Role of the Parasight-F Test in the Diagnosis of Complicated *Plasmodium falciparum* Malarial Infection

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An evaluation was made of the diagnostic efficacy and utility of the Parasight-F test in diagnosing *Plasmodium falciparum* malaria, compared with conventional microscopy, particularly in severe and complicated cases. This study was designed as a prospective, case control hospital-based study. Febrile patients suspected to be suffering from malaria were selected randomly and were subjected to peripheral smear examinations (thick and thin) and Parasight-F tests till the required number of at least 30 cases of *P. falciparum* infection were identified, including at least 15 complicated cases. In addition 20 cases of *P. vivax* malarial infection as well as 20 healthy age and sex-matched individuals were taken as two control groups. The outcome measure was the number of cases with positive Parasight-F test results compared with conventional microscopy. Thirty-two patients with *P. falciparum* malaria were identified, with 15 severe and complicated cases. Peripheral smears were positive in 29 (91%) of these, while parasight-F test was positive in 31 out of 32 (97%) cases. Parasites were detected only by bone marrow examination in one case. Diagnostic sensitivity and specificity of peripheral smears for detecting falciparum infection were 90.6% and 100% respectively while that of the Parasight-F test were 96.8% and 100%, respectively (P>.05). The Parasight-F test has high sensitivity and specificity in diagnosing *P. falciparum* malarial infection, comparable to or even higher than microscopy exams, particularly in severe and complicated cases, with additional advantages of speed, simplicity and objectivity.

Key Words: Malaria, diagnosis, *Plasmodium falciparum*, parasight-F test.

Malaria continues to be a major global health problem, with over 2 billion people being exposed to varying degree of malaria risk in some 100 countries. Almost all deaths and severe disease are caused by *Plasmodium falciparum* malaria. Delay in diagnosis and treatment of *P. falciparum* malaria can result in severe deterioration of patient conditions, together with the development of a number of life threatening complications. The severe nature of infection, along with its potential for outbreaks, emphasizes the importance of rapid diagnosis to combat the related complications and thereby avoid significant mortality. Microscopic examination of the conventional Giemsa-stained blood smear has been the most reliable and inexpensive technique for the diagnosis of malaria. Although the procedure is relatively simple and sufficiently sensitive if properly preformed, it is time consuming and requires a skilled technician and adequate instrumentation and methodology, including staining procedures. Alternatives to traditional microscopy for the detection of malarial parasites have long been sought. One such alternative is a quantitative buffy coat assay, a diagnostic test based on fluorescent microscopic examination of the centrifuged parasite in a capillary tube, stained by acridine orange, reported by Rickman et al [1]. Also, a few scientists have shown that acridine orange staining of thin blood films is an appropriate technique for the laboratory diagnosis of malaria in developing countries [2]. However, both these fluorescent microscopic

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techniques are expensive and require considerable expertise. Newer molecular techniques, such as hybridization with specific DNA probes and polymerase chain reaction (PCR) for the diagnosis of *P. falciparum* malaria, are too sophisticated for routine diagnosis of malaria [3].

Many researchers in the recent past have obtained favorable results using a rapid manual antigen capture assay (Parasight-F test) for the diagnosis of *P. falciparum* malarial infection. The Parasight-F test is an immunodiagnostic test based on the detection of a specific soluble glycoprotein antigen, histidine rich protein II in the blood of patients with *P. falciparum* malaria. This antigen is secreted early during the erythrocytic cycle of the parasite, with a peak during schizont rupture. Various clinical trials done to evaluate the diagnostic sensitivity and specificity of this test have shown a sensitivity varying from 86.7% to 93.4% and a specificity of from 98.2% to 99.3% [4,5,6]. In India, Pinto et al. have reported that this technique can be superior to peripheral smear staining and helps in making an early diagnosis [7].

It has been seen that a proportion of severe and complicated cases of *P. falciparum* infection are aparasitaemic by microscopy, by the time they are admitted to a tertiary care institution, either because they already have been partially treated, or for other, as yet poorly understood, reasons. The etiology of such clinical status is often in doubt, causing major problems for clinical management. Presumably the Parasight-F test could be very useful for identifying or excluding a malarial etiology in such patients. For the sake of simplicity, speed, capability of early diagnosis, and specificity, this test would be very useful in patients with life threatening complications of *P. falciparum* infection. We evaluated the usefulness of the Parasight-F test and compared it's utility with conventional peripheral thick and thin smears for the diagnosis of *P. falciparum* malaria, including severe and complicated cases.

Materials and Methods

Patients. This study was conducted prospectively in the departments of Medicine and Pathology, Maulana Azad Medical College and the associated Lok Nayak Hospital. The clinical material was comprised of febrile patients suspected clinically as cases of malarial infection selected randomly from patients attending both the outpatient department and those severe cases admitted to the hospital with any complication related to malaria. Selection criteria for the study group include patients with acute febrile illness and no obvious source or focus of bacterial, viral or fungal infection, malignancy or systemic illness and those patients admitted to the hospital suspected clinically as having severe and complicated cases of P. falciparum infection, as defined by the working group convened by WHO [8]. We excluded patients with febrile illness who had obvious foci of bacterial or viral infection, malignancy or other etiological factors for fever, and those patients who had a relapse of fever within 14 days of being diagnosed and treated for *P. falciparum* malaria. This was due to the fact that the Parasight-F test can remain positive for 14 days after the treatment has started, limiting its usefulness for diagnosing reinfection in patients who have a relapse of fever within 14 days. All such patients fulfilling the above-mentioned criteria were subjected to detailed history and clinical examination, including previous antimalarial medication used, and were evaluated for any associated complication of *falciparum* malarial infection, such as cerebral malaria, renal failure etc.

Methods. All the patients were submitted to the following investigations: peripheral blood film examination, including both thick and thin smears with a parasite count; the Parasight-F test; complete haemogram and platelet count; blood sugar; liver function tests; kidney function tests; chest X-ray; urine analysis; ECG; and serum electrolytes. Special investigations in the severe and complicated cases included arterial blood gas analysis, CT scan of the head, lumbar puncture, and other relevant investigations. Bone marrow examination was done selectively for patients with a strong suspicion of malaria and negative peripheral smears. Both thick and thin blood smears were stained with Giemsa and were initially screened with a high-power optical microscope

for the presence of parasites and later on, under oil immersion for species identification. At least 100 oil immersion fields were examined, taking at least 10 minutes before reporting a negative. Parasite counts were routinely expressed as per microlitre of blood. The Parasight-F diagnostic kit was obtained from Becton Dickinson, India. The test was performed as per the instructions provided in the manual. A positive test is evident if a distinct pink line appears on a test dash in the middle of the strip (Figure 3). The control line appears thereafter in all the samples, to indicate the reagent control.

Patients with febrile illness were screened for malarial infection till the required number of at least 30 cases of *P. falciparum* infection, with at least 15 of them belonging to the category of severe and complicated cases as defined by WHO, were met. A positive case of *P. falciparum* infection was defined as a patient with acute febrile illness accompanied by positive peripheral smear/ bone marrow examination and/ or a febrile patient with strong clinical suspicion of malarial infection, a positive Parasight-F test along with dramatic improvement when treated with standard antimalarial therapy, and exclusion of other etiological agents by appropriate investigations. The latter assumption is based on the very high specificity of the Parasight-F test in detecting *P. falciparum* cases, as shown by various clinical trials and field studies [4-7,9-11]. Also, It has been postulated and argued by several researchers that the Parasight-F test can be positive in a proportion of severe and complicated cases of patient infection with *P. falciparum*, who may be aparasitemic by the time they are admitted to a hospital, due to inadequate antimalarial treatment or low grade chloroquine resistance, resulting in negative smears. The Parasight-F test, due to its ability to detect the soluble circulating antigens in the blood, rather than the parasite itself, may be positive in such cases and may in fact be a better marker of total parasite load than peripheral blood smear examination. Apart from the 30 cases of *P. falciparum* infection as defined above, 20 cases of *P. vivax* malarial infection diagnosed by peripheral smear examination as well as 20 healthy age and sex matched subjects were taken as two control groups and were labeled as control groups I and II, respectively.

Results

On the basis of the above-mentioned case definition, 32 cases of *P. falciparum* malarial infection were identified, with 15 of them having severe disease or complications resulting from the falciparum infection. Age and sex distributions of the uncomplicated *P. falciparum* group, and the complicated falciparum group along with both control groups of (*P. vivax* - control I and healthy subjects - control II) were comparable (age and sex matched). The mean age of the uncomplicated group was 30.2 ± 8.6 years; in the complicated group it was 28.9 ± 9.8 years; in the *P. vivax* control group it was 31.2 ± 10 years while the healthy control group was 30.2 ± 9.6 years ($P = 0.78$). The male to female ratio was also uniform, ranging from 3:1 to 4.6:1 across the four groups ($P=0.98$). The mean duration of illness was 8.1 ± 4.0 days in the uncomplicated falciparum cases; 7.4 ± 2.4 days in the complicated cases and 6.5 ± 2.6 days in the *P. vivax* control group ($P = 0.30$). Fever was present in all cases of uncomplicated falciparum malaria, while 20% of the cases of complicated falciparum malarial infection and 5% of the cases of *P. vivax* malaria did not experience any fever. Similarly a higher proportion of the cases (40%) with complicated falciparum infection demonstrated anemia, , while it was found in only 11.8% and 5% of the cases of uncomplicated falciparum and *P. vivax* malaria, respectively ($P = 0.019$). Seizures developed in 7 out of 15 cases (47%) of complicated falciparum malaria, while none of the patients of the uncomplicated group or the vivax control group had seizures.

Mean haemoglobin concentration and mean platelet count was comparable in the four groups (Table 1). Mean parasite density was 952.9 ± 168.2 / µl of blood in uncomplicated cases of falciparum group, while the complicated group had a mean density of 8951.3 ± 629.3 (range 1,280 – 516,320/µl) which was significantly higher than in the uncomplicated group. Asexual ring forms were not detectable in the peripheral smears of three complicated cases of *P. falciparum* two of which had cerebral malaria. A third case having disseminated intravascular coagulation (DIC), with

Table 1. Clinical, Biochemical and hematological profile of *Plasmodium falciparum* malaria cases and controls

Variables	Uncomplicated *P. falciparum* (n=17)	Complicated *P. falciparum* (n=15)	*P. vivax* cases control-I (n=20)	Healthy control-II (n=20)	P
Mean Age (yr)	30.2 ± 8.6	28.9 ± 9.8	31.2 ± 10.0	30.2 ± 9.6	.78
Male: female	4.6:1	4:1	4:1	3:1	.98
Fever (%)	100%	80%	95%	-	.09
Anemia (%)	11.76	40	5	-	.01
Seizure (%)	0	46.6	0	-	.00
Hemoglobinuria (%)	0	6.6	0		>.05
Mean hemoglobin (gm/dl)	10.6 ± .99	10.06 ± 1.65	10.8 ± 1.14	11.0 ± .8	>.05
Mean platelet count (lacs/mm^3)	1.75 ± .46	1.40 ± .51	1.74 ± .38	1.6 ± .42	>.05
Parasite density (per µl blood)	952.9 ± 168.2	8951.3 ± 629.3	2160.2 ± 190.7	-	<.05
Microscopy positive for ring forms	17	12	20	0	
Parasight–F positive	17	14	0	0	

Figure 1. Ring forms of *Plasmodium falciparum*. Giemsa stain, under oil immersion (X 1200).

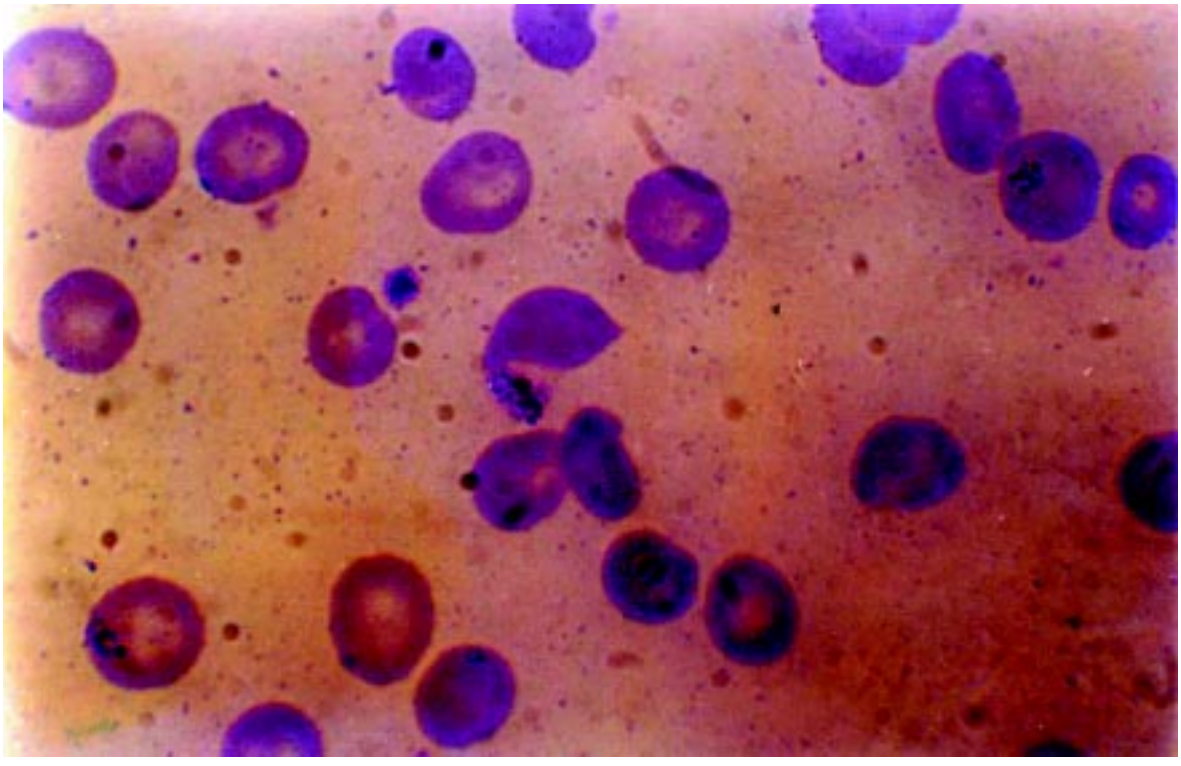


Figure 2. Gametocyte of *Plasmodium falciparum*. Giemsa stain, under oil immersion (X 1200).

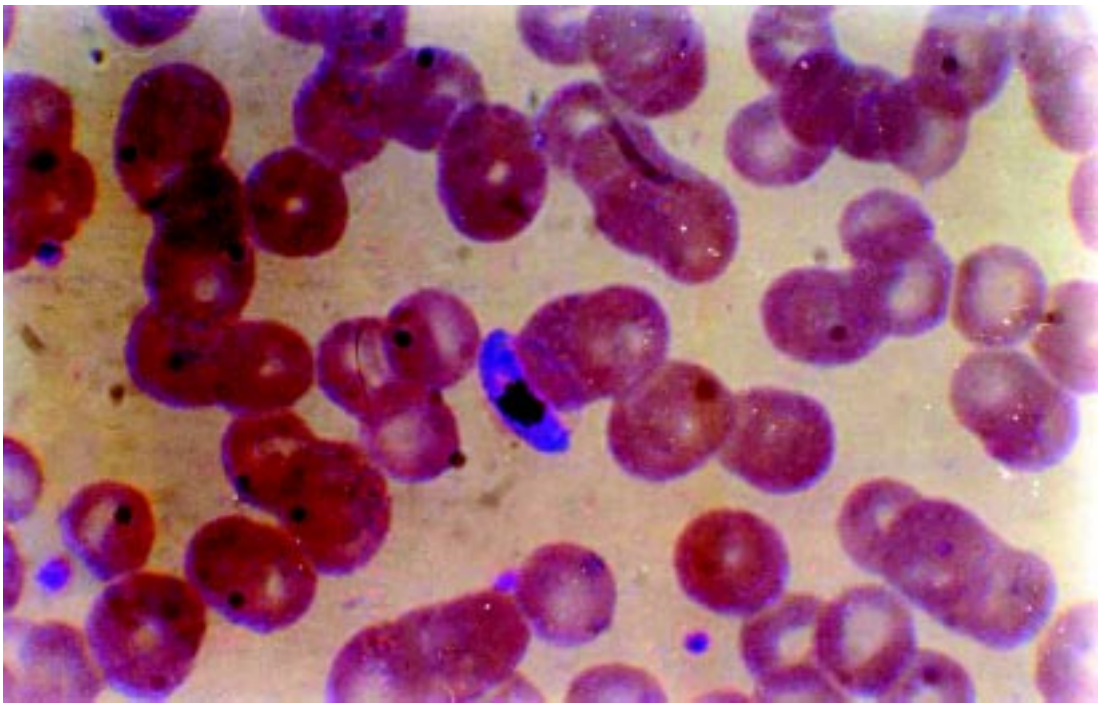
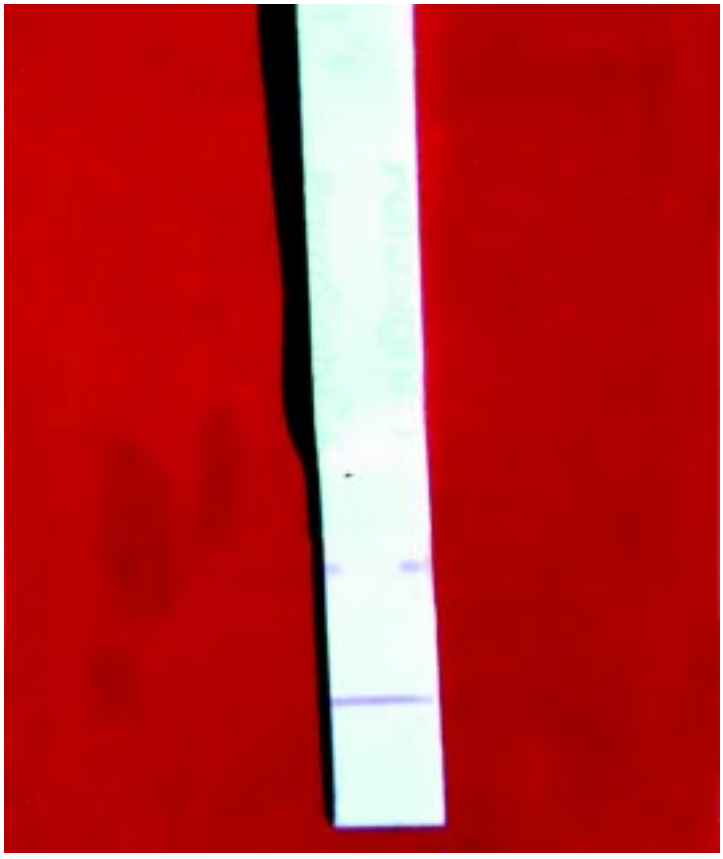


Figure 3. Parasight-F test – positive result.



spontaneous bleeding, had only gametocytes in the peripheral blood smear; a bone marrow examination of this patient also revealed ring forms. The minimum parasite density detectable by microscopy was 20/µl. Mean blood sugar levels were significantly lower in the complicated falciparum group when compared with the uncomplicated group and controls (P = 0.04). Similarly liver enzymes, viz. AST and ALT, and blood urea concentration, were significantly higher in the complicated group when compared to the uncomplicated group or the controls (P< 0.05).

Eleven out of 15 complicated cases of *P. falciparum* had cerebral malaria, while there was one patient each with severe anemia, subacute renal failure, hemoglobinuria and disseminated intravascular coagulation with spontaneous bleeding. Peripheral smears were positive for the ring forms in 29(91%) out of 32 cases (Figure 1), while Parasight – F was positive in 31 cases (97%). The Parasight-F test was able to detect two cases of *P. falciparum* malaria in which peripheral smears did not demonstrate the parasites. However, it failed to detect the antigen in one case presenting with DIC and spontaneous bleeding, whose peripheral smear showed only gametocytes (Figure 2). None of the 40 patients of the two control groups demonstrated a positive Parasight-F test or positive smears for the *P. falciparum* ring forms. Diagnostic sensitivity and specificity of the Parasight–F test were 96.8% and 100%, respectively, compared to 90.6% and 100% with conventional microscopy. Moreover for the Parasight-F test the predictive value of a positive test was 100% while that for a negative test was 97.5%. An additional feature that was noted was that the color band of the Parasight-F test was significantly darker in complicated cases when compared to uncomplicated cases, suggesting a direct correlation with the clinical severity of illness in such cases.

Discussion

Based on our study the Parasight-F test is an objective and rapid antigen detection assay for the detection of *P. falciparum* infection, with very high sensitivity and specificity, comparable to or possibly even better than conventional microscopy. This is in concordance with hospital and field studies done in various part of the world [4,10-13]. It is especially true for severe and complicated cases, as the test was able to detect the antigen in two cases of cerebral malaria, which were negative by conventional microscopy. The reason for smear negativity in these cases could have been prior administration of antimalarial drugs in inadequate doses before presenting to the hospital, causing partial clearance of the parasite as both the patients had a history of being treated by antimalarials. It is also possible that the two cases had a low-grade resistance to chloroquine, resulting in negative smears, while the circulating antigen was detectable in the blood. Response to quinine by both of them supports this possibility. Moreover, other factors, such as low levels of parasitemia, below that detectable by conventional microscopy could also have contributed to smear negativity in these patients, probably due to the sequestration of the asexual stages in deep vasculature, as described by Kodisinghe et al. [4]. One of the patients in our study, out of 32 cases, had disseminated intravascular coagulation, with spontaneous bleeding, and was Parasight–F test negative. Peripheral smears from that patient revealed only gametocytes (Figure 2). As the antigen is secreted by the trophozoite stage of the parasite and neither the peripheral smears nor the bone marrow examination demonstrated the trophozoite stage, with peripheral smears showing only sexual forms, the resulting insufficient antigenemia could have been the reason for the false negative antigen detection test, as suggested by Pinto et al [7]. Another alternative explanation could be blocking antibodies, as the patient could have developed these antibodies due to being ill for more than a month. Antigenic variation in HRP-II could also have contributed to a negative test in this case.

The Parasight–F test showed no cross reaction with other species of plasmodium and none of the of the *P. vivax* infected patients had a positive test. This could be attributed to the detection of specific histidine rich protein HRP-II antigen secreted by *P. falciparum* only. Owing to the very high specificity of this test, its

potential for being used as a test to exclude pathologies other than malaria needs no emphasis. Also the intensity of the color band of the test was much more positively correlated with the severity of illness than peripheral parasitemia, though a subjective bias in interpreting the color band cannot be ruled out. This suggests that the Parasight-F test is a better prognostic indicator than peripheral microscopy in complicated *P. falciparum* infection. This hypthesisis in agreement with views expressed by Kodisinghe et al., who suggested that as the test detects the soluble antigen rather than the parasite itself, which may be sequestered in deep vasculature, it might therefore be a better indicator of total parasite load than peripheral parasitemia [4].

Although we did not demonstrate that the diagnostic sensitivity and specificity of the Parasight–F test is significantly higher than conventional microscopy, it nevertheless was superior to the latter in a few aspects. When compared with conventional microscopy, this test was simple to perform and could be done by semi-skilled personnel. It is a rapid test, with results being available within five minutes. This not only helped in making an early diagnosis but also obviated the need for expensive and invasive interventions such as CT scans and lumbar puncture. Unlike microscopy, the results of the tests were objective and reproducible, with no subjective variation in interpretation of the results of the qualitative test. Last but not the least, it helped with the prognosis of complicated cases of *P. falciparum* infection.

Conclusions

In conclusion, the Parasight-F test has very high sensitivity and specificity in diagnosing *P. falciparum* malarial infection, comparable or maybe even higher than that of conventional microscopy especially in severe and complicated cases. Moreover the Parasight-F test offers advantages over microscopy in terms of speed, simplicity, objectivity, and possibly better prognostic implications.

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