement of more expensive drugs unaffordable to resource poor countries (Barmish *et al.*, 2004). Traditional practice for outpatient has been to treat presumptively for malaria based on a history of fever but a significant proportionof those treated may not have parasites (over 50% in many settings) and hence waste a considerable amount of drugs (Shillcut *et al*., 2008). This old clinical based practice is still relevant today especially in infants where time spent on getting a confirmatory laboratory diagnosis could lead to increased fatality.

WHO currently makes the tentative recommendation that parasite based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high- prevalence areas and certain other situations (WHO, 2006). The traditional method of microscopic identification of parasites, however, is not only daunting in poor power setting but also time consuming and requiring a lot of expertise/ training. Thus microscopy in Africa is generally limited to larger clinics. This peripheral blood smears/ microscopy, however, still remains the gold standard in laboratory diagnosis of malaria (Nandwani *et al*., 2005).

A relatively new and easy to perform tests has been developed to diagnose P. falciparum rapidly without recourse to a microscope. Most recently, the test could detect P. vivax infections (Cheesbrough, 1998). The diagnosis is based on the immunochromatographic detection of antigen HRP2 (histidine – rich protein 2) or specific pLDH (parasite lactate dehydrogenase). Both HRP2 and pLDH are produced by the parasites during their growth and multiplication in red cells (Cheesbrough, 1998). It is against this back drop that the study aims at comparing the rapid test to microscopy the gold standard in the diagnosis of malaria in Abuja, north central Nigeria.

MATERIALS AND METHOD

Abuja the Federal Capital Territory (FCT) is located geographically at the centre of Nigeria. It lies between latitude 8º02" and 9º25"N; longitude 6º45" and 7º45"E. The F.C.T falls in the semi-seasonal equatorial climate zone with associated contrasting wet and dry periods.

A total of 251 blood samples were collected randomly into EDTA bottles and taken to the diagnostic laboratory in NIPRD clinic for processing and analysis.

Thick and thin films were prepared by the method of Menderietta *et al.,* (2007). The serum was separated from the blood samples into cryotubes for RDT.

2µL of blood was placed using an applicator on a new glass slide, another new slide was run forward against the old slide with blood sample, creating a head, body and tail, Slides were placed on a rack to dry for about 30 minutes. Slides were fixed in 70% alcohol and allowed to dry to avoid washing off. Giemsa stain was used to flood the slide and allowed to dry for 30 minutes. Slides were rinsed with distilled water with pH of 7.2 then allowed to dry. Oil immersion was used on slide and then mounted on a microscope with X100 objective lens.

12µL of blood was placed on a new glass slide, another slide was used to smear blood sample in a ring form. Slides were placed on a rack to dry for about 45 minutes. Slides were flooded using Giemsa stain and allowed to dry for about 35 minutes then rinsed with distilled water pH of 7.2, then allowed to dry. Oil immersion was applied and slides were mounted on a microscope with X100 objective lens and viewed.

Procedure for Rapid Diagnostic Test (RDT) Kits

5µL of separated serum of each blood sample was placed into sample well, 80µL of assay buffer was added into developer well and results were read within 10 – 15 minutes.

Parasites observed in microscopy were identified by their morphological characteristics according to illustrations from Coatney *et al.,*(1971).

Chi-square test was used to identify any significant differences in malaria prevalence among age groups and student t-test was used to calculate any significant difference using microscopy and RDT in malaria diagnosis. Values of $P \geq 0.05$ were considered not significant.

RESULTS AND DISCUSSION

Two hundred and fifty-one clients with clinical suspicion of malaria, based on fever from Idu and surrounding communities had their blood samples tested with both methods. Of the 251 clients 110(43.82%) were positive by RDT, compared to 87(34.66%) by microscopy, (Fig. 1). Though this difference was noted for the two methods, it was not statistically significant (Tab. $t = 2.306 > Cal\ t = 0.3833$; $P > 0.05$) between microscopy and RDT detection techniques.

Rapid diagnostic technique RDT test(fig, 2) detected

malaria more in age group 11-20 years 43 (17.13%) and 21-30 years old 38(15.14%) respectively, but lower in older ages 31 - ≥50 years (<7%) with no significant difference (Tab $\chi 2$=9.488 > Cal χ 2 df.4,1 = 3.32, p > 0.05) in the detection of malaria parasite in the age groups (Fig, 2). In relation to sex, seroprevalence of malaria parasite was 41(35.96%) in males compared to 46(35.58%) in females using microscopy while the RDT detected 51(44.74%) in males than 59(43.07%) prevalence in females respectively (Table 1). There was no significant difference in detecting malaria with either of the method (Tab $\chi 2$ = 9.488 > Cal $\chi 2$ =df.4, 1 = 4.58; p > 0.05). In all, RDT detected higher values in both sex 51(44.74%) and 59(43.07%), for males and females respectively.

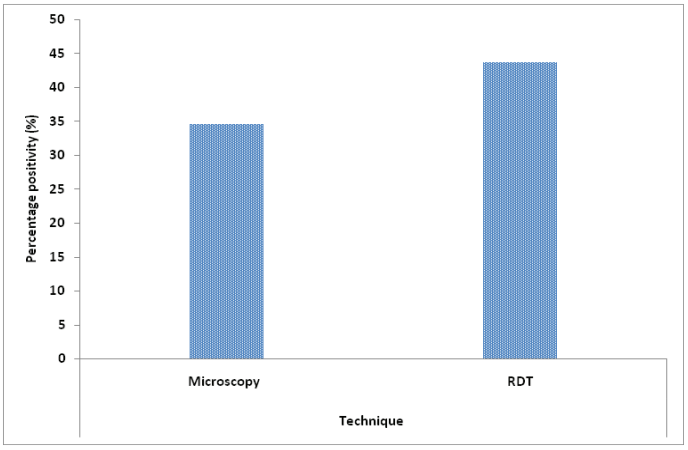

Figure 1: Comparative Diagnosis of Malaria Using RDT and Microscopic Techniques

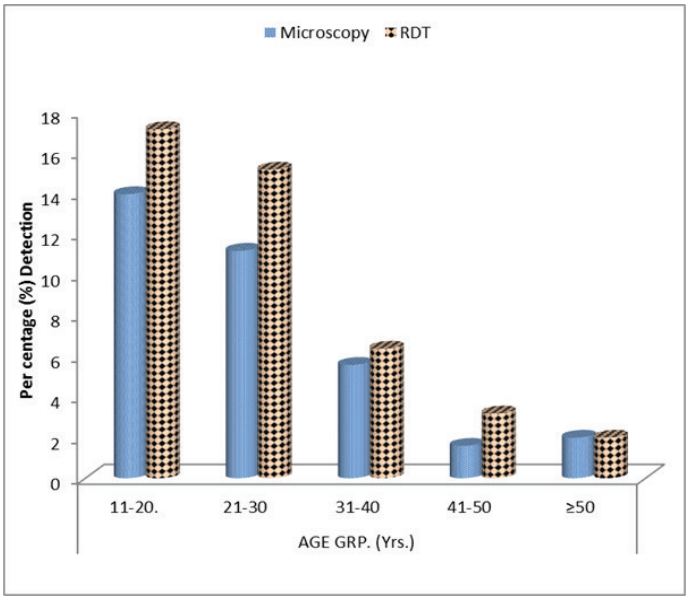

Fig 2: The comparative diagnosis of malaria using RDT and microscopy based on age.

Table 1:Comparative diagnosis of malaria using rapid diagnostictechnique(RDT) and microscopy test based on sex.

DIAGNOSTIC TEST	MALE		FEMALE		TOTAL	
	Number sampled	Number (%) positive	Number sampled	Number (%) positive	Number sampled	Number (%) positive
RDT	114	51 (44.74)	137	59 (43.07)	251	110 (43.82)
MICROSCOPY	114	41 (35.96)	137	46 (35.58)	251	87 (34.66)

Mendiratta, *et al.,* (2007) reported the usefulness of HRP2 antigen detection kit Paracheck Pf for the rapid diagnosis of falciparum malaria with 92.6% sensitivity and a specificity of 98.68%. Besides the financial savings from unnecessary treatments, the use of non – microscopical rapid malaria tests is of value in the early investigation and management of malaria epidemics. It is worthy to note also that these rapid tests are also of value in the diagnosis of severe and complicated falciparum malaria in those who have taken antimalarials (Cheesbrough, 1998).The diagnostic accuracy of this method was measured against routine microscopy as gold standard. Some earlier studies have also compared RDTs using microscopy as gold standard (McMorrow *et al.,* 2008; Bell *et al*., 2005). In this study RDT gave a higher detection value than microscopy. Similar report has been recorded for HRP-2 based rapid diagnostic technique (Tangpukdee *et al.*, 2009; Ruiz *et al*., 2002). A number of factors are known and associated with low sensitivity of microscopy including the inherent limitations of microscopy (Bell *et al.,* 2005), existence of low density infections from indiscriminate treatment of malaria and inappropriate use of anti-malarial (Kyabayinze *et al*., 2008) which result in low parasitaemia. The observation of low detection of malaria with microscopy reveals possible limitation of the method in diagnosis of malaria infection in patients. With RDTs, it is possible forclinicians' to see both negative and positive malaria results (Reyburn *et al.*, 2007; WHO, 2000).

RDTs have been known to identify more positive cases of malaria in excess of microscopy gold standard. This has been attributed to patients with persistently circulating antigen due to prior use of anti-malarials as well as the level of immunity (Batwala *et al.*, 2010). Earlier report indicated that RDT detect majority of malaria cases but also led to treatment of a small percentage of patients without malaria infection (Batwala *et al*., 2010). However, HRP2-based RDTs remain positive after treatment. HRP2 signal has been noted to persist during the first week of treatment (Karbwang *et al.*, 1996; Pullan *et al*., 2010; Mayxay *et al*., 2001). This is an inherent weakness in HRP2-based tests. Some countries have adopted RDT as a method for parasitological diagnosis of malaria in addition to microscopy (Uganda Ministry of Health, 2010). Due to large number of malaria

patients in Africa, many are treated presumptively. Although microscopy has its limitations (Mens e*t al.*, 2007), it is known to detect low level of parasitaemia in a blood sample with different *Plasmodium* species infections (Batwala *et al.,*2010).

This study has been able to show that RDT detected higher malaria values in both sexes 51(44.74%) and 59(43.07%) for males and females respectively. The Pf showed higher sensitivity compared to microscopy in detection of malaria and is recommended for screening of malaria infections and for large scale assessment for Primary Health Care (PHC) centres, and field studies on malaria.

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