

Characterization of Human Malaria Parasites

Ultrastructural Studies on *Plasmodium vivax*

For the first time a detailed ultrastructural study was carried out on *P. vivax*. Fine structural analysis of growth and differentiation of successive stages involved in erythrocytic and sporogonic phases of development of this parasite was done and compared with those of other malaria parasites.

In the erythrocytic phase, asynchrony during merozoite formation within the schizont and caveola-vesicle complexes and cytoplasmic clefts observed in all infected erythrocytes (Fig. 6) were important features of *P. vivax* (Nanda, 1990). During sporogonic phase, oocysts on a single midgut exhibited differential rate of development. The invasion of sporozoites into the acinal cells of salivary gland resulted in depletion of rhoptries and changes in pellicular membranes (Nanda *et al.*, 1985). Morphologically two types of sporozoites were observed in salivary gland cells. Apart from providing better understanding of the parasite morphology this study may provide basis for other investigations like mechanism of drug action, host-parasite interactions, etc.

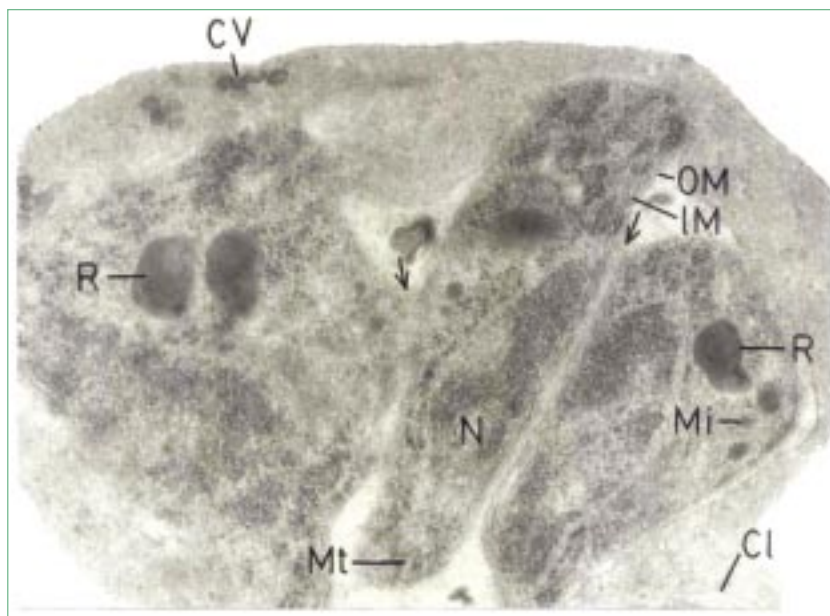


Fig. 6: Fully differentiated *P. vivax* merozoites, prior to host cell lysis, showing well developed pellicular membranes (OM, IM), microtubules (Mt), rhoptries (R), micronemes (Mi), nucleus (N) and surface strands to bridge the adjacent merozoites (arrows). Note the caveola-vesicle complexes (CV) and cytoplasmic clefts (Cl) in the host cell

Genetic Diversity Studies

To develop suitable and novel control strategies against the parasite, it is important to know the extent of genetic polymorphism existing in the parasite population. Data generated on the extent of genetic diversity existing in *P. vivax* and *P. falciparum* populations from different regions of the country will be important for the development and testing of new drugs and malaria vaccines. Therefore, with an objective to understand genetic structure and to estimate the type and the extent of genetic diversity existing among *P. falciparum* and *P. vivax* populations, studies on enzyme and DNA size

polymorphism have been carried out in relation to space and time. Fig. 7 shows the sites from where isolates have been characterized.

Polymorphism at glucose phosphate isomerase (GPI), glutamate dehydrogenase (GDH) and adenosine deaminase (ADA) enzyme loci (Joshi *et al.*, 1989, 1996, 1997; Biswas *et al.*, 1996) and merozoite surface proteins (MSP), circumsporozoite protein (CSP), glutamate rich protein (GLURP), GAM₁ (transmission blocking candidate antigen) genes were examined. The markers used to study polymorphism in human malaria parasites are given in Table 6.

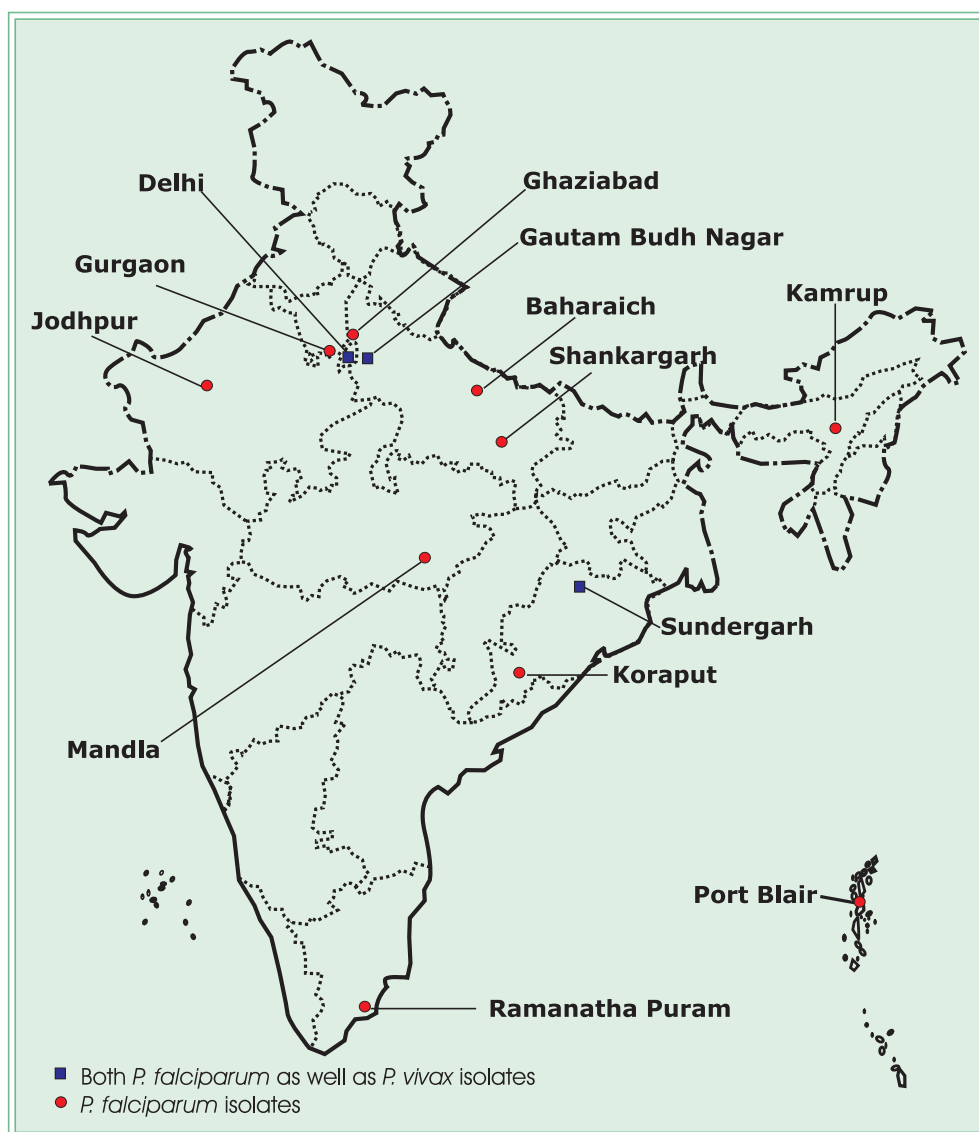


Fig. 7: Map showing study sites for genetic diversity in malaria parasites

Table 6. Markers and polymorphism in human plasmodia

Markers	<i>P. falciparum</i>	<i>P. vivax</i>
<i>Enzyme</i>		
GPI	6 alleles	6 alleles
GDH	5 alleles	7 alleles
ADA	3 alleles	5 alleles
LDH	1 allele	1 allele
<i>DNA</i>		
<i>size variations</i>	MSP-1: 9 alleles in block II region 22 alleles in 3 families – RO 33, MAD 20 & K1	GAM-1: 9 alleles
	MSP-2: 8 alleles in central region 15 alleles in 2 families-3D7 & FC27	MSP-3α : Alu I : 14 alleles Hha I : 11 alleles
	GLURP: 11 alleles	
	MSP-1₁₉ : EBA 175 rII, TRAP	CSP: 3 alleles MSP-3α : 16 genotypes
<i>Sequence variations</i>		

Studies carried out on the isoenzyme typing of *P. vivax* isolates collected from patients using GPI, GDH, ADA and LDH for the first time showed the random mating nature of *P. vivax* isolates in India (Joshi *et al.*, 1989). Further studies continued in relation to time and space for both *P. vivax* and *P. falciparum* species (Joshi *et al.*, 1996, 1997 & Shukla *et al.*, 1996) generated following important information: (i) high genotype diversity exists among isolates of India; (ii) similar population structure exists in different geographical areas; (iii) a high proportion of isolates are comprised at least of two genetically distinct clones of parasites—multiclonal, mean number of clones per isolate being 1.1 to 1.4; (iv) loci for GPI, GDH and ADA enzyme are not linked; and (v) no differences were observed in the proportions of single and multiclonal isolates during low and high transmission periods of *P. vivax*. Identical allelic forms observed among *P. vivax* isolates studied from Delhi in relation to time are shown in Fig. 8.

Studies on the gene polymorphism of CSP (circumsporozoite protein), GAM-1 (gene encoding

a *P. vivax* transmission blocking candidate antigen) and MSP-3 α (merozoite surface protein 3 alpha) in Indian *P. vivax* isolates have revealed two variants of CSP differing in nucleotide sequences and nine size variants in GAM-1 in isolates of Delhi. CSP variants were detected using PCR amplification using CSP specific primers and hybridizing with allele specific DNA probes using digoxigenin labelling systems. GAM-1 gene has been assayed using nested PCR amplification with specific primers. *Pv* MSP-3 α locus was investigated in Indian field isolates using a combined polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) protocol. Isolates collected from symptomatic patients attending malaria clinic of Malaria Research Centre, Delhi showed both size and sequence polymorphism. Size variations of PCR amplified product ranged between 1.2 and 1.8 kb. RFLP pattern with Alu I and Hha I has shown 14 and 11 alleles respectively. Sixteen different genotype combination of RFLP patterns were observed in a total of 19 isolates analyzed (Fig. 9) suggesting extensive parasite diversity within *P. vivax* population of this region.

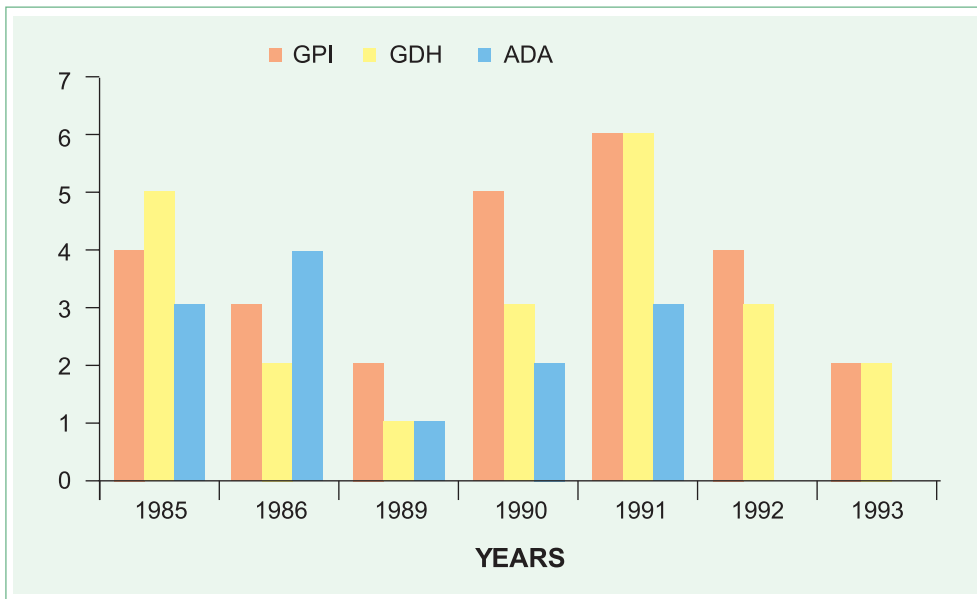


Fig. 8: Allelic polymorphism in *P. vivax* isolates

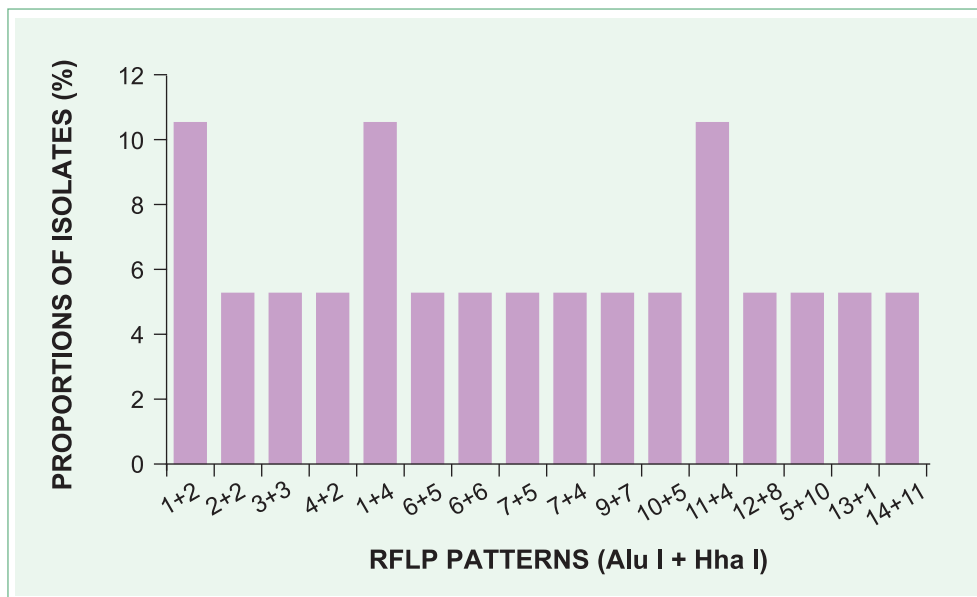


Fig. 9: Genotype combinations of MSP-3α observed among *P. vivax* isolates

In *P. falciparum*, studies carried out by us on the MSP-1 and MSP-2 polymorphism have shown 18 different genotypes (Fig. 10) among *P. falciparum* isolates studied from Haryana, Delhi, Orissa and U.P. Analysis of isoates from different parts of the country using family specific assay revealed presence of all the three families of MSP-1, namely K1, MAD20 and R033 and both of MSP-2—3, D7 and FC27 with prevalence of K1 and MAD20 families in mainland while that of R033 in the samples analyzed from Andaman and Nicobar Islands.

Random combination of alleles at different loci of *P.*

falciparum and *P. vivax* populations in India suggest that these isolates belong to a random mating population. Presence of multiple infections in an isolate with different genetic make-up may have serious implications in malaria control programme as different genotypes may have different response to various drugs/vaccines and may exhibit differential clinical phenotype.

Markers developed were assessed for parasite surveillance studies. Analysis of *P. falciparum* positive blood spots collected on Day 0 and day of recrudescence indicated that MSP-1, MSP-2 and

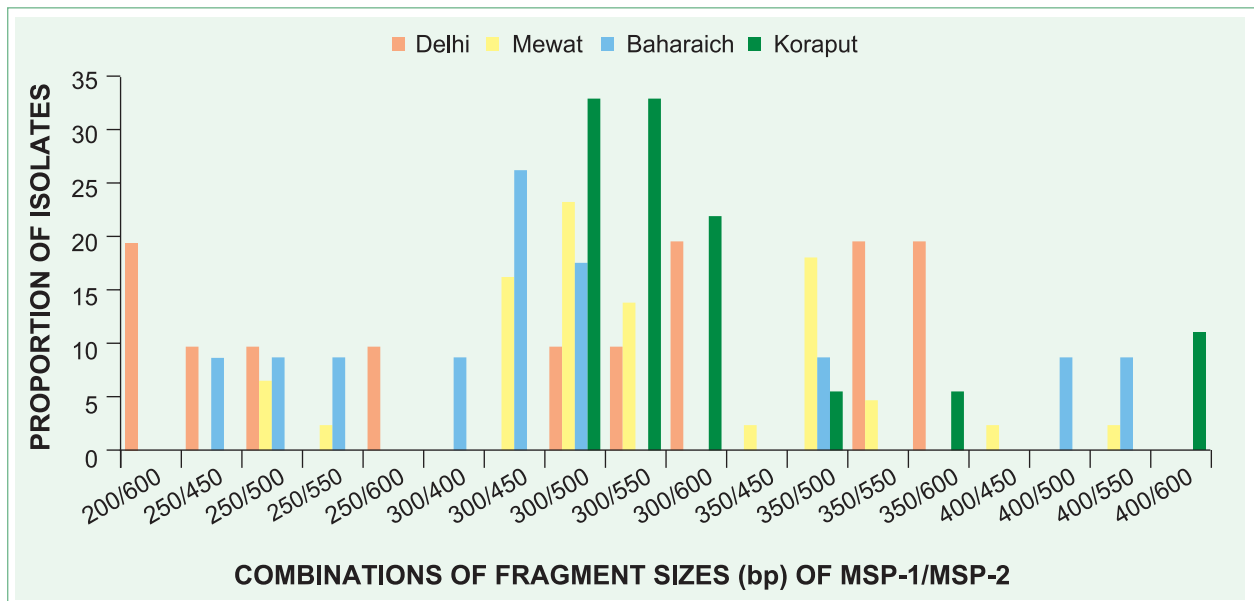


Fig. 10: Genotypes observed in *P. falciparum* isolates

GLURP size markers can be used for differentiating recrudescence from fresh infection on the basis of genotypes of parasites.

Studies on Genetic Variations in T Helper Cell Epitopic Regions (Th2R and Th3R) of Circumsporozoite Protein (CSP) of *P. falciparum* Isolates from India

The role of T cells in malaria immunity has been appreciated for a long time. CSP has two T helper cell epitopes flanking the highly conserved region RII and spanning amino acid residue 326 to 343 (Th2R) and 361 to 380 (Th3R). However, these regions show polymorphism. Studies were performed to find out whether the genetic variations are regionally unbiased, polymorphism is restricted and can be categorized into groups because the T cell domains could be included in a polyvalent sporozoite vaccine and such a strategy might largely depend on the extent of polymorphism in these epitopes. So far we have studied 51 *P. falciparum* isolates and found that the majority of the Indian isolates are regionally unbiased and could be categorized into four groups and sequences of the two groups showed similarity with the sequences of *P. falciparum* isolates from other geographical regions of the world, although some of the isolates showed wide sequence variation and could not be categorized into any group. Therefore, the

prototype variants from each group could be included in a subunit polyvalent vaccine against sporozoites.

Molecular Analysis of Invasion of Indian *P. falciparum* Field Isolates and Cytoadherent Properties of Infected Erythrocytes

The invasion of erythrocytes by *Plasmodium* merozoites is mediated by specific molecular interactions between host receptors and parasite ligands. Most laboratory strains of *P. falciparum* use sialic acid residues on glycophorin A as receptors for erythrocyte invasion. A 175 kD *P. falciparum* protein known as EBA-175 (for erythrocyte binding antigen-175kD), mediates binding to sialic acid residues of glycophorin A during invasion. Some *P. falciparum* laboratory strains are known to possess alternate invasion pathways and can invade neuraminidase-treated RBCs. It is not known how commonly such alternate pathways are used by *P. falciparum* field isolates. We have studied the invasion phenotypes of *P. falciparum* field isolates collected from different regions of India (Okoyeh *et al.*, 1999). Out of 15 *P. falciparum* isolates tested, 5 showed invasion and multiplication in both neuraminidase and trypsin treated erythrocytes, 3 in neuraminidase treated but not in trypsin treated erythrocytes and 4 in trypsin treated but not in neuraminidase treated erythrocytes. These studies

indicate that *P. falciparum* field isolates commonly use alternate invasion pathways that do not depend on sialic acid residues of glyophorin A.

Cytoadherence refers to the ability of erythrocytes infected with blood stage parasites, trophozoites and schizonts, to adhere to the vascular endothelium in the human host and bind to uninfected erythrocytes to form rosettes. Cytoadherence of *P. falciparum*-infected erythrocytes in brain capillaries have been implicated in cerebral malaria and sequestration in the placenta results in complications in pregnancy. The endothelial receptors used for cytoadherence include ICAM-I, CD36, VCAM, E-selectin, CD31 and chondroitin sulfate A (CSA). Cytoadherent

phenotypes of Indian *P. falciparum* field isolates collected from different regions of India have been studied. In a preliminary study, out of 13 isolates screened, 9 showed binding property and 4 did not. JDP-2 (*P. falciparum* isolates collected from tribal areas of Jagdalpur (Chhattisgarh) showed high binding with CD36 and JDP-8 showed high binding with ICAM-I and also forms rosettes. Another *P. falciparum* isolate RAJ-86 collected during Rajasthan epidemic in 1994, showed binding with CD36 and CSA (Chitnis *et al.*, 1998). Information is important for the development of novel strategies that block cytoadherence to receptors such as ICAM-I and prevent or reverse pathological outcomes such as cerebral malaria.

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