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COMPARISON OF RAPID DIAGNOSTIC TESTS AND MICROSCOPY FOR MALARIA

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ABSTRACT

Presumptive treatment of malaria results in significant overuse of antimalarials. This study compared the diagnostic accuracy of Histidine Rich Protein II and plasmodium lactate dehydrogenase (pLDH)-based Rapid Kits(RDTs)and using expert microscopy as the gold standard for the detection of falciparum and non-falciparum in 200 individuals suffering from fever episodes over a period 8months in a malaria-endemic area in Osogbo, Osun State. 99(44.5%) of these patients were microscopically parasitaemic with three *Plasmodium species* identified expect *P.ovale*. 25 (12.5%) of the study population had temperature < 37.5^{0}C at the time of presentation in the clinic among which 16 (64%) were parasitaemic. Furthermore, 148 (74%) of the study population had fever episode of which 65(44%) were positive for malaria. The sensitivity and specificity of pLDH (*Pf*) were 84.7% and 78.3% respectively and HRP-2 were 72.7% and 90.9% respectively. Both had high detection (94.7%) at parasite density ≥ 10,000 parasite/µl of blood. Microscopy still remains the 'Gold Standard' since both are not 95% sensitive and cannot determine parasites quantification.

Keywords: Plasmodium, Microscopy, Rapid Kits, Osogbo, Nigeria, LAUTECH

COMPARAISON DES TESTS DE DIAGNOSTIC RAPIDE ET MICROSCOPIE POUR LE PALUDISME

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RÉSUMÉ

Le traitement présomptif de paludisme résulte de l'usage abusif considérable des antipaludiques. Cette étude a pour but de comparer l'efficacité de diagnostic de l'histidine RichProtein II et de test de diagnostic rapide (TDR) à base de kits plasmodium lactate déshydrogénase (pLDH) et en utilisant la microscopie experte comme «gold standard» pour la détection de *P. falciparum* et non-*falciparum* chez 200 personnes souffrant d'épisodes de fièvre sur une période de huit mois dans une région où le paludisme est endémique dans Osogbo, l'Etat d'Osun. 99 (44,5%) de ces patients étaient parasitémiques à la microscopie à trois espèces de *Plasmodium*identifiées différentes de *P. ovale*attendu. 25 (12,5%) de la population étudiée avait une température <37,5°C au moment de leur arrivée à la clinique parmi lesquels, 16 (64%) étaient parasitémiques. En outre, 148 (74%) de la population d'étude avait un épisode de fièvre dont 65 (44%) étaient positifs pour le paludisme. La sensibilité et la spécificité de pLDH (Pf) étaient respectivement de 84,7% et 78,3% et celles de HRP-2 étaient respectivement de 72,7% et 90,9%. Tous les deux

tests avaient une bonne détection (94,7%) à densité parasitaire ≥ 10000 parasite/ul de sang. La microscopie reste le «Gold Standard» puisque les deux autres tests ne sont pas sensibles à 95% et ne peut pas déterminer la quantité parasitaire.

Mots clés: Plasmodium, microscopie, kits de test rapide, Osogbo, Nigeria, LAUTECH

INTRODUCTION

Malaria remains an important public health concern in countries where transmission occur regularly as well as in areas where transmission has been largely controlled or eliminated. An estimated 40% of the World population today is at risk of malaria infection and the World Health Organization (WHO) estimates that each year there are more than 300 millions episodes of acute illness and at least two million deaths due to Malaria (1). Malaria is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. Variable factors such as distribution and efficiency of mosquito vector, climate and other environmental conditions and the behavior and level of acquired immunity of the exposed human population contributes to wide distribution of malaria(1).Methods for diagnosis of malaria in endemic countries include microscopy, RDT, polymerase chain Reaction (PCR), and clinical methods. Microscopy remains the gold standard diagnostic technique of choice for malaria. It is less costly and sensitive to a threshold of 5 to 50 parasite /µl (depending on the microscopist expertise). It can also characterize the infecting species and their relative densities (2). Above all, microscopy requires considerable technical expertise for optimal blood film preparation, examination and interpretation. Immunochromatographic capture procedure is an RDT based on the detection of malaria antigen and was developed to improve the timeless, sensitivity and objectivity of malaria diagnosis through less reliance on expert microscopy, (2). Preferred targeted antigens are those which are abundant in all asexual and sexual stage of the parasite.

Currently interest is focused on the detection of histidine-rich protein2 (HRP-2) from *Plasmodium falciparum* and parasite-specific lactate dehydrogenase (pLDH) or Plasmodium aldolase from the parasite glycolytic pathway found in all species. However, several factors in the manufacturing process as well as environmental conditions may affect RDT performance. These include sub-optimal sensitivity at low parasite densities, an inability to accurately identifying parasites to the species level or quantify infection density, and a higher unit cost relative to microscopy (2).

Presumptive / clinical diagnosis is the least expensive and most commonly used method and is the basis for self-tropical diseases like typhoid fever, respiratory tract infections and viral infections impairs its specificity and therefore encourages the indiscriminate use of antimalarials for managing febrile conditions in endemic areas. Accuracy of a clinical diagnosis varies with the level of endemicity, malaria season and age group. No single clinical algorithm is a universal predictor (3).Changing patterns of accepted morphological appearance of malaria species, possibly due to drug pressure, strain variation, or approaches to blood collection, have created diagnostic problems that cannot easily be resolved merely by reference to an Atlas of Parasitology (4).

WHO currently recommends that parasite based diagnosis should be used in all cases of suspected malaria with the possible exception of children in high prevalence areas and return traveler from endemic zones (Samuel *et al.,* 2008 5).Prompt and accurate diagnosis is a key to effective treatment and management of patients with malaria parastemia which will eventually reduce malaria morbidity and mortality. This work is designed to compare the current methodologies and approaches in the diagnosis of malaria in a practical and helpful way for the laboratory and for the physician caring for the patients.

MATERIALS AND METHODS

Study area: This study was conducted in LAUTECH teaching hospital, Osogbo.Osogbo metropolis is in Osun state and is located in South-west of Nigeria.Malaria is endemic in these areas and predominant during the raining season. Mean annual rainfall 1250-2000mm with relative humidity of 60-70% and temperature of 28-32°C and a population density of 448,000 ***(2009 census)***.

Subject selection: The symptomatic individual of different ages were recruited in this study. Febrile patients with typical malaria symptoms (headache, joint pains, body weakness) both inpatients and outpatient of these hospitals were recruited into the study. Inclusion criteria include fever (temperature ≥37.5°C,) and other malaria symptoms like headache, joint pains, body weakness and diarrhoea.

Ethical issues and clearance: Ethical approval was obtained from ethical committee of LAUTECH Teaching hospital, Osogbo.

Patients and Sample collection: The period of sample collection was 7months January-August,2013 (ending of dry season and beginning of raining season) The biodata of 200 subjects were noted and included age,

sex. Also clinical data such as history of fever in the past 24 hours, headache, generalized body pain, joint pains, chills and rigor were noted. Consenting febrile patients with auxiliary temperature of >37.5^{0}C were recruited into the study and five millimeters of blood was collected from the antecubital vein area of the patients after cleaning the area with methylated spirit. Part of the blood was used for microscopic examination of malaria parasite.

Laboratory procedure

Blood film Microscopy: Thick and Thin blood films were made following standard laboratory procedure and the malarial parasite were counted in relation to number of white blood cells (WBC) usually 200WBC (or 500WBC) when the number of parasites is less than 10 per 200 WBC counted and multiplying this by the average of the total WBC counted in such individual as earlier described by Onile and Taiwo , 2005.(6)

Rapid Diagnostic Tests

The SD BIOLINE kit (**SD** Bioline System Korea) which contains a plastic cassettes which is pre-coated with two monoclonal antibodies as two separate lines across the test cassettes. One monoclonal antibody (test line 2) is pan-specific to lactate dehydrogenase (pLDH) of the Plasmodium species(*P.falciparium, P.vivax, P.malariae, P.ovale*) and the other line (test line 1) consists of a monoclonal antibody specific to histidine-rich protein 2 (HRP2) of the *P. falciparum* species and *one step malaria Histidine-rich protein II(*P.f)test were used for the study. This was carried out and interpreted following manufacturer's instructions.Internal procedural controls were included in the test.

Method for presumptive Diagnosis

Method for clinical diagnosis was based solely on clinician's judgment who based the diagnosis on presenting complaints and from physical examination of the patient without any reference to laboratory test/analysis. Some of the symptoms presented are, fever which is the chief complain, vomiting, headache, anorexia, abdominal pain, body pain, diarrhea, Tiredness, sweating, diarrhea

The temperature of each patient was noted together with other symptoms presented. Majority of patients with temperature ≥37.5°C were recruited in to the study.

Analysis of Results

Data were analyzed using SPSS packaged version 16.0. Sensitivity refers to the proportion of the samples with positive result. Specificity refers to the proportion of the samples with negative result.The present data were calculated using the formulas:

Sensitivity = TP/(TP + FN) 100%

Specificity = TN/(TN + FP) 100% : where TN represents true negative, TP true positive, FN false negative and FP false positive .

RESULTS

A total of 200 patients with clinical symptoms of malaria were enrolled in this study. It was observed in the age and gender distribution among the study population;that the female gender was more represented in all the age groups, with the 1-20 years old group having the highest frequency as shown in figure 1. These differences were not significant (X^2 = 3.47, df =3, p = 0.433) as shown in Table 1. Figure 2 compares the age group with parasitaemia, 137 (68.5%) within the age group 1-20 had the highest parasitaemia 82 (59.9%). However the differences were significant (X^2= 18.93, df =3, p= 0.001). Table 2 shows the clinical presentation among the patients in which fever is prevalent. 65 (44%) of the total number 148 that complained of having fever were actually observed to be parasitaemic.

A body temperature of ≥37.5^{0}C was recorded in 175 cases (87.5 %) of which 83 (45%) were positive for malaria while the rest were negative. Those withtemperature ≤37.5^{0}C were 25 (12.5%9) among which 16 (64%) were positive for malaria. The difference in the number of patients having either of the two temperature group significantly affect those that were parasitaemic (X^2 = 7.39, p= 0.002).

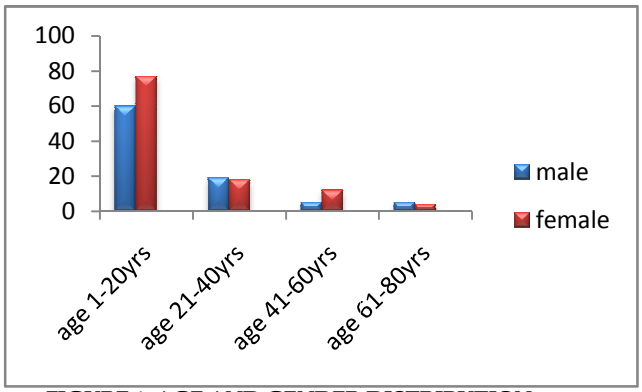


FIGURE 1: AGE AND GENDER DISTRIBUTION AMONG THE STUDY POPULATION

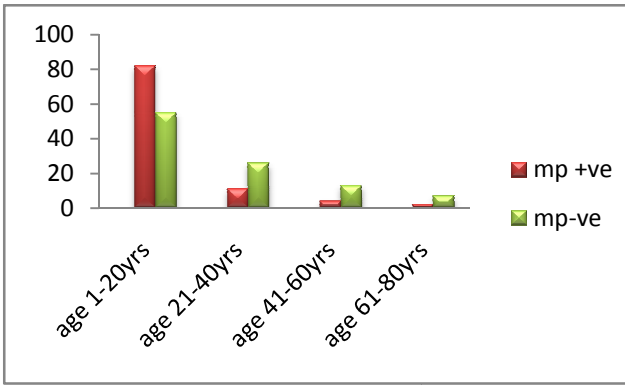


FIGURE 2: RELATIONSHIP BETWEEN AGE GROUP AND PARASITAEMIA

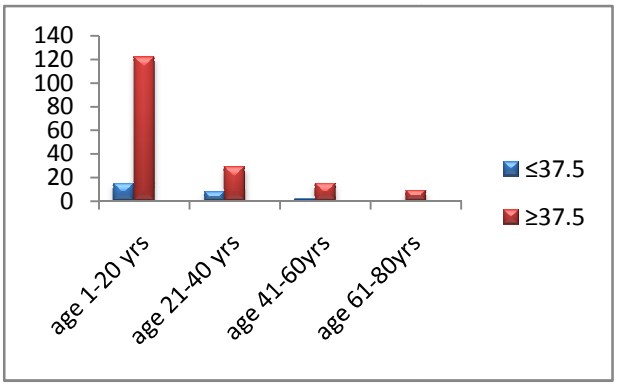
The relationship between clinical body temperature among the study gender within age groups as shown in figure 3.The difference between age and temperature was not statistically significant (X^2= 4.41, df= 3 p= 0.220) even though there was no significant difference in temperature presentation and sex group (X^2= 0.142, df= 1, p= 0.707).

The species of *Plasmodium falciparum* was detected in 84 (40%) of the total blood samples collected with density ranging between 43 and 227,857 parasites/µl of blood (mean= 3712.07).From figure 4 9 (9.09%) of the positive samples had a mixed infection of *P.vivax*and *P.falciparum* having a density of 94-680/µl (mean= 5.91/µl). Onlysix samples had a *P.malariae*and *P.falciparum* withdensity ranging between 57and 331parasites/µl of blood (mean= 2.77/µl).

Comparison Of Microscopy With Rdts

The result of the performance of the RDTs with the reference standard of microscopy is indicated in table 3, 4 and 5. The prevalence of Plasmodium species using microscopy and RDTs was 81 (40.5 %), 85 (42.5 %), 41 (%) and 99 (44.5 %) respectively as indicated in Table 3.A composite reference was generated and used as the gold standard to assess the sensitivity of each method used in the analysis. This was defined as true positive if all the two methods tested positive and as true negative, if all the two methods tested negative. Sensitivity is defined as the probability that a truly infected individual will test positive, and specificity as the probability that a truly uninfected individual will test negative (Table4).

TABLE 3 : RELATIONSHIP AGE GROUP & TEMPERATURE



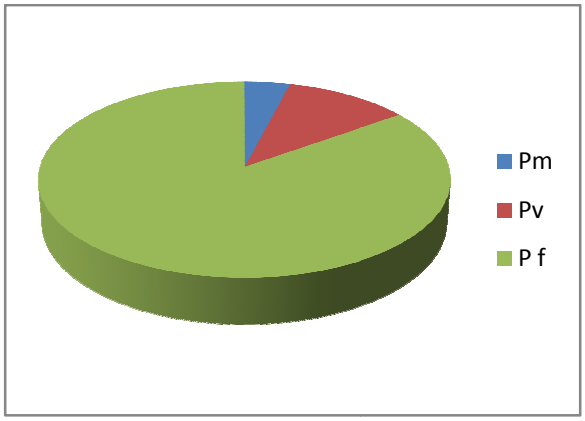


FIGURE 4: *PLASMODIUM SPECIES* POPULATIONS

The pLDH (*pf*) was more sensitive than SD Bioline (84.7%) although the latter was more specific (90.9%). SD Bioline showed more false negative result (29) than pLDH ( ) (Table 5).

pLDH was commercially made to detect other plasmodium species causing malaria infection. Even though made specifically to detect HRP-2 antigen expressed by *P. falciparum and Pan* ,having high percentage detection (90%) and 0% at parasite density of < 100 and where as SD Bioline has percentage detection of 55 %. Both assays are very sensitive at higher parasitaemia.

TABLE 1: AGE GROUP AND SEX DISTRIBUTION OF PATIENTS AMONG THE STUDY POPULATION

Age group (years)	Gender Female	Male	Total	P value
1-20	**77**	**60**	**137**	**0.433**
21-40	**18**	**19**	**37**	
41-60	**12**	**5**	**17**	
61-80	**4**	**5**	**9**	
Total	**111**	**89**	**200**	

TABLE 2: PREVALENCE AND CLINICAL PRESENTATION OF MALARIA AMONG THE STUDY POPULATION

Clinical Symptoms	Number Observed	No. of microscopy positve (%)	No. of RDTS positive	
			HRP 2 (%)	**pLDH (%)**
Fever	148	65 (44)	54 (37)	56 (38)
Headache	121	38 (32)	31 (27)	35 (29)
Chills	113	47 (42)	39 (35)	43 (38)
Cough	36	5 (19)	4 (11)	7 (19)
Tiredness	27	9 (33)	4 (15)	6 (22)
Diarrhoea	18	12 (67)	5 (28)	3 (17)
Anorexia	32	16(50)	11(34)	8 (25)
Vomiting	31	12 (39)	7 (23)	14 (45)
Abdominal pain	10	2 (22)	3 (30)	4 (40)
Body pain	43	15 (35)	6 (14)	5 (12)
Myalgia	12	7 (58)	5 (42)	8 (67)
Sweating	41	13(32)	7 (17)	10 (24)

TABLE 3: PREVALENCE OF MALARIA ACCORDING TO DIFFERENT DIAGNOSTIC METHODS USED.

	No Positve	% Positive	No Negative	% Negative
Microscopy	99	44.5	101	55.5
HRP II	81	40.5	119	59.5
pLDH (pan)	41	20.5	159	79.5
(*Pf*)	85	42.5	115	57.5

TABLE 4: COMPARISON OF DIAGNOSTIC TEST RESULTS OF THE HRP2, PLDH AND MICROSCOPY

Result/Methods	Microscopy	HRP II	pLDH	Composite Reference
True positive	72	72	72	72
False positive	27	9	13	_
True Negative	90	90	90	90
False Negative	10	29	25	_

TABLE 5: SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF THE THREE TESTS.

	Microscopy	HRP 2	pLDH
Sensitivity(%)	87.8	72.7	84.7
Specificity(%)	76.9	90.9	78.3
Positive Predictive value(PPV%)	72.7	88.9	84.7
Positive Predictive value(NPV%)	90	75.6	78.3

DISCUSSION

The people suffering from malaria among the study population were almost average although they had clinical manifestation, which may presumably be symptoms of malaria infection (Table 1). From this study, the female gender were more than the male (Figure 1) because birth statistic shows that female children are more than male. Among other age groups female presented themselves for malaria for malaria diagnosis more than male counterpart due to cultural believe and drug abuse (concoctions). In addition female had temperature ≥ 37.5^{0}C within the gender age group of 1-20 and 41-60. This could be as a result of physiological changes especially during their menstruation periods and menopause (Hot flushes). The high prevalence of parasitaemia observed among the children of age group 1-20 is suggestive of underdeveloped immunological status.

Fever, headache, chill and sweating in order of prevalence which was clearly observed among the study population confirmed the febrile paroxysm of malaria presentation which is likely to coincide with schizogonic cycle of *Plasmodium* in the human host (7).Although many had temperature greater than 37.5^{0}C at the time of presentation in the clinic for diagnosis, this may be due immunological response to presence of antigens such as *Plasmodium* and other disease causative agents (co-infection). But the few that had temperature < 37.5^{0}C could be as a result of typical periodicity of paroxysm which lasts for 72 hours in *P.malariae* and 48 hours in other species, although sometimes continuous in *Falciparum* malaria (7).

The presence of Plasmodium species in the body system elicits host immune responses in which TNF-α, IL- 1 and other cytokines were secreted which in the process of mounting up resistance, up-regulates endothelia cell and cause fever (8). Hence, the higher prevalence in fever for both parasitaemia and antigenaemia. However, fever symptom cannot categorically be allied as a measure for clinical diagnosis of malaria as it serves as a generalized symptom for most diseases.

The accuracy and urgency in the diagnosis of malaria made the use of rapid diagnostic method imperative especially in rural areas where health facilities and electric power supply are inadequate, two rapid diagnostic kits of the same product were (SD Bioline pLDH for the diagnosis of *Plasmodium falciparum* andother speciesand HRP-2) were evaluated for their efficacy.

From the study SD Bioline pLDH (*Pf / Pan*) and HRP-2 were found to be very easy to use and the result can be visibly read in 15 minutes. The limitation of this kit is that there is a greater risk of infection, if there should be spill from the mixture in the well unlike other diagnostic kit in which the blood and diluents are applied into the cellulose pad directly. However, the ability of both kits to detect parasite antigens (Histidine Rich Protein -2 and Plasmodium Lactase dehydrogenase respectively) from finger prick blood allow efficient handling for use by non-technician with less training in agreement with findings from other studies (9,10).

The prevalence (20.5%) of pLDH (*Pan)* test for non-falciparum species was low compare with *Pf* (42.5%) and HRP -2 (44.5%) this is because other species of plasmodium are not common in this area however *P. falciparum* demands particular attention because it is the species of human malaria parasite that carries the most severe form of the disease and can kill with stunning speed.

Gametocytes were detected by microscopy in only 3 of the 200 cases (1.5%) no HRP 2 and pLDH results were positive in a case with the smear that showed gametocytes. *P.vivax* and *P.malariae* was also detected by microscopy which were picked by the PLDH. From this finding, SD Bioline was found to be more sensitive (Table 5) and pLDH made specifically to detect HRP-2 antigen expressed by *P. falciparum has* high percentage detection at parasite density of < 100 than HRP-2. However both performed excellently at higher parasite density > 10,000/µl .

The specificity of both assays are encouraging despite the occurrence of occasional false positive *P. falciparum* detection. This could probably be due to previous recent infection with malaria or the presence of circulating rheumatoid factor and some report has also establish the decreased activity assays with antimalaria therapy (11).

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