CHAPTER

6

Diagnostic Challenges of Malaria in India

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INTRODUCTION

Malaria is a life threatening protozoal disease, transmitted by the infected Anopheline mosquitoes, caused by parasites known as Plasmodium (P) falciparum, P.vivax, P.malarie and P. ovale. The fifth species of plasmodium detected infecting humans in South-Eastern Asian countries since 2004 is P.knowlesi, commonly known as "monkey malaria parasite".[1]

GLOBAL SCENARIO

It is a major public health problem responsible for substantial morbidity, mortality and economic loss. Its transmission is ongoing in 108 countries containing 3billion people, out of which 1.2 billion are high risk cases and causing 1 million deaths each year.[2] In 2015 WHO documented 214 million symptomatic cases and 438,000 deaths from malaria.[2] P.falciparum malaria is the predominant infectious disease in tropical and sub-tropical countries with an estimated global incidence of 207 million cases and 627,000 deaths reported in 2012. (WHO)[3]

INDIAN SCENARIO

At present official figures for malaria in India, available at National Vector Borne Disease Control Programme (NVBDCP) indicate 0.7-1.6 million confirmed cases and 400-1000 deaths annually.

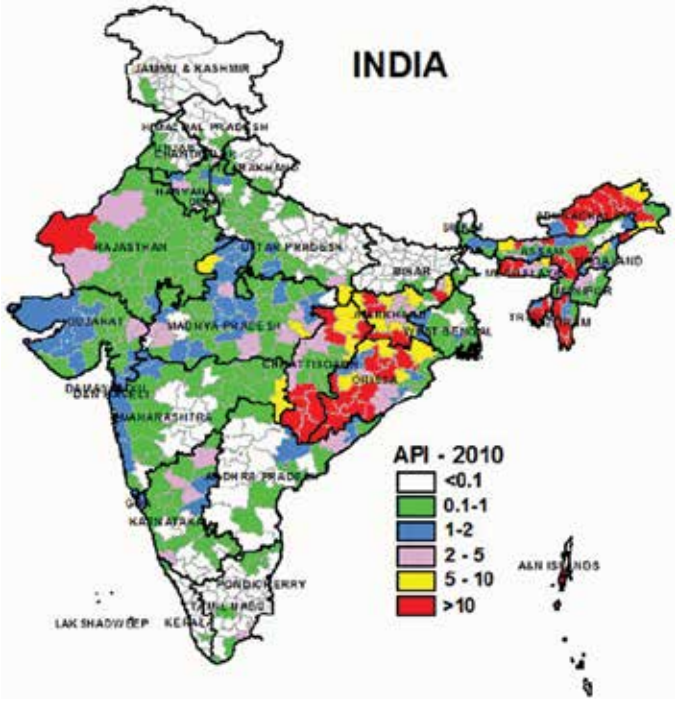

Fig. 1: Malaria endemic areas in India

According to World Malarial Report 2014, 22% (275.5m) of India's population, live in high transmission (>1 per 1000 population) areas, 67% (858.9m) live in low transmission (0-1cases per 1000 population) areas and 11% (137.7m) live in malaria free (0 cases) areas[4] (Figure 1).

With a population of 36.7 million (3.5%) the state of Odisha contributes about 25% of the total annual malaria cases, more than 40% of P. falciparum malaria cases and nearly 20-30% of deaths caused by malaria in India, followed by Meghalaya, Mizoram, Maharashtra, Rajasthan, Gujarat, Karnataka, Goa, Southern Madhya Pradesh, Chattisgarh and Jharkhand that also report significant number of malaria cases and deaths.[4]

DIAGNOSIS OF MALARIA

Malaria cases are diagnosed clinically and supported by laboratory investigations.

Clinical Diagnosis

The earliest symptoms of malaria are very non-specific and variable that includes fever with chill and rigor, headache, dizziness, anorexia, nausea, vomiting, loose motion etc. and very often they are received in secondary and tertiary care centres with complications like cerebral malaria, renal failure, hypoglycaemia, anaemia, respiratory distress or septicaemia. Clinical diagnosis of malaria is challenging in most tropical countries as it needs high degree of suspicion in both endemic and non-endemic areas, because malaria alone and with complications can mimic many other diseases like dengue fever, leptospirosis, influenza, hepatitis, enteric fever, scrub typhus, all types of viral encephalitis and gastroenteritis. It must be considered in the differential diagnosis of sepsis in pregnant woman arising in the uterus or urinary tract. The non-specific nature of clinical signs and symptoms of malaria may promote indiscriminate use of antimalarials due to over-treatment or non-treatment of other diseases in malaria endemic areas.

Laboratory Diagnosis

Rapid and effective malaria diagnosis decreases both patient's suffering and community transmission. In the laboratory, malaria is diagnosed using different techniques e.g.conventional microscopic diagnosis by staining thin and thick peripheral blood smears, quantitative buffy coat (QBC) method, and rapid diagnostic tests (RDT) and

Table 1: Comparison of Rapid Diagnostic Tests for Malaria Antigens

	PfHRP2 tests	PfHRP2 and PMA test	pLDH test
Target antigen	Histidine rich protein 2 of P. *falciparum*,water soluble protein expressed on RBC membrane	Pan-specific *Plasmodium* aldolase. Parasite glycolytic enzyme produced by all species and PfHRP2	Parasite lactate dehydrogenase. Parasite glycolytic enzyme produced by all species
General test format	2 lines	3 lines	3 lines
Capability	Detects *P. falciparum*only	Can detect all 4 species	Can detect all 4 species
Non-falciparum species	Not detected	Detected; differentiation between the 3 not possible	Detected; differentiation between the 3 not possible
Mixed infections of P. *falciparum* with non-falciparum species	Appear as *P. falciparum;*differentiation not possible	Appear as *P. falciparum;* differentiation not possible	Appear as *P. falciparum;* differentiation not possible
Detection limit	>40-100 parasites/µL	Higher for P. *vivax* and other non-falciparum species	> 100-200 parasites/µL for *P. falciparum* and*P. vivax;* may be higher for*P. malariae* and*P. ovale*
Post-treatment persistence of antigens	Reported up to 31 days	Reported; longer for pan specific antigenemia than for PfHRP2	Reported up to 1 -3 weeks
Cross-reactivity between malarial species	Reported	Reported	Reported
Cross-reactivity with auto antibodies	Reported, high (up to 83% with rheumatoid factor)	Not known	Reported. low (3.3% with rheumatoid factor)
Indication of viability of parasites	No	No	Positive test indicates presence of viable parasitemia

molecular diagnostic methods such as polymerase chain reaction (PCR) amplification of parasitic nucleic acid.

Peripheral Blood Smear Examinations

Accurate and timely diagnosis of malaria infections in febrile patients is a critical part of case management. Microscopic examination of stained blood smear remains the gold standard practice for malaria till date. Though considered as advantageous for accurate identification, species detection, parasite density estimation, low cost test, its parasite detection limitation is estimated to be 4-20 parasites/µl blood but under field conditions a threshold of about 50-100 parasites/µl is more justified.[5] More over its accuracy depends upon quality of microscope experience and training of microscopists and staining procedure and time consuming observations, added to that PHC's clinic examine blood smears from a large no. of clinically suspected patients with the help of one or two trained microscopists resulting in misleading interpretation and under estimation of malaria parasite.[6]

At any parasitaemia, more is the no. of mature stage parasites poorer is the prognosis. In general if more than 50% of peripheral blood parasites are at the tiny ring stage (i.e diameter of nucleus is less than 50% of the diameter of cytoplasm rim), the prognosis is relatively good. If more than 20% of parasite contain visible pigment (i.e. mature trophozoites or schizonts), the prognosis is relatively bad.

The presence of malaria pigment on polymorphonuclear leucocytes (PMN) (neutrophils) is a useful indication of the diagnosis of malaria, especially in anaemic children and in patients of sever malaria with absent or low parasitaemia.[1] If more than 5% PMN's contains malaria pigment then prognosis worsens.

QBC Test

These technique uses epi-fluorescent microscope that detects malaria parasite DNA when stained with fluorescent dyes e.g. acridine-orange. It is a simple, rapid and sensitive test for diagnosing malaria but it requires specialised instrumentations which is more costly then conventional light microscopy and is poor at determining species and numbers of parasites.

Rapid Diagnostic Test (RDT)

Over past two decades RDTs for malaria case identification has been developed and tested as an alternative to microscopy. This is based on the principles of detection of antigen released from parasitized RBCs.The result is a coloured test line obtained in 5-20minutes.RDTs incorporate antibodies against Histidine rich protein2 (HRP2) specific for P.falciparum, Plasmodium specific LDH (pLDH) for all malaria species and also Plasmodium aldolase.

Several studies compared RDTs based on these antigens, with different results in terms of sensitivity, specificity,

Table 2: Comparison of Peripheral Blood Smear Examination and RDTs for Malaria

	Peripheral Smear	Rapid Diagnostic Tests
Format	Slides with blood smear	Test strip
Equipment	Microscope	Kit only
Training	Trained microscopist	'Anyone with a little training'
Test duration	20-60 minutes or more	5-30 minutes
Test result	Direct visualization of the parasites	Color changes on antibody coated lines
Capability	Detects and differentiates all plasmodia at different stages	Detects malaria antigens (PfHRP2/ PMA/pLDH) from asexual and/or sexual forms of the parasite
Detection threshold	5-10 parasites/μL of blood	1 00-500/μL for P. *falciparum,* higher for non-falciparum
Species differentiation	Possible	Cannot differentiate among non-falciparum species; mixed infections of *P.falciparum* and non-falciparum appear as *P. falciparum*
Quantification	Possible	Not possible
Differentiation between sexual and asexual stages	Possible	Not possible
Disadvantages	Availability of equipment and skilled microscopists, particularly at remote areas and odd hours	Unpredictable efficiency at low and very high parasitemia; cross reactions among plasmodial species and with auto-antibodies; persistence of antigens
Status	Gold standard	Not yet approved by the FDA
Cost per test	US$ 0.12-0.40	US$ 1 .20-13.50

positive and negative predictive values. Since 2010, WHO has recommended either RDT or microscopy for confirmation of suspected malaria cases before treatment.[7] The RDT consumption has increased in developing countries for the past few years. The WHO has recommended a minimum standard of 95% sensitivity at parasite densities of 100/μl. Most RDTs today have achieved >95% sensitivity goal for P.falciparum but not for non P. falciparum[5] (Table 1). RDTs, in field conditions require to be stable under extremes of temperature and humidity during use and storage.

In 2010, P.falciparum lacking pfhrp2 and/or pfhrp3 genes were first isolated from infected human subjects in the Amazon region of Peru.Other endemic regions also reported false negative results using RDTs based on pfhrp2 due to gene deletions.[7]

A lot of RDT negative cases often responding to anti-malarias questions not only to establish the genetic make-up of Indian population but also to the humidity, temperature resistant RDT Kit's standardisation.

Both microscopy and RDT cannot detect parasite densities of less than 100 parasites per ml particularly in field conditions where a symptomatic carriers have a much lower parasite density[8] (Table 2).

Polymerised Chain Reaction (PCR)

During the past decade highly sensitive and specific nucleic acid amplification techniques have been developed to detect malaria parasite those are PCR, quantitative PCR (qPCR), Reverse transcriptase PCR (RT-PCR).

But PCR & Loop mediated isothermal amplification (LAMP) are the techniques used for detecting parasite DNA. These are highly sensitive and vary useful for detectingmixed infections in particular at low parasitaemia where conventional microscopy or RDT will be of no help.

PCR detects< 10 parasite/μl, LAMP detects 5-10 parasite/μl and NASBA's (Nucleic Acid Sequence Based Amplification) selection limit is < 1 parasite/μl. For field based epidemiological study PCR based methods are most useful to quantify, detect and diagnose low density parasitaemia whether asexual or gametocyte forms. In developing countries these tests are performed in reference centres as it is impractical to use for diagnosis of malaria in standard clinical settings. At present according to WHO, molecular diagnostic tools based on nucleic acid amplification techniques don't have a role in the clinical management of malaria.[2]

CONCLUSION

The foremost point in the malaria case management is prompt and accurate diagnosis that can be obtained by proper travel history, clinical diagnosis, confirmation by specific diagnostic procedures having high accuracy. The diagnostic challenges in India can be alleviated

if a suitable, simple, sensitive and low cost diagnostic procedure with high specificity would be available.

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