Assessing the Parasight-F® Test for the Rapid Diagnosis of Malaria in Parts of the South Eastern Nigeria

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User-friendly, reliable and inexpensive methods for diagnosing malaria are needed at the primary health care level. During a 28 days randomized trial, the Parasight®-F test was assessed on against standard light microscopy of Giemsa-stained thick blood smears for diagnosing Plasmodium falciparum parasitemia in patients with P. falciparum (n = 84) or P. vivax (n = 59) malaria. The median P. falciparum parasite count on day 1 was 2,373/mL (range = 20 – 74,432/mL). At the start of treatment, the Parasight-F® test had a sensitivity of 95.2% (80 of 84; 95% confidence interval [CI] = 88.2 – 98.7), and a specificity of 94.9% (56 of 59; 95%CI = 85.8 – 98.9). On day 7, this test showed false-positive results in 17 (16.3%) of 104 patients (95%CI = 9.8-24.9). The Parasight-F® test performed well when compared with light microscopy in detecting P. falciparum parasitemia in patients presenting with clinical malaria. However, the high false-positive rate on day 7 limits its use for patient follow-up.

1. Introduction

Plasmodium falciparum is a global problem resulting in considerable morbidity and mortality [1,2]. Plasmodium vivax also causes appreciable morbidity and has become chloroquine – resistant in some endemic areas [3,4]. Therefore, the accurate diagnosis of malaria in febrile patients is essential for optimal patient management and the rational use of antimalarial drugs in malaria control programs. However, because standard light microscopy is often lacking in malaria-endemic areas, malaria is often diagnosed clinically by detecting the signs, a practice that is known to be inaccurate [5,7]. Over diagnosis of malaria and the excessive use of antimalarial drugs are common in tropics and result in inappropriate treatment of non-malaria fevers, wasted resources, potentially avoidable drug toxicity, and drug pressure that promotes the development of drug-resistant malaria [8,9].

The Parasight-F® test (Becton Dickinson, Sparks, MD) is an immunochromatographic dipstick test that specifically detects P. falciparum histidine-rich protein II (Pf HRP II) in whole blood by antibody agglutination, resulting in the appearance of a red band on the dipstick [11]. In previous trials of patients from areas of transmission of P. falciparum, this test has generally demonstrated high sensitivity and specificity when compared with light microscopy, the current gold standard for diagnosing malaria [12-24]. High sensitivity and specificity values have also been documented for detecting P. falciparum malaria in areas of mixed P. falciparum and P. vivax transmission in Brazil (91% and 97%, respectively), Sri Lanka (90.2% and 99.1%, respectively), and Sumba Island in eastern Indonesia, (95.5% and 89.8% respectively) [25-27]. However, in most of Sub Saharan Africa, the use of the Parasight-F® test in detecting P. falciparum in asymptomatic, individuals has not been documented.

We report the results of the Parasight-F® test in patients with confirmed malaria infections acquired in parts of the Imo River Basin of South Eastern Nigeria.

2. Materials and Methods

The Parasight- F® test was assessed during a ran-
A randomized trial in rural communities in Ezinihitte, Ahiazu and Aboh Mbaise Local Government areas which form part of the Imo River Basin of South Eastern Nigeria. Malaria is highly endemic in this area and transmission occurs year round, but it intensifies during the rainy season from April to October. All enrolled patients had confirmed malaria parasitemia and were monitored for 28 days. Study end points were the parasitologic end points of the World Health Organization (WHO) 28-day in vivo test: parasite sensitivity (S = complete and sustained clearance of asexual parasitemia by day 7 to day 28) or resistance (RI = complete asexual parasite clearance by day 7 but recrudescence within 28 days; RII = marked reduction [75%] of asexual parasitemia within 48 hours but no clearance by day 7; and RIII = no marked reduction of asexual parasitemia by 48 hours) [34]. Thick and thin malaria blood smears were stained with Giemsa, read, and reported according to standard methods [35]. Parasite counts were quantified using the measured total white blood cell count (Beckman Coulter, Inc., Fullerton, CA or the quantitative buffy coat method; Becton Dickinson); if not measured, a white blood cell count of 8,000/mL was assumed.

The Parasight-F® test was performed on days 1, 3, 7 and 28. Data were analysed (chi-square test for proportional data and Mann-Whitney U test for continuous data) using Epi Info 6.04b (Centers for Disease Control and Prevention, Atlanta, GA). Standard diagnostic test values (sensitivity and specificity) were calculated, as well as the test efficiency (the proportion of correct test results): TP + TN/TP + FP + TN + FN and Youden’s misclassification index (a measure of test reliability): 1 – (a + b), where TP = true positive, TN = true negative, FP = false positive, FN = false negative, a = probability of a false-positive result (FP/TP + FP), and b = probability of a false-negative result (FN/TN + FN).

Informed oral consent was obtained from all patients involved in the study. The study received ethical approval from the Aboh Mbaise General Hospital Ethical committee. The Institutional Review Board of Imo State University, Owerri, Imo State also reviewed and approved the study.

### 3. Results

There were 152 enrolled patients with *P. falciparum* parasitemia; all had symptoms or signs consistent with malaria at presentation. Of these, 143 (94%) had Parasight-F® testing done, and 142 (93.4%) had measured total white blood cell counts (median = 6,300/mL, range = 2,100 –13,600/mL).

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
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<tbody>
<tr>
<td>Specificity</td>
<td>94.9%(56/59)[85.8-98.9]</td>
<td>83.1%(69/83)[73.3-90.5]</td>
</tr>
<tr>
<td>Efficiency</td>
<td>95.1%(136/143)[90.2-98]</td>
<td>84.1%(106/126)[76.5-90]</td>
</tr>
<tr>
<td>Youden’s index</td>
<td>90.1%(89.3-91)</td>
<td>69.2%(67.6-70.7)</td>
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* values in brackets are 95% confidence intervals

The sensitivity and specificity of the Parasight-F® test on days 1, 3 and 7 are shown in Table 1 while none was recorded on day 28. On days 1,3,7 and 28, the respective proportions of false-positive tests were 3 of 59 (5.1%; 95% confidence interval [CI = 1.1-14.1%], 14 of 83 (16.9%; 95% CI = 9.5 – 26.7)], 17 of 104 (16.3%; 95% CI = 9.8 –24.9), and 3 of 54 (5.5%; 95% CI = 1.2 –15.4). The three false-positive results on day 1 occurred in patients with microscopically confirmed *P. vivax* infections. The f
four patients with false-negative Parasight-F® test results on day 1 had low *P. falciparum* parasite counts (20 –175/mL). Male and female patients had similar test results (Table 2).

At follow-up, the proportion of patients infected with *P. falciparum* who had a positive Parasight-F® test result decreased with time; these proportions were similar in cases of sensitive or RI resistant parasitemia (Table 3). The median day 1 *P. falciparum* counts were higher in patients with positive Parasight-F® test results on day 3 (3,904.5/mL versus 693.75/mL; *P* = 0.02) and day 7 (6,084/mL versus 1,989/mL; *P* = 0.013). Patients with day 1 *P. falciparum* parasitemias > 2,373/mL (the median) were significantly more likely to be test positive on day 3 (33 of 40 [82.5%] versus 17 of 37 [45.6%] relative risk [RR] = 1.8, 95% CI = 1.2 –2.6, *P* = 0.007), but not on day 7 (14 of 35 [40%] versus 7 of 33 [21.2%], RR = 1.9, 95% CI = 0.9 –4.0, *P* = 0.09).

**Table 2:** Comparison of day Parasight-F test results between Males and females in parts of the Imo River Basin, Nigeria.

<table>
<thead>
<tr>
<th></th>
<th>Parasite count</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>2,359.5 (20-64,998)</td>
<td>93.9% (31/33)</td>
<td>93.8% (15/16)</td>
</tr>
<tr>
<td>Females</td>
<td>2,856 (38-74,432)</td>
<td>96.1% (49/51)</td>
<td>95.3% (41/43)</td>
</tr>
</tbody>
</table>

* Value are the day 0 median (range) Plasmodium falciparum counts per microliter

**4. Discussion**

This study has shown that the Parasight-F® test performed well when compared with light microscopy for detecting and comparison of day 1 Parasight-F test results between males and female patients in the study area identifying *P. falciparum* infections in symptomatic adults with low to moderate parasitemias at the time of disease presentation. The results of this test and those of light microscopy were discordant on days 3, 7 and 28. There were no differences in the test values between male and female patients.

Our data are broadly consistent with the results of a growing number of clinical studies conducted in epidemiologically diverse malaria-endemic regions and in travelers [12-16, 19, 22-27], but contrast with those of Fryauff and others[28], who assessed the Parasight®-F test as an epidemiologic tool for the detection of asymptomatic infections in

**Table 3:** Proportions of positive/negative Parasight-F test results at follow-up in patients with sensitive or RI resistant malaria parasitemias

<table>
<thead>
<tr>
<th>Parasite-F</th>
<th>Sensitive no. (%)</th>
<th>RI resistance no. (%)</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Day 1</td>
<td>37 (92.5)</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Day 3</td>
<td>23 (59.0)</td>
<td>16 (41.0)</td>
</tr>
<tr>
<td>Day 7</td>
<td>10 (25.6)</td>
<td>29 (74.4)</td>
</tr>
<tr>
<td>Day 28</td>
<td>3 (8.1)</td>
<td>34 (91.9)</td>
</tr>
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</table>
children and adults with life-long exposure to malaria. They found that the Parasight®-F test had an overall sensitivity of 60.3% (41 of 68) in all ages, but in patients less than 10 years old the sensitivity was only 40% (12 of 30). They postulated that the high degree of malaria immunity of these individuals may have blocked the reaction between the PfHRP II protein and the monoclonal antibody on the dipstick. They also found a low specificity of 79% (108 of 136) in transmigrants more than 10 years old; the false-positive test results (21%) were predominantly associated with microscopic diagnoses of *P. vivax*.

The high specificity (95%) we obtained is comparable with results from other areas of significant *P. vivax* transmission [25-27]. We had false-positive test results that were associated with *P. vivax* parasitemia on day 1, and negative slides on days 3, 7 and 28. *Plasmodium falciparum* parasitemia may be missed if *P. vivax* is the predominant species. The negative slide results are consistent with continuing production of Pf HRP II (asexual forms/young gametocytes) or persistence after parasitologic cure. *Plasmodium falciparum* HRP II has been detected for up to four weeks in patients and travelers following parasitologic cure [14,15,22,26,36]. We found that a positive day 3 test result was not an early predictor of recrudescent parasitemia, although the number of RI cases was low (n = 9). In Thailand, one study showed that test positivity on day 4 was predictive of recrudescence, while another did not. A small number of our patients with low *P. falciparum* parasitemias had false-negative test results, a finding consistent with other studies that have documented sensitivities of 7-40% in patients with parasiteamias <120/mL [15,19,22,28].

The Parasight®-F test and other immunochromatographic tests have clear advantages over light microscopy. They are low technology, user friendly, and yield rapid results. Clinicians should be aware that the results obtained must be interpreted in light of the symptoms and signs of the patients, and the epidemiologic context of patient presentation. A positive Parasight®-F test result in high transmission areas merely indicates the presence of *P. falciparum* parasitemia and may not explain the patient’s illness.

Cost is a major impediment for the widespread deployment in malaria control programs [19,23,24]. There may be utility in the Integrated Management of Childhood Illnesses, a UNICEF/WHO clinical system designed to assist health care workers in differentiating and managing serious febrile illnesses in children less than five years old. Limited operational experience exists with the panmalarial antigen immunochromatographic test for detecting non-falciparum parasitemia; they may have advantages over rapid tests that only detect *P. falciparum* in specific settings [27,38,39].

References


