TECHNICAL SERIES

Malaria and its diagnosis

Rapid tests for Malaria detection

...Setting trends

Introduction

Malaria diagnosis has gained importance with the availability of rapid diagnostic tests (RDTs) either antigen or antibody based. Medical organization like WHO, National institute of malaria research (NIMR) have advocated the use of Antigen detection RDTs for correct and timely diagnosis of malaria. Antibody based tests are used but they have no clinical relevance and do not help in correct and timely diagnosis.

Antigen detection RDTs are therefore most suitable for ensuring accurate diagnosis and quick initiation of therapy to prevent unnecessary use of drugs that could lead several complications including drug resistance.

Clinical symptoms of malaria

Malaria is a febrile illness characterized by fever and related symptoms like chills, Sweat, Headache, body ache, malaise etc; followed by enlargement of liver or spleen.

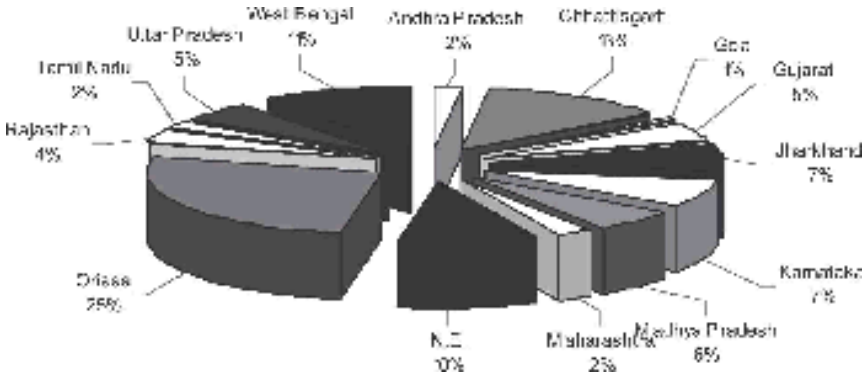
Malaria is a great imitator of other febrile symptom and it can present with such varied and dramatic manifestation. In endemic areas malaria should be incorporated as a part of differential diagnosis for almost all the clinical illness!

Malaria is apparently a simple disease. Yet it kills million every year – why?

Malaria is probably the only infection that can be treated in just three days, yet it kills millions every year worldwide. Without prompt diagnosis and appropriate treatment, malaria can become a medical emergency by rapidly progressing to complications and death. Most cases of severe malaria are caused by *P. falciparum* infection. Rarely, *P. vivax* or *P. ovale* produce serious complications, or death.

What is the Indian picture?

India is highly endemic and contributed about 76% of South-east Asia's total cases. In an independent study on malaria mortality, it has been estimated that malaria attributed death (including diagnosed and undiagnosed) rate is about 200,000 per year in India. Only early and proper diagnosis and correct therapy can prevent of the severity, and death. State wise distribution of malaria incidences in India has been described in below figure (data of 2007)



Immune response to Malaria Infection

Let's take an example of an individual who has never been infected with malaria. When this person gets infected for the first time the malaria antigens would be released in his/her blood stream. In the first infection only the malaria antigens are found in the blood stream. The body recognizes the malaria antigens as "foreign" and mounts an immune response.

As a result of this immune response, antibodies to malaria antigen would be formed in the individual. It must be noted that the malaria antigens are found in the individual's blood only during an ongoing infection with malaria. Once the person is cured of malaria, the antigens are also eliminated from his/her system. However the antibodies formed in the individual would continue to persist in his/her blood stream for a few years. As the individual gets re-infected the concentration of antibodies in the body continues to increase. While the presence of malaria parasites and its antigens in the blood of an individual confirm a current ongoing infection, the presence of antibodies can only indicate a past infection with the malaria parasites.

Presence of Antibodies to Malaria Antigens to General Population

From the above example of an individual patient, when the aforesaid sequence of events is extended to a larger population living in malaria endemic area such as India, due to persistent infection, re-infection, relapse cycles, the general population continues to be challenged with malaria infection. As a result population in malaria endemic countries harbor higher and higher level of antibodies to malaria. Even though they are healthy ambulatory and free from malaria infection.

Diagnosis of malaria – scenario

Diagnosis of malaria involves identification of malarial parasite or its antigens or antibodies in the blood of the patient. There are two types of diagnostic test for malaria- Microscopic tests and rapid tests. Microscopic examination for identification of parasites in peripheral smear is considered to be "gold standard method" as parasites have been indentified directly. Although this seems simple, the efficacy of the diagnosis is subject to many factors like blood collection time, density of parasites, quality of stains used, technical skill of microscopist etc.

In view of the above limitations of microscopy, what is the alternative available for malaria diagnosis?

Because of the above limitations of microscopy; rapid test for malaria has become the most admired test for diagnosis of malaria and has played a crucial role where microscopy is impractical or impossible, mainly in peripheral or remote areas.
In fact uses of malaria antigen detection RDTs are now a global WHO mandate for the detection of the types of malaria and its resultant treatment.

Strategy of malaria detection using RDTs

Two aspects are very important for using RDTs for malaria diagnosis,

- **Early detection** – It should detect malaria in the early stage of infection because as we discussed malaria especially *P. falciparum* infection can become a medical crisis by hastily progressing to fatality if not diagnosed in early stage.
- **Specific detection** – Since clinical symptoms of malaria are often confused with other febrile illness it is very important to detect the disease specifically to avoid improper therapy. The test should not show positive result in other febrile infection cases.

What are different types of RDTs available in market for malaria diagnosis?

There two types of RDTs available in the market.

- Malarial antigen detection tests – For detection of different parasite specific antigen such as Pf HRP II, pLDH and malarial aldolase. These antigens are released by parasites in blood stream of infected person during asexual (Erythrocytic schizogony- in RBC) as well as sexual stage in human (Gametogony). These antigens are present only during the malarial infection stage and disappear soon after successful treatment.
- Anti-malarial antibodies detection tests - after certain time of exposure of malaria, an infected individual's immune system produces antibodies against the parasite proteins. These antibodies serve as a marker to detect presence of malarial infection.

What are basic limitations of antibody detection test?

There are two basic limitations of antibody detection,

- Low Sensitivity or delayed detection: Antibodies to the malarial parasite proteins appear a few days after malarial infection, increasing in titer over few weeks. So detection of malaria is only possible after few days of appearance of clinical symptoms. But in malaria diagnosis early detection is very critical, belated diagnosis and delayed treatment may lead to severe complications even death of the patient.
- Compromised Specificity or false result: Prolonged persistence of malarial antibodies depends upon the endemicity, demographical characteristics, repeated exposure and therapeutic factors. Re-infection or relapse induces a secondary immune-response with a rapid increase of antibody titer. These antibodies persist in the patients' blood stream for a long time (some times more than 10 years) even after successful therapy.

These tests may show positive results in patients who have high antibody titer due to past/treated malarial infection; but are harboring other febrile infections such as typhoid, dengue, flu etc. In such cases it may lead to incorrect therapy.

Is an antibody detection RDT for malaria viable in our country?

Malaria is highly endemic in most parts of our country. Persons belonging to an endemic area may show a positive result with antibody detection test in normal healthy conditions or in other febrile illness, which can lead to wrong therapy.
So antibody detection is neither clinically viable for diagnosis nor appropriate even as screening tool in our country where anti-malarial antibodies are usually present in common population.

Advantage of Malarial antigen detection RDTs

- They detect the circulating antigen released by parasites during erythrocytic phase in the blood stream of an infected individual.
- They can detect malarial infection immediately after appearance of clinical symptoms.
- They may detect malarial infection even when parasites are sequestered in deep vascular compartment thereby missed by microscopic examination.
- Antigens are released by live parasites only and are eliminated from the blood stream soon after successful treatment. So in case of cured patients, antigen detection RDTs show negative result within a couple of days.
- Detection of ongoing malarial infection helps in timely and accurate diagnosis of febrile illness (malaria or non malarial infection) and initiation of correct therapy, thereby saving life and reducing the treatment cost.
- It eliminates the possibilities of drug induced complications of the patient due to unnecessary anti malarial therapy in non malaria cases.

What are the complications caused by anti-malarial drugs?

Study has reported some complications among patients due to side effects caused by anti malarial drugs. These complications include vomiting, abdominal pain delirium, hallucination, anemia, convulsion, even coma due to hypoglycemia. Serious complication like Jaundice, hemoglobinuria may occur in certain cases, where the liver or kidney gets affected.

The complications and the name of respective causative anti malarial drugs are listed below,

Drug/s	Complications
Chloroquine, Quinine, Mefloquine, Halofantrine, Tetracyclines, Primaquine	**Vomiting, Faintness, Abdomen pain, Convulsion**
Chloroquine	**Itching**
Chloroquine, Quinine, Mefloquine	**Altered behavior, confusion, delirium, Hallucinations, etc.**
Quinine	**Coma:** due to hypoglycemia, Hypoglycemia
Primaquine	**Anemia :** hemolysis in patients with Glucose 6-phosphate dehydrogenase deficiency, **Jaundice :** Hemolytic jaundice due to G6PD deficiency or jaundice due to liver malfunction. **Hemoglobinuria :** blood in urine - hemolysis due to G6PD deficiency.

What do the experts has to say about malaria RDTs?

Some experts' opinions and recommendations on use of appropriate RDTs for diagnosis of malaria are stated below,

WHO/UNICEF (A guide for selecting rapid diagnostic test kit: 1st edition) –
Malaria morbidity, mortality and transmission can be reduced if prompt diagnosis and adequate treatment is available. Rapid diagnostic tests (RDTs) offer the potential to provide accurate and timely diagnosis to everyone at risk, reaching those previously unable to access good quality microscopy services.
A malaria RDT, sometimes called "dipstick" or "malaria rapid diagnostic device" detects specific antigens (proteins) produced by malaria parasites. These antigens are present in the blood of infected or recently infected people.
RDTs detecting anti-malaria antibodies also exist, but have indications other than case management.

National Institute of Malaria Research – NVBDCP (Govt. of India) -
Rapid Diagnostic Tests are based on the detection of circulating parasite antigens. Several types of RDTs are available. Some of them can only detect *P. falciparum*, while others can detect other parasite species also……. Presently, NVBDCP supplies RDT kits for detection of *P. falciparum* at locations where microscopy results are not obtainable within 24 hours of sample collection.

Ann of Saudi Med. Vol.22;2002: (Malaria screening of...) Saeed A.A. et al. –
In malaria endemic countries like Saudi Arabia, excluding antibody-positive donations would result in too much wastage of blood units. However antigen malaria testing appears to offer a potential utility, as only few donations would be rejected.

Malaria – a focus on screening: Knox C. & Appleton N. (CLI Feb-Mar 2004) –
Indirect methods rely on the detection of antibodies to malarial parasites….. Indirect methods are not suitable for the diagnosis of malaria. …..They are less appropriate as a screening tool in countries where malaria is endemic because of very high incidence of malaria antibodies in the population.

At a glance comparison between Malarial antigen detection RDTs & anti-malarial antibody detection RDTS

Antigen detection RDTs	Antibody detection RDTs
Can detect malaria infection immediately after appearance of clinical symptoms.	Antibodies may be detectable after certain days of appearance of clinical symptoms.
Detect antigens released by live parasites during infection.	Detect antibodies to malaria which are produced by infected individual's immune system.
Show positive result only in active and ongoing infection cases.	May show false positive result in healthy individuals and patient representing other febrile illnesses.
Recommended by WHO and several national /international healthcare research organizations for malaria diagnosis.	Not recommended for diagnosis or malaria case management.
Possibility of cross reactivity or false result is almost nil.	Cross activity with other parasite like Babesia specific antibodies may show false positive result. Cross reactivity between two malarial parasite (Pf &Pv) antibodies has also been observed.

Need of the hour – A true choice for malaria detection

Timely and correct therapy is only way to reduce the socio economic burden of malaria of our country. It is now established from the above discussion that microscopy and antibody detection RDTs with their limitations fail to provide requirements of early and accurate diagnosis. It is now evident that the True Choice for malaria diagnosis is based on antigen detection. Antigen detection RDTs offer quick effective and timely diagnosis of malaria.

References and data sources

1. Malaria Diagnosis: Guide for selecting rapid diagnostic test kits 1st edition 2007 UNICEF.
2. Adult child malaria mortality in India: a nationally representative mortality survey. Dhingra Neeraj et al.
3. Ann of Saudi Med. Vol.22;2002: (Malaria screening of...) Saeed A.A. et al.
4. Update on rapid diagnostic testing for malaria. Murray et al.
5. Profiling antibody immune response against blood stage malaria vaccine candidates. Gray J. Corran P.H. et al.
6. Dr. B. S. Kakkilya. www.malariasite.com
7. Malaria – a focus on screening. Knox C. Appelon N.
8. Secretion of malaria histidine rich protein from plasmodium falciparum infected erythrocyte. Howard et al.
9. Burden of malaria in India: retrospective and prospective view. Kumar et al.