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A COMPARATIVE STUDY OF DIFFERENT MALARIA DIAGNOSTIC ASSAY IN INDIA

Debadrita Modak

Research Scholar, Department of Zoology, CMJ University, Jorabat, Meghalaya.

Supervisor Name – Dr Kartik Maiti (Assistant Professor)

Abstract

When an infected female Anopheles mosquito bites you, you will acquire malaria, which is spread by the protozoan parasite that causes it. Malaria can only be effectively treated and prevented if it is diagnosed early and accurately. Misdiagnosis of malaria can lead to increased morbidity and death, hence a high-quality diagnosis is essential. Early diagnosis of malaria may be based on signs and symptoms with inadequate specificity, which may lead to overtreatment. Many health centers and hospitals have long regarded microscopy to be the primary method of diagnosing malaria, although the quality of this diagnosis is often poor. Rapid diagnostic testing (RDT) kits for malaria are capable of reaching distant places where microscopy cannot and providing results within 10-15 minutes. When it comes to diagnosing malaria, polymerase chain reaction (PCR) testing is the most accurate and sensitive approach since it can identify both low-density malaria as well as a variety of other forms of the disease.

1. INTRODUCTION

Of all the contagious disease caused by protozoa, malaria is the most destructive for man. Malaria a most serious vector - borne disease is one of the major causes of illness and death in tropical and subtropical regions of the world. In the past, malaria was thought to be caused by foul gases emanating from onrushes, hence the disease, was named 'malaria' (Italian, mala = bad; aria = air) meaning 'bad air'. In 1716, Lanciriestablisheda connection between abundance of mosquitoes and malaria. In 1880, French doctor, Charles Laveran, discovered the malarial parasite Plasmodium in human blood. In 1885-86, Golgi studies the erythrocyticschizogony of malaria parasite in man. In 1897, Ronald Ross discovered the Oocystof plasmodium on the

stomach of female *Anopheles* mosquito. In 1898, Ross worked out the life cycle of avian malaria parasite (*P. praecox*) in mosquito, while Grassi, Bignamiet.al., demonstrated the life cycle of human malarial parasite (*Plasmodium vivax*).In Addition to the direct effect it exerts by increasing premature mortality and morbidity. These constitute a serious increasing premature economic development of countries in which this disease is econ.

Symptoms and characteristics of malaria have been documented in historical writings from ancient times. Writings from Ebers Papyrus from 1570 B.C.. Chinese medical book Neiching(2700 B.C.);Neghina, (2010), Indus valley in northern India (Vedic period 1500-800 B.C.) and Brahmanic (800 B.C. - 100 B.C.) contain many references to repeated paroxysmal fever associated with enlarged spleen known as "King of disease". This suggests the occurrence of tertian malaria (*Plasmodium vivax*) and quartan malaria (*Plasmodium malariae*) infection in India. Much later time around 1000 B.C., *Plasmodium falciparum* had reached India. The prevalence of disease in early civilization was confirmed withmodern methods which detected malaria antigens in the skin and lungs of Egyptian mummies dating back to 3200 B.C. and 1340 B.C.

2. COMPARISON OF DIFFERENT MALARIA DIAGNOSTIC ASSAY IN INDIA

In order to study the malaria diagnosis in Indian population, total of 2333 blood samples were collected at different time periods from February 2012 to October 2015, by both active and passive surveillance. The samples were collected from 11 different geographical locations (consisting of nine states) that spanned vertically from Delhi, in the North to Tamil Nadu, in the South and horizontally from Gujarat, in the West to Assam, in the East and covering most of the malaria-endemic regions in India. The collected samples were diagnosed using three different methods to detect the malaria parasite, *P. falciparum*, *P. vivax* or mixed species, causing the infection and disease.

2.1 Diagnosis by Rapid Diagnostic Test

During the collection of field samples, an RDT kit, Falci-vax (Zephyr, Biomedicals, India), was utilized to diagnose malaria. If just the red band in the control is visible, the sample is free of

malaria parasites. The presence of a red band in the P. falciparum lane indicates that the sample is positive for P. falciparum and P. vivax detection, together with the presence of the control band. This indicates a mixed infection if the sample is positive for both P. falciparum and P. vivax, as seen by the presence of red bands in the respective lanes. Over 2333 samples were tested using the bivalent RDT kit. There were 793 confirmed instances of malaria, of which 355 (44 percent) were caused by Plasmodium falciparum, 320 (48 percent) were caused by Plasmodium vivax, and 58 (8 percent) were caused by a mixed infection.

2.2 Microscopic Diagnosis

EGB lab technician employees at the NIMR in New Delhi generated microscopic slides from samples collected in the field as well as the RDT test using both thick and thin smears. Slides from the Malaria Parasite Bank at the National Institute of Medical Research (NIMR) in New Delhi were then transferred and stained with JSB Stain by our technical personnel. Using a microscope, these slides were tested for the presence of malaria parasites. Among the 2333 blood smear samples analyzed by microscopy, 764 samples were positive for malaria parasites, including 275 (50 percent) instances of P. falciparum mono-infection, 322 (43 percent) cases of P. vivax mono-infection, and 42 (7 percent) cases of mixed malaria infection.

2.3 Molecular Diagnosis

The 18s rRNA gene was used as a target for nested PCR in the molecular diagnosis of malaria infection. Table 1 lists the ideal annealing temperatures for nested PCR. In the Nested PCR assay, three distinct primer pairs were employed, one for each Plasmodium genus in the first PCR stage and two different primer pairs for each P. falciparum and P. vivax parasite in the second PCR phase. P. falciparum and P. vivax samples exhibit obvious bands at 205 and 120 bp, respectively, but mixed malaria-infected samples display bands at both the sites. A total of 2333 samples were examined by nested PCR, and 827 instances of malaria parasite infection were determined to be positive. The P. falciparum mono-infection is represented by 423 samples, the P. vivax mono-infection by 372 samples, and the mixed infection by 105 (12.6 percent) samples (Table 1).

Table 1: Optimized annealing temperature of three primers to amplify 18S rRNA gene in Indian malaria samples.

Primers	Annealing Temperature (˚C)	Fragment Size (bp)
First Step		
Ps	60	1100
Second Step		
P. falciparum	55	205
P. vivax	55	120

2.4 Prevalence of malaria infections on the basis of PCR assay in Indianpopulations

Our research shows that the prevalence of malaria infections varies by region in India, with ODS2 (Rourkela) having the greatest proportion of P. falciparum and GUJ having the lowest (Nadiad). Also, DEL (Delhi) and TND (Chennai) had the greatest and MAH had the lowest percentages of P. vivax, respectively (Gadchiroli). There were 29% of instances in MAH, followed by 17% in MDP (Betul), 16% in KNT (Mangalore), 13% in ODS2, 6% in UTP (Shankargarh), 5% in ODS1 (Kendujhar), and 4% in GUJ, which had the lowest percentage of cases. DEL, TND, ASM1 (Diphu), and ASM2 areas have no incidences of mixed infection (Guwahati). Our investigation identified only two populations (DEL and TND) to be mono-infected with P. vivax out of 11; ASM1 and ASM2 had both P. falciparum and P. vivax mono-infection but no cases of mixed infection, we thus came to this conclusion. We observed that the remaining seven communities studied were infected with varied amounts of P. vivax, P. falciparum, and other malaria parasites (Table 2).

Table 2: Details of locations of 11 malaria sample collection sites from India with details results on three different diagnostic tests (microscopy, RDT and PCR assay).

Samplel ocation	TotalSa mple	Microscopy			RDT			PCR Assay		
		Pf	*Pv*	Mixed	*Pf*	*Pv*	Mixed	*Pf*	*Pv*	Mixed
DEL	16	0	10	0	0	14	0	0	11	0
ASM1	79	4	2	0	5	5	0	5	4	0
ASM2	155	9	15	0	10	20	0	9	16	0
UTP	344	24	83	2	17	96	4	28	91	9
	570	42	77	2	43	73	2	58	75	6
GUJ	28	3	19	0	4	24	1	2	21	1
MDP	64	5	30	1	8	23	0	2	26	7
	355	41	26	6	52	14	9	45	18	12
ODS1	100	3	15	0	4	16	0	3	17	1
ODS2	140	62	18	7	72	14	9	69	13	12
MAH	118	66	32	17	73	9	22	64	12	40
	34	29	0	0	28	0	0	27	0	1
TND	41	0	18	0	0	19	0	0	21	0
KNT	289	36	53	7	39	53	11	38	47	16
Totalsam ple	2333	764			793			827		

3. CONLCUSION

Malaria parasites are present in nearly all endemic countries as either mono or mixed infections, as evidenced by the epidemiological picture in terms of the incidence of various mono and mixed parasite infections across the world. Malaria parasites may be found on humans and mosquito vectors all around the world, whether in a temperate or tropical climate. Local malaria control programs must be assessed whether they can break the adaptive relationships between mosquitoes, parasites, and humans impacted by the local environment and meet their goal to eliminating malaria.

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