PREVALENCE OF FALSE NEGATIVE PARASIGHT F TEST COMPARED WITH MICROSCOPY TECHNIQUE IN MALARIA DIAGNOSIS, AS SEEN IN BENIN CITY; NIGERIA

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ABSTRACT

Objective: This study determined the prevalence of false negative *Plasmodium falciparum* paraSight F (Histidine rich protein 2) enzymatic test in patients who attended the Outpatient and Emergency departments of the University of Benin Teaching Hospital (UBTH) Benin City, Nigeria.

Patient and Method: This was a prospective study involving 8,824 patients aged 6 months to 85 years with clinical features suggestive of malaria who attended the outpatient and emergency departments of UBTH, from June 2006 to May 2014.

Result: During the study period of eight years, a total of 8,824 patients were seen, with mean age of 47.2 -+ 6.8 years. There were 3,921 males, and 4,903 females giving a male to female ratio of 1:1.2 under five years' constituted 5,383 (61%); 2, 112 (23.9%) were paraSight F false negative, out of this, under five years made up 1,377 (65.2%). Infection with *Plasmodium* ovale was 269 (3.1%) while *Plasmodium malariae* was 157 (1.8%).

Conclusion: This study showed that microscopic technique for malaria parasite detection is and remains the gold standard, with other auxiliary test recommended as screening test.

Keywords: **Malaria, *Plasmodium falciparum*, False Negative ParaSight F Test, HRP2, Benin City**

INTRODUCTION

Malaria is a major public health problem and it is the second most common cause of death in Africa (after AIDS) and subtropical countries.[1]

Each year there are 300-500 million clinical cases of malaria (90% of them in Africa) resulting in 1.5-2.7 million deaths, mostly children less than five years[2]

Plasmodium falciparum is the most malignant specie causing malaria in the hotter and more humid regions of the world.[2] It is the main species found in tropical and subtropical Africa and parts of central America and South America, Bangladesh, Pakistan, Afghanistan, Nepal, Sri Lanka, South East Asia, Indonesia, Philippines, Haiti, Solomon Islands, Papua New Guinea and many Islands in Malanesia. It also occurs in parts of India, the middle East, and eastern Mediterranean.[2]

The species *Plasmodium falciparum* contains several varieties HRP2 which show differences in geographical distribution, vector susceptibility, human infection pattern, drug susceptibility, morphology antigenic composition and content of enzymes like histidine-rich protein.[2]

The diagnosis of malaria is by detecting and identifying malaria parasites microscopically in blood films.[1] More recently, rapid malaria antigen or enzyme test have been introduced to rapidly diagnose malaria.[4,6,10]

ParaSight F was the first rapid immunochromographic malaria antigen test to be developed.[5,6,8,10] Rural health workers were found to perform the test as well as laboratory personnel.[7] The evaluations have shown paraSight F to be both sensitive and specific, performing as well as and sometimes better than microscopy in field situations.[1] Workers in Mali found a small percentage (2 to 3%) of false negative paraSight F test result due to local strains of *Plasmodium falciparum* lacking the hrp2 gene which produces HRP2.[4] The sensitivity of paraSight F test before now

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is not known in Benin City. This study was conducted to find out the prevalence of false negative paraSight F test compared with microscopy Field's staining technique among patients with clinical diagnosis of malaria in Benin City Nigeria.

PATIENTS AND METHODS

A total of 8,998 patients were diagnosed as having clinical malaria, 174 did not give consent to be included in this study and hence were excluded from this study. Capillary blood samples were collected prospectively from patients attending the Outpatient, and Accident and Emergency clinics of the University of Benin Teaching Hospital during an eight- year period, from June 2006 to May 2014. Age range of 7 months to 78 years; with mean age of 47.2 ± 6.8 years.

Capillary blood samples were screened for malaria (*P,. falciparum*) by the paraSight F test method (Becton Dickson Diagnostics, USA). The rapid test was done by collecting capillary blood and dispensing into a small tube containing saponin saline. A test strip was placed in the tube which has antibody specific for *Plasmodium falciparum* HRP 2 impregnated in a line across the strip. The reaction was visualised by adding a pink-red colour detector reagent containing sulphorhodamine B dye and polyclonal antibody raised against *Plasmodium falciparum* HRP 2. As the reagent travelled up the strip it attached to the captured HRP 2 antigen, producing a pink coloured line. After adding a wash solution to clear the blood, the pink line was seen against a white background, indicating a positive test for *Plasmodium falciparum* malaria.

Control: A positive control was contained on the strip. It was seen as a broken pink line above the test line. When the patient's test was negative only the broken pink line of the control is seen.

For *Plasmodium falciparum* malaria microscopic test a thin film was prepared on a glass slide using the patient's capillary blood, allowed to dry and fixed–stained with Field stain B dye for 30 seconds followed by Fields stain A for another 30 seconds and the stain decanted, wash with water, decanted again, allowed to dry again with the back of the slide wiped with cotton wool. *Plasmodium falciparum* was diagnosed by observing on the microscope using x7 eyepiece and x100 oil immersion of the objecting lenses characteristic numerous Schufner's dots (red), Maurer's dots (clefts- red mauve) in intact red blood cells containing also chromatin (dark-red) and Cytoplasm (blue) of *Plasmodium falciparum* parasite with the white blood cells (which were mainly monocytes) containing brown black malaria pigments. Note that all negative samples from paraSight F test were first subjected to a microscopy Fields staining technique for thick and thin blood films (HD, Merck/BDH Diagnostics, UK), with positive and negative control samples for quality control.

A detailed clinical history and physical examination was elicited from all paraSight F false negative and microscopy Fields stain positive patients for the presence of fever, bitter taste, anaemia, anorexia, chills and rigors, headache, back and joint pains, vomiting and diarrhoea, splenomegaly, jaundice, cough, and haemoglobinuria.[11]

A certificate of approval to carry out this study was obtained from University of Benin Teaching Hospital Benin City (UBTH) Ethical Committee.

RESULTS

Of the 8,825 patients 3,921 were males and 4,903 females (giving a male to female ratio of 1:1.2) under five years was 5,382 (61%), from the total of 8,825, 2,117 (23.99%) were paraSight F false negative. Positive clinical features of malaria were elicited from 2,101 out of the 2,117 paraSight F false negative patients such as fever 2,010 (99.5%), bitter taste 1,810 (85.7%), headache 2,080 (98.5%) back and joint pains 1,121 (53.1%), vomiting 1,101(52%), diarrhoea 598(28.3%), splenomegaly 1,092(51.7%) jaundice 879(41.6%), cough 1,995(94.5%) chills and rigors 1,787(84.6%) and haemoglobinuria

(coca cola coloured urine with broken down red blood cells on microscopy) 364(17.2%).

Also, of note were those with false negative paraSight F test and positive microscopy for *P. ovale* 269(12.7%) and *P. malariae* 157(7.4%), but 6,712(76.1%) were positive for *Plasmodium falciparum* using the paraSight F test.

Of note was that patient's with samples with false negative result from rapid test had their clinical features of suspected malaria results after use of antimalaria confirmed after a one week appointment clinically.

DISCUSSION

It was shown from this study that microscopy technique for malaria parasite test still remains as confirmed by previous studies the best and gold standard.

Benin City Nigeria prevalence of 23.99% may also be postulated that more percentage of *Plasmodium falciparum* parasites in this region lack the HRP 2 gene i.e hrp2 gene.

In earlier studies conducted in Mali, to investigate prevalence of false negative paraSight F test results, the prevalence ranged from 2-3%,[4] in this study it was 23.99%.

The workers in Mali showed that this false negative test results were due to local strains of *P. falciparum* lacking the hrp2 gene which produces HR P2.[4]

The sensitivity of paraSight F test is reported as between 84.2 to 96.6% most studies have found the lower limit of detection for paraSight F to be equivalent to 25-60 parasites/µL detected in a thick film.[8] Although an experienced microscopist working under optimal conditions is able to detect as few as 10-20 parasite/µL in a thick blood film, this level of sensitivity is rarely achieved in most district laboratories, Craig and Sharp found a sensitivity of 84 parasites/µL for Giemsa thick films and a sensitivity of 30 parasite/µL for paraSight F.[4,8]

Also, for paraSight F test specificity is

TABLE I: AGE DISTRIBUTION VERSUS SEX

AGE(YEARS)	MALES(%)	FEMALES (%)
<5	2,240(25.4)	3,142(35.6)
6-15	134(1.5)	113(1.3)
16-25	114(1.3)	621(7.0)
26-35	401(4.5)	634(7.2)
36-45	103(1.2)	101(1.1)
46-55	425(4.8)	143(1.6)
55-78	504(5.7)	149(1.7)

TABLE 2: AGE DISTRIBUTION VERSUS FREQUENCY OF FALSE NEGATIVE RAPID TEST (paraSight F test)

Age(years)	Frequency of false negative ParaSight F test	Percentage false negative
< 5	1668	31 %
6-15	140	19%
16-25	24	3.2%
26-35	42	4.1%
36-45	9	4.5%
46-55	103	18.1%
55-78	131	20.1%

TABLE 3: AGE DISTRIBUTION VERSUS RESULTS OF THE TWO MALARIA PARASITE TEST

Age	Rapid test (paraSight F test)				Thin film stain malaria parasite microscopy test			
	+ve	+ve %	-ve	-ve %	+ve	+ve %	-ve	-ve %
< 5	3,470	39.3	1,912	21.7	5,302	60.1	80	0.9
6-15	84	1.0	163	1.8	201	2.3	46	0.5
16-25	686	7.8	52	0.6	728	8.2	7	0.08
26-35	907	10.3	68	0.8	997	11.3	38	0.4
36-45	185	2.1	19	0.2	196	2.2	8	0.09
46-55	422	4.8	146	1.7	512	5.8	56	0.6
55-78	475	5.4	178	2.0	607	6.9	46	0.5

estimated to be between 81- 99.5% with variations being found in different areas of malaria transmission.[1] Positive predictive values ranged from 80-98.7% although lower values have been found in hypoendemic areas while negative predictive values ranged from 72-100%.[1,4]

Limitations to this study, HPR2 gene was not determined for which I recommend further research work to be done on.

The strength of this study is that it agrees with others done elsewhere.[4, 8]

CONCLUSION

The purpose of this study was to show the prevalence of false negative paraSight F test as compared to microscopic technique using Field staining of thick and thin blood films for malaria parasite detection.

The main finding was that microscopy technique remains the gold standard for malaria parasite detection.

The implication for laboratory and clinical practice is that more microscopist should be trained in malaria parasite detection, while other methods of diagnosis should be screening tests only. [15, 16, 17]

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