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Original Research Article

Comparative analysis of the diagnostic accuracy of malaria parasite by microscopy, RDTs (PfHRP-2 & PLDH) and PCR

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ABSTRACT

Background: Blood smear is considered as the gold standard test to diagnose Malaria parasite. The newer RDTs (malaria antigen) are reported to be highly sensitive, specific and time saving as compared to other diagnostic modalities. This test is undertaken to compare the efficacy of PfHRP-2 tests, PLDH and manual technique.

Results: A total of 252 cases of malaria as diagnosed by Composite reference technique were studied. The sensitivity of TFM, RDTs and PCR is 71.5%, 84.3% and 82.6% respectively and the specificity is 81.9%, 77.2% and 78.2% respectively.

Conclusion: The fact that the PCR & RDTs are costly, cannot assess the response of patients to treatment and inability to assess parasitic stage and density, makes the old dictum "Blood smears are the gold standard for the diagnosis of Malaria" to still hold truth.

1. Introduction

Malaria is the most common, highly endemic, fatal disease affecting over 500 million people worldwide and responsible for over a million pediatric deaths. Malaria is caused by a protozoan parasite of the genus plasmodium. Among the 4 Plasmodium species, P. falciparum is the most pathogenic and fatal if not timely treated.[1] The Hazardous nature of infection can be assessed by the following statement that malaria is endemic in 107 countries inhabited by half of world's population (WHO 2013).

Microscopy remains the gold standard for the diagnosis of malaria with a threshold sensitivity of 5 to 50 parasite/μl (depending on the expertise). Thick smears as compared to thin smears, gives a higher percentage of positive diagnosis in much less time since it has ten times the thickness of normal smears. Five minutes spent in examining a thick blood film is equivalent to one hour spent in scanning whole length of a thin blood film.[2]

2. Materials and Methods

This is a 3-year study from June 2015 to May 2018 conducted in Dept. of Pathology, in Deemed Medical college, University, Hospital and Research centre India.

EDTA anticoagulated blood was used for smearing thick & thin blood films and unfixed dried film was placed in buffered water (pH-7.2) and stained in giemsa for 10-15 min. MP cytoplasm stained blue and the nuclear chromatin red.

Malaria parasitic density was calculated by the below formula

$$\%\ Malaria\ parasitaemia\ = \frac{No\ of\ MP}{Total\ no.\ of\ WBC} \times 100$$

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(On Thick smears Ring forms or trophozoites should be counted per 100 WBC, gametocytes are excluded).

PCR was done in a reference Laboratory as per standard protocols.

3. Results

A total of 750 clinically diagnosed cases of malaria were studied. A composite standard reference method was formulated by using these 4 diagnostic modalities and in collaboration with other labs

3.1. Number of positive cases

1. Thick film microscopy = 265
2. RDTs = 287
3. PCR = 262
4. Composite reference technique = 252

Composite reference technique showed total of 348 true negative cases,

P. falciparum = 155

Non falciparum= 97

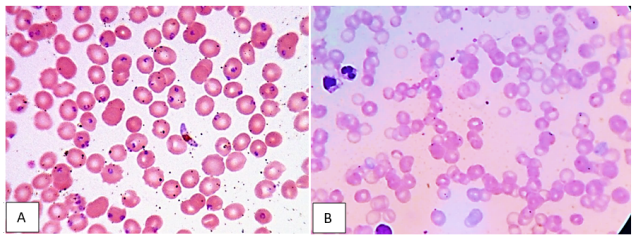

Fig. 1: Plasmodium falciparum (gametocyte and ring forms)

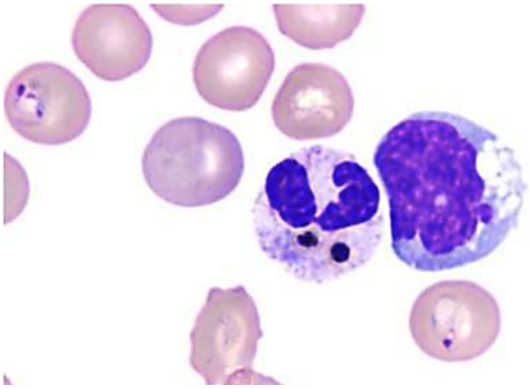

Fig. 2: Malaria pigment in neutrophil

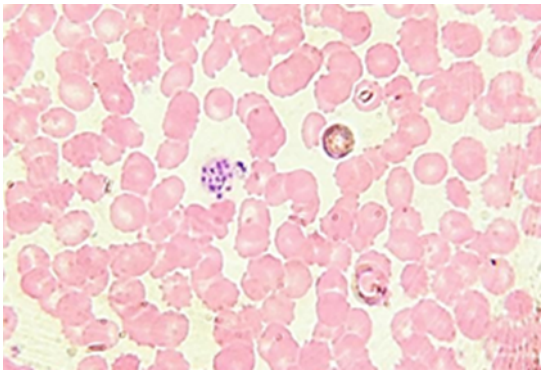

Fig. 3: Schizont (Plasmodium falciparum)

4. Discussion

4.1. Malaria parasite can be diagnosed by these 4 principal techniques

1. Microscopy
2. Antigen
3. Antibodies against MP
4. PCR

4.2. Malaria serology tests (antibody detection)

1. Positive test indicates past infection
2. Not useful for treatment decisions
3. Investigating congenital malaria
4. Diagnosing, or ruling out, tropical splenomegaly syndrome.

The antibody based method as anticipated showed good level of sensitivity but is very unspecific.

4.3. Malaria antigen detection – RDTs

1. Commercial kits are available as immunochromatographic rapid diagnostic test.
2. The sensitivity of these dipstick strip tests approaches that of thick film microscopy (i.e. 0.002% parasitaemia equivalent to 100 – 200 parasites/ μL of blood).

4.4. PfHRP-2 tests (histidine rich protein)

1. Uses monoclonal Abs to detect a histidine rich protein of P. falciparum.
2. Threshold for parasite detection $\geq$ 100 parasites/μl (less sensitive than pLDH).
3. Can differentiate between P.falciparum and non-falciparum malaria.
4. May remain positive up to 14 days post treatment, inspite of asexual and sexual parasite clearance, due to circulating antigens
5. Cannot detect mixed infections.
6. May give false positives due to rheumatoid factor.

Table 1: Comparison of sensitivity and specificity of different methods

Methods	TP	FP	TN	FN	Sensitivity%	Specificity%
TFM	252	77	348	73	77.5	81.9
RDTs	252	103	348	47	84.3	77.2
PCR	252	97	348	53	82.6	78.2
Composite Reference	252	00	348	00	100	100

TP = True positive, FP= False positive, TN= True negative, FN= False negative
Sensitivity =TP/ TP + FN**, Specificity** =TN/TN + FP

Table 2: Comparative analysis of different studies

Reference study	Sensitivity %			Specificity %		
	TFM	RDT	PCR	TFM	RDT	PCR
Present study 2018	71.5	84.3	82.6	81.9	77.2	78.2
Olusola Ojurongbe 2013 [3]	77.2	62.3	97.3	72	87.4	62.5
S Gatti M. 2006 [4]	99	100	98.9		92.9	100
Nandwani et al [5]			96.8			

TFM – Thick film microscopy

Table 3: Comparison of P. Falciparum sensitivity

	P. Falciparum	SensitivityPresent	S Gatti
Composite reference	155	100	
Thick and thin blood smear	140	90	88
PfHRP -2	150	96.8	100
pLDH	145	93.5	
PCR	155	100	

4.5. Parasite lactate dehydrogenase (pLDH)

1. Use of monoclonal and polyclonal Ab.
2. pLDH is only produced by viable parasites, so it becomes negative 2-3 days after successful treatment.
3. Monitoring response to treatment (not HRP2- based tests).
4. Threshold for parasite detection as low as 10 parasites/μl i.e. more sensitive.
5. Does not cross-react with other species – P. Vivax, P. Ovale, P. Malariae.

4.6. Microscopic review of PBS → Gold standard for the diagnosis of Malaria (Moody 2000)

1. Detect MP with a threshold sensitivity of 5 to 50 parasite/μl (Trampuz et al 2003)
2. Precisely detect and differentiate MP species and parasitic density
3. Monitor the response of treatment and hence drug efficacy
4. Cost effective and precise (useful in endemic areas and developing countries)
5. The major draw back is the TAT (40 min)

In this study, Smears for MP detection showed a sensitivity of 77.5% and specificity of 81.9% which is in comparison with other studies.

PCR detects specific nucleic acid sequence and its ability to detect <5 parasite/μl of blood. PCR is useful both for initial parasite diagnosis and for monitoring the efficacy of treatment. PCR product analysis is done by Gel electrophoresis but PCR requires about 10–11 hours to complete whereas microscopy took an average of 40–45 min. PCR detects the presence of malaria parasites on/in the red blood cells. PCR is expensive, requires electric power and time consuming& hence less affordable in developing countries. [6]

RDT (84.3%) is more sensitive than PCR and TFM in diagnosing malaria but lacks specificity (77.2%) and the major drawback is RDT remains positive during treatment and hence response

to treatment cannot be assessed. The total number of false positive cases by RDT is 103 as the patient were tested positive for MP by RDTs even through there was no sexual or asexual forms see in PBS. In this study PfHRP -2 is found to be more sensitive than pLDH for detection of p.falciparum infection but pLDH is found to be more reliable for monitoring efficacy of drug.

4.7. Major drawback of RDTs

1. Suboptimal sensitivity to low parasite density
2. Inability to accurately differentiate parasitic species and density
3. Expensive

Table 4: Comparative analysis of MP diagnostic techniques in India [10]

	BFM	RDT HfHRP-2 pLDH		PCR
MP species detection	yes	Only P.F	yes	yes
MP test result (No parasitemia)	Negative	+ ve	+ve	+ve
Sensitivity (per μl)	50- 500	100-200	100-200	1-5
TAT	40 min	15 min	15 min	10 hrs
Accessibility in developing countries	Easy	Little difficult		Rare

RDT is a malaria diagnostic tool used for early diagnosis of the disease & it has greatly improved the control & management of the disease. Though reliable, their challenging performance demands for continuous quality control monitoring. This has prompted WHO to recommend QC of RDT by monitoring their test performance using microscopy for at least 20 malaria positive and negative RDT samples. [7–9]

5. Conclusion

Microscopy is the most widely used tool to diagnose malaria and if done meticulously is very sensitive and can detect a parasite level of $\leq$50/μL (0.001%), moreover it also gives important information to the clinician like species, parasites stages and parasite density.

RDTs are costly when compared to blood smears, cannot assess the response of patients to treatment, are unable to assess parasitic stage and density and also test positive even when the patient is on antimalarial drugs and even with no parasitemia in blood. PCR is also expensive and its TAT is around 10 to 12 hours. These facts limits their use as a screening test for MP in developing countries and makes the old dictum "Blood Smears are the Gold standard for the diagnosis of Malaria" to still hold truth.

6. Source of Funding

No funding sources

7. Conflict of Interest

None declared.

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