COMPARATIVE ANALYSIS OF DIFFERENT DIAGNOSTIC TECHNIQUES FOR PLASMODIASIS

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Abstract

The effectiveness in detecting malaria, of two immunochromatographic rapid diagnostic test (RDT) kits namely: "Global Devices One Step Rapid Test" and "SD-Bioline Malaria Pf/Pv" were evaluated by comparison with stained blood film microscopy. Blood samples of 202 patients with provisional diagnosis of malaria were tested in the laboratory for malaria parasites. The results of the stained film microscopy showed that 156 (77.2%) were posited, while the RDT kits – SD-Bioline and Global Devices showed positive results of 127 (62.9%) and 53(26.2%) respectively. The performance characteristics of the RDT kits, SD-Bioline and Global Devices, respectively were: sensitivity – 76.3% and 33.3%; specificity – 82.6% and 97.8%; test accuracy – 77.7% and 48.0%; positive predictive value – 93.7% and 98.1%; negative predictive value – 50.7% and 30.2%. Statistical tests of the effectiveness of the different techniques at P>0.05 and P>0.01 showed significant difference between the RDT kits and among the three tests. Comparison of plasmodiasis infection rates between the sexes and among 4 age groups: 0 – 11, 12 – 25, 26 – 49 and ≥50 showed no statistically significant difference at 5% and 1% alpha levels. The SD-Bioline Malaria Pf/Pv kit showed an appreciable effectiveness. It is therefore recommended for use in field screening, and in the absence of microscopist in the laboratory.

Introdution

Malaria remains a public health problem in over 90 countries worldwide, especially in Africa, where it is the leading cause of mortality in children under the age of 5. It accounts for 40% of public health expenditure, 30-50% in-patients admission and up to 50% of outpatients visits (WHO, 2002). The direct financial and indirect costs such as loss of productivity, earning, and school days due to absenteeism have major impacts on both social and economic development in malaria-endemic areas (Louise, 2003). Prompt and early diagnosis is therefore the key to effective management and control of the disease (Muktar, Isyaku, Abubakar & Aliyu, 1999)

Microscopy of stained films is one of the established reliable methods of diagnosis of malaria, and it is a valuable technique when correctly carried out. Unfortunately, technical requirement and trained personnel as well as the need for

Emokpae, Sarkin-Fada, Kwaru, Ofolu, Imoru, Magaji, Arzai, Musa, Schott & Takalmawa, 2004). Due to the technicalities involved in the diagnosis of plasmodiasis by the microscopy method, the interest of scientists was raised in the development of more rapid diagnostic methods, which also would require less expert training. The clinical diagnosis, otherwise called prognosis, of malaria, which is more widely used based on the symptoms of malaria. This is rather non-satisfactory because the symptoms of malaria are very "non-specific" and overlaps with those of other febrile (feverish) illnesses (WHO 1999).

Current interest in rapid diagnosis of malaria is focused primarily on detection of Histidine-rich protein-II ($PfHRP_2$) from *Plasmodium falciparum* (WHO, 1999 and Bonchogakson, Yanogkul, Panyin & Vichers 1996). There is also the detection of parasite Lactate dehydrogenase (pLDH) produced by all the *Plasmodium* species (WHO, 2001 & Becton 1996). The advantage of the chromatographic diagnoses is their ability to give rapid results, which are similar to that of microscopy (Abubakar *et al* 2004). Another major advantage is that they do not require high-level manpower training. The need for a rapid diagnosis of malaria requiring less comprehensive technical training led to the manufacture of various types of chromatographic kits for malaria diagnosis. Although the immunochromatographic tests are exceptionally easy to perform, there are some inherent problems of sensitivity & specificity, which measure accuracy of the diagnoses. Another factor is the cost of the rapid diagnostic kits when compared to microscopy. The aim of this study is to compare the diagnosis of plasmodiasis by microscopy with two rapid diagnostic test (RDT) kits: **Global Devices** and **SD-Bioline**

Materials and Methods

Study design.

This was an experimental based study, designed to evaluate some rapid diagnostic test (RDT) kits for their ability to detect malaria. The evaluation used the stained blood film microscopy as "gold standard".

Patients and samples

A total of 202 patients clinically diagnosed of malaria from three hospitals in Nnewi were chosen for the study. The hospitals were Myles specialist hospital, Uchenna hospital and Dominion hospital.

Blood samples (2ml) were collected from each patient through venepuncture; the blood samples were delivered into ethylene diamine tetra-acetic acid (EDTA) bottles and mixed well by inversion. Samples were collected from both sexes and for age groups: 0 – 11, 12 – 25, 26 – 49 & ≥50. The analyses were done in October and November 2007.

Blood film examination

Blood thick films were made on slides, labeled for each patient and stained following the methodology described by Cheesbrough (1998). Each film was systematically and carefully examined under the microscope using oil immersion

objective lens. Presence or absence of any of the stages of malaria parasites was carefully noted. Where any of the stages of the parasites is not seen, malaria pigments were looked for as a further guide as noted by Abubakar *et al* (2004). Results were adequately noted.

Procedure for Rapid Diagnostic Tests.

Two diagnostic test kits were used: Global Devices One Step Rapid Test and SD-Bioline Malaria Pf/Pv.

Global Devices One Step Rapid Test

A test device was removed from the foil pouch and used immediately, not latter than 30 minutes so as to obtain reliable result. The test device was placed on a clean and level surface; anticoagulated whole blood was transferred, using a custom-made pipette with 10µl mark, to the specimen well of the test device. Also 3 drops of a provided buffer (diluent) was added, avoiding trapping of air bubbles in the specimen well. The result was read after one minute.

SD-Bioline Malaria Pf/Pv

The procedure was the same as above except that plasma was used in place of whole blood.

Performance Ccharacteristics (tests) of the RDT

Performance characteristics were calculated using the standard World Health Organization (WHO, 1999) format and (Mbarakurwa, 1997).

Results

Table 1: Summary of Microscopy results.

Criteria	Total Tested	No. Positive	% Positive
Total	202	156	77.2
Male	93	69	74.2
Female	109	87	79.8
0-11 yrs	47	33	70.2
12-25 yrs	51	43	84.3
26-49 yrs	62	51	82.3
≥ 50 yrs	42	29	69.0

Out of a total of 202 people, 156 tested positive, representing 77.2%. The 202 people included 93 males and 109 females of whom 69(74.2%) and 87(79.8%) respectively were positive (table 1). Statistical analysis showed that there in no significant difference in the infection rates between the sexes at $P>0.05$ & $P>0.01$. A further breakdown of participants into 4 age groups was done: 0-11 yrs (47), 12-25 yrs (51), 26-49 yrs (62) and ≥ 50 yrs (42). The results of the age groups respectively showed the following positive results: 33 (70.2%), 43 (84.3%), 51(82.3%) and 29 (69.0%). The 12-25yrs age group showed the highest prevalence rate of 84.3% with the ≥ 50 yrs age showing the least (69.0%). A statistical analysis at 1% and 5% alpha levels show that there is no significant difference in the prevalence rates among the age groups.

Table 2: Summary of results of Global Devices

Criteria	**Total Tested**	**No Positive**	**% Positive**
Total	202	53	26.2
Male	93	23	24.7
Female	109	30	27.5
0-11 yrs	47	7	15.0
12-25 yrs	51	17	33.3
26-49 yrs	62	20	32.3
≥ 50 yrs	42	12	28.6

53 of the 202 participants, who represents 26.2% tested positive in the diagnosis (Table 2). 23 (23.7%) and 30 (27.5%) of males and females respectively tested positive from total of 93 and 109 respectively.

The infection rates among 4 age groups as shown by this diagnostic tool as included in the table above shows that the least infected age group is 0-11 yrs (15.0%) with 12-25 yrs age group topping the list with 33.3% infection rate. The later is in agreement with the result from microscopy, but not the former. Statistical analyses of infection rates between sexes and among age groups show no significant differences

Table 3: Summary of results of SD-Bioline kit

Criteria	**Total Tested**	**No Positive**	**% Positive**
Total	202	127	62.9
Male	93	55	59.1
Female	109	72	70.6
0-11 yrs	47	23	48.9
12-25 yrs	51	36	70.6
26-49 yrs	62	41	66.1
≥ 50 yrs	42	23	54.8

The result by this test kit showed that out of 202 participants, 127 (62.9%) tested positive for plasmodiasis (see table 3). Between the sexes, 55 (59.1%) out of 93 males and 72 (70.6%) out of 109 females were positive.

From the table 3 above, a look through the age groups shows that the highest infected is 12-25 yrs in agreement with the other diagnostic tools while the least is 0-11 yrs age group in agreement with Global devices.

Performance Characteristics of the RDT Kits.

The result data were sorted to obtain the variables in table 4 used to calculate the performance characteristics.

Table 4: Presentation of variables used in calculation of performance C**haracteristics of the RDTs in relation to microscopy**

Variables	**Global Devices**	**SD-Bioline**
True positive	52	119
False Positive	1	8
True Negative	45	38
False Negative	104	37

The performance characteristics of the two rapid diagnostic test (RDT) kits calculated shows that the sensitivities of the kits are 33.3% and 76.3% for Global Devices and SD-Bioline respectively. Other performance characteristics as in table 5 are specificity: 97.8% and 82.6%; test accuracy: 48.0% and 77.7%; Positive predictive value; 98.1% and 93.7%; Negative Predictive value; 30.2% and 50.7%; false positive rate; 2.2% and 17.4% and false negative rate; 69.8% and 49.3% respectively.

Table 5: Performance Characteristics of the RDTs relative to those of Microscopy (mp film).

	Sensitivity%	**Specificity %**	**Test accuracy %**	**Positive predictive value %**	**Negative Predictive value %**	**False positive rate %**	**False Negative Rate %**
SD-Bioloine	76.3	82.6	77.7	93.7	50.7	17.4	49.3
Global Device	33.3	97.8	48.0	98.1	30.2	2.2	69.8

Discussion

The development of easy, sensitive, specific, accurate and cost affordable rapid diagnostic test (RDT) kits for the detection of malaria infection is highly desirable. The general performances of the kits are shown in table 5. The SD-Bioline has a sensitivity of 76.3% while Global device has 33.3%, then specificity are 82.6% and 97.8% respectively. Their respective test accuracy is 77.7% and 48%. These results are comparable with the work of Mbarakurwa *et al* (1997) in Zimbabwe, the team evaluated the performance of a RDT kits– Parasight F and found a sensitivity of 85% and specificity of 93%. Other researches like Agbomo *et al* (1998) found the rapid diagnostic test kits useful in quick assessment of plasmodiasis. Van-den broek, Hill, Gordillo, Angarita, Hamade, counihan & Guthmann (2006) assessed the diagnostic capacity of three rapid diagnostic kits against expert microscopy in Colombia. The sensitivity they obtained for the three kits were 90.0%, 83.6% and 81.4% for Paracheck – PF, NOW – malaria ICT and Optimal – IT respectively.

The RDTs, however, detected malaria in some patients, who were tested negative by the microscopy (see table 4), these were the denoted as false positive. Although false positives have been reported in other investigations as Van-denBroek *et al* (2006), Noedl, Yingyuen, Laoboonchai, Fukuda, Sirichaisinthop and Miller (2006) and Agbomo *et al* (1995), but they appear to play a minor role in the usefulness of malaria test for clinical settings. False positive reactions may occur in individuals who have been recently treated for malaria as reported by Shiff *et al* (1993), Singh *et al* 1997 and Beadle *et al* 1994.

Other causes of false positive result are circulating rheumatoid factors (Agbomo *et al* 1995), self-medication resulting in partial treatment (WHO 1996). Sequestration of the malaria parasites at the time of blood collection is a factor that is quite interesting. There is evidence of parasitemia clinically, the RDT kits also test positive

whereas microscopy couldn't detect the parasite of any stage. Abubakar *et al* (2004) discovered in a research that 5 patients tested positive by RDT kits and negative with microscopy. It was further discovered by thorough microscopic examination that malaria pigments were seen in the peripheral blood leucocytes of these patients. This finding added weights to the usefulness of the RDTs – its advantage over microscopy when the parasites are sequestered in the deep musculature of the vital organs as seen in cerebral and placental malaria (White, Chapman and Watt. 1992). The said finding of Abubakar *et al* 2004 is comparable to a finding in this research of two patients that tested positive with SD-Bioline, but negative with microscopy. A careful examination of the film in the microscopy revealed malaria pigments, hence, although the parasite stages sought for in the peripheral blood were not seen, the indication by the RDT kit is not misleading at all. The work of Abubakar, *et al* (2004) solicits the careful examination for malarial pigments in the peripheral blood leucocytes in diagnosis of malaria especially when the blood stages of the parasites are not seen.

The detection of a sample as negative by the immunochromatographhic tests whereas its microscopy was positive was encountered. These tests, i.e. false negatives were encountered by many researches as: Beadle *et al* (1994), VanderJagt *et al* (2005), Abubakar *et al* (2005), Noedl *et al* (2006) among many other researchers. This could be as a result of level of parasitaemia; sensitivity of rapid diagnostic tests decrease with the reduced or low parasitaemia levels. The false negative rate of the Global Devices kit is quite alarming (tables 4 and 5) compared to that of SD-Bioline kit. This can be potentially dangerous, as it will definitely fail in diagnosis of malaria in an infected individual, until the infection gets to a critical point or development of complications.

Another possible cause of false negative results is the activity of the immune system of individual, which combats the antigens of the parasite in the peripheral blood. This makes the parasites antigen to be absent, while the parasites are present and seen in a stained film at microscopic examination.

Statistical analyses, using Random Block Design and Chi-Square Analysis, to compare the results of the RDTs with the microscopy showed that there is significant difference among the techniques, hence suggesting a strong need for improvement on the effectiveness of the rapid diagnostic test kits. The SD-Bioline Malaria Pf/Pv showed an appreciable effectiveness. It is therefore recommended for use in field screening and in the absence of microscopists in the laboratory.

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