Diagnostic Tests to Distinguish Between Malarial Parasites

Gandhi Anilkumar Surendrakumar
M. Pharm. Sci.

The control of malaria presents a major challenge, as 1/3 rd of the world's population inhabits endemic areas with more than a 100 million cases being reported per year. Malaria was nearly eradicated from India during the early 1960s but unfortunately it has struck back with a vengeance. This is attributed to the development of drug resistance by the causative organisms, Plasmodium vivax and Plasmodium falciparum. Studies indicate that an increase of three degrees in the average global temperature during the next century could increase malaria carrying mosquitoes by such an extent that 50 to 60 million more cases are expected each year. Thus intense research work in malaria is necessary to find newer methods of diagnosis and newer drugs for its treatment.

Symptoms

Malaria is a protozoan infection characterised by paroxysms of chills, fever, sweating and by anaemia, splenomegaly and a chronic relapse course.

Classification

Sir Ronald Ross discovered the parasites causing malaria in humans for which he was awarded the Nobel Prize in 1902. There are atleast four parasites identified to be responsible for causing malaria in human beings, 1) *Plasmodium vivax*, 2) *Plasmodium falciparum*, 3) *Plasmodium ovale*, 4) *Plasmodium malariae.*

Out of these, the first two species P. vivax and *P. falciparum* account for more than 95% of malaria cases. *P. falciparum* malaria is also known as the malignant tertian malaria, or tropical malaria, or pernicious malaria and *P. vivax* malaria as benign tertian malaria, or simple tertian malaria.

Diagnosis

Diagnosis of malaria is by studying the symptoms like periodic attacks of chills and fever without apparent cause, enlargement of spleen, etc. The physician also take the advantage of the differntial diagnosis of malarial parasites. The presence of the parasite is confirmed in the thick blood smear stained with one of Ramanowsky stains (usually Giemsas).Thin smears are used for species differentiation.

Table 1 : Diffential diagnosis of malarial parasites.

No.	Characteristics	*P. vivax*	*P. falciparum*
1.	Parasitemia (mm^3)		
	Average :	20,000	50,000 - 500,000
	Maximum :	50,000	upto 2,500,000.
2.	Stages of parasites usually observed.	Trophozoites, schizonts and gametocytes.	Young trophozoites and gametocytes.
3.	Changes in infected R.B.C.s	Enlarged	Not changed.
4.	Trophozoites ring stage.	Large with one chromatin dot.	Small with two chromatin dots.
5.	Schizonts (mature)	12-24 merozoites	Not usually seen.

6.	Maurer's dots.	Absent	Present.
7.	Schuffner's dots	Present	Absent.
8.	Gametocytes.	Large and rounded fills up the red cell	Kidney - bean shaped round or pointed ends.

However, these tests are inadequate to specifically distinguish these parasites.

Treatment

In absence of specific tests, physicians employ the following treatment :

1. Chloroquine is used for all forms of malaria ; however P. falciparum has developed resistance to it.
2. Primaquine is used for radical cure.
3. Resistant strains are treated with Quinine (I.V. in severe cases), with Pyrimethamine and either Sulfadiazine or Dapsone or Sulfadoxine.
4. In pregnancy, Chloroquine is given (no Pyrimethamine or Sulfonamides) due to transplacental transmission of malarial agents to the foetus.
5. For *P. vivax*, anti-relapse drug is used which consists of Chloroquine phosphate and Primaquine phosphate.
6. Recent trend in the treatment of *P. falciparum* malaria is to use Fluoroquinolones.

Thus many drugs like Chloroquine, Amodiaquine, Quinine, etc. may lead to clinical cure but for radical cure, treatments are to be given which generally involve combination therapy.

As mentioned earlier special treatment is required to prevent relapse in P. vivax malaria. Due to the high level of parasitemia with *P. falciparum* there is a high degree of infection which is dangerous, demanding quick and specific treatment. Trophozoites of this parasite may invade the capillaries of internal organs resulting in several other manifestations like Jaundice, renal failure, diarrhoea, Early foetal death (in pregnant women) which complicates the disease. The organism has developed resistance against many of the existing drugs, hence if normal treatment is continued it may not respond resulting in severe infection and complications. Since it sequestrates in deeper organs, it is widely distributed and may not give positive results in the amount of blood taken, for which frequent tests are required.

Very specific tests are thus required to distinguish between two parasites so that proper line of treatment is followed to prevent relapse in *P. vivax* malaria and to prevent complications and seriousness of malaria due to *P. falciparum*.

Existing methods of detection suffer from disadvantages such as frequent testing being required to detect *P.falciparum* malaria which leads to patients noncompliance due to frequent withdrawal of blood samples.The method is not handy and depends on the ability of a person to make smears, to observe slides etc. which may lead to human errors as thickness and evenness of film may vary giving improper results. The test may fail to detect mixed infections. The method is difficult to reproduce quantitatively.

These difficulties are overcome by following techniques which are unequivocally species specific and the techniques of sample preparation and hybridisation are simple and rapid.

A) Diagnosis of *P. vivax* malaria using a Specific Deoxyribonucleic acid Probe :

A DNA probe which specifically distinguishes *P. vivax* malaria from *P. falciparum* malaria has been derived from a P. vivax DNA library. This probe VPL101 consists of 3.2 kilobase pairs and does not hybridise with upto 6µg. of human or P. falciparum DNA. VPL101 contains atleast

two copies of a 205 base pair repeat sequence.

The subcloned repeat probe VPL101/5, reacted with 73 of the 76 microscopically diagnosed P. vivax samples but not with any of the human DNA samples or any of 8 4P. falciparum DNA samples from a cultured parasite. It was possible to detect P. vivax in mixed infections in which only P. falciparum parasites were identified by microscopy.

B) Synthetic Antigens useful in the Diagnosis of Malaria Induced by *P. vivax*:

Peptides were synthesized that consisted of atleast 5 nonapeptides with an amino acid sequence corresponding to that of varient a (DRAAGQPAG) or varient d (DRADGQPAG) of the repeating monomer of circumsporozoite protein of P. vivax. The peptides were,

1. $(DRADGQPAG)_2$ - $(DRAAGQPAG)_2$ - DRADGQPAG.
2. $(DRAAGQPAG)_2$ - $(DRADGQPAG)_2$ - DRAAGQPAG.
3. DRADGQPAG - $(DRAAGQPAG)_2$ - $(DRADGQPAG)_2$.
4. DRAAGQPAG - $(DRADGQPAG)_2$ - $(DRAAGQPAG)_2$.

These peptides have utility in the detection of anti- *P. vivax* antibodies in the blood of infected persons by immunoassys such as ELISA.

C) Measurement of Lactate Dehydrogenase (LDH) Activity of *P. falciparum* as an Assessment of Parasitemia :

The assay is based on the observation that the LDH enzyme of P. falciparum has the ability to rapidly use 3- acetylpyridine NAD (NAPD) as a coenzyme in the reaction leading to the formation of pyruvate from lactate. Human red blood cell LDH carries out this reaction at a very slow rate in presence of APAD. The development of APAD was measured and the formation of this product established the basis of an assay that detected the presence of *P. falciparum in vitro* cultures at parasitemia levels of 0.02%. A correlation was found between levels of parasitemia and activity of parasite LDH (PLDH). PLDH activity can be measured in blood hemolyzates or in plasmas or serums from a patient with malaria.

D) Cloning of a DNA Probe and its Application in the Detection of *P. falciparum.*

The clones containing parasite DNA fragments were screened from a genomic library of *P. falciparum.* A DNA probe derived from clone pBF4 consisting of 3 kilo base pairs hybridises specifically with P. falciparum DNA but not with human DNA. The nick translated radiolabelled probe can detect 10pg purified *P. falciparum* DNA and a 0.001% parasitemia after 24 hr. of film exposure. The probe reacts with all microscopically diagnosed *P. falciparum* samples and 3 of *P. vivax* samples but not with any of 10 human DNA samples.

Another method involves spotting lysed blood from malaria infected patient directly onto nitrocellulose paper and identifying the malaria species on the basis of hybridisation of parasite DNA with species specific probe.

E) Evaluation of Synthetic Oligonucleotide Probe for Diagnosis of *P. falciparum* Infection :

A 21 nucleotide repeat (5' - AGGTCTTAACTTGACTAACAF-3') present in P. falciparum genome was chemically synthesized commercially and used as the probe. The oligonucleotide was end labelled with γ - ^{32}P ATP.

The sensitivity of the synthetic oligonucleotide in detection of P. falciparum was evaluated using DNA slot-blots. The radiolabelled oligonucleotide specifically hybridised to small quantities of homologous DNA. After 2 hr. film exposure to hybridisation as low as 100 pg of purified DNA was detected indicating sensitivity of the test. No cross-hybridisation of the oligomer was observed with as much as 3 µg of human DNA. It did not hybridise with any of the 21 unidentified samples. Out of 50 patients samples studied, 7 were infected with P. vivax and only one of these gave a positive hybridisation signal that too after keeping for 18 hrs.

Conclusion

DNA probe methods are more efficient and allow rapid handling and screening of a large number of samples even by untrained personnel in field situations. In these methods it is possible to detect mixed infections as well as very low levels of infections.

Thus these techniques have proved to be important tools for more accurate epidemological analysis and control programme monitoring where microscopic methods tend to give lower results due to low parasitemia and mixed infection. They may also be useful in screening blood donors especially in malaria endemic countries.

References

1. Barker, Robert H. Jr. , Laksami et al ; *Science,* 1986, 231(4744), 1434-6.
2. Liu, Keyi, Huang , Bingcheng etc. ; *Chinese Medical Journal* 1993, 106(1), 31-4 (Chemical Abstract 119, 175130.)
3. Markler, Michael T., Hinrich, David J.; *American Journal of Tropical Medicine and Hygiene,* 1993, 48(2), 205-10.
4. Malariology vol. 1 and 2 Nov. 1993.
5. Mucenki, cathleen M. David etc. ; *American Journal of Tropical Medicine and Hygiene* - 1986, 35(5), 912-20.
6. Relf. Wendy A., Taravanij, Savant etc.; *Transactions of Royal Society of Tropical Medicine and Hygiene,* 1990, 84(5), 630-4.

❑❑❑