patients suffering from other diseases. High anti-GPL antibody was found in case of *P. falciparum* and *P. vivax* infection but not with normal or nonmalarial sera. Blood sera of people from different malaria endemic zones with different history of malaria exposure in recent past were examined by ELISA against both the GPL and RESA Pf/155 (AR1) antigen. The GPL antigen was found to be a glycophospholipid having galactose, mannose, xylose and glucose moiety in the glycogen part and also contain phosphate group and lipid (Tables 3–4).

Table 3. Comparison of ELISA O.D. value between glycophospholipid isolated from parasitized and nonparasitized *Pf* culture supernatant

Ag	Pooled serum	Dilutions		
		100	200	400
GPL_1(Parasitized)	*Pv*	0.57	0.50	0.36
	Pf	0.66	0.54	0.23
	Neg	0.34	0.28	0.19
GPL_2(Nonparasitized)	*Pv*	0.48	0.36	0.28
	Pf	0.47	0.35	0.32
	Neg	0.42	0.35	0.32

Circulating Histidine Rich Protein II Antigen and Specific Antibody Responses in *P. falciparum* Patients during Acute Infection and after Treatment

P. falciparum synthesizes three histidine rich proteins, HRP-1, HRP-2 and HRP-3. HRP-1 was identified as parasite knob associated antigen, whereas HRP-2 was identified as surface exposed protein complex available in all natural isolates irrespective of knob phenotype. HRP-3 is available on parasite surface at the lowest abundance compared to HRP-1 and HRP-2. *P. falciparum* HRP-2 plays a crucial role in parasite development and growth. *Pf*-HRP-2 circulates in blood even after 14 days post infection. Its C-terminal half induces a partially protective response. It is proposed that HRP-2 may facilitate transport of haemoglobin to the food vacuole and catalyze the reaction. HRP-2 is a structurally well-characterized molecule present in all natural isolates of *P. falciparum*. It shows potential effects on the host immune system and has proven its worth as an antigen for specific diagnosis of malaria.

The study was conducted in a group of patients suffering from uncomplicated falciparum malaria to monitor how long circulating HRP-2 antigen could be detected in blood by sandwich ELISA and to determine the profile of antigen-specific antibodies following antimalarial treatment. Finger prick blood samples from a group of 40 malaria

Table 4. Antibody against glycolipid antigen and its response under different malariogenic conditions

Area	ELISA O.D.				Known status
	No.	R1	*Pf*	Glycolipid	
Raigarh	44	0.44 ± 0.18	0.42 ± 0.20	0.59 ± 0.29	Appearing
Haldwani	42	0.30 ± 0.09	0.34 ± 0.12	0.27 ± 0.09	Disappearing
Rajasthan	46	0.35 ± 0.15	0.47 ± 0.20	0.47 ± 0.17	Epidemic
Control	10	0.21 ± 0.14	0.22 ± 0.13	0.06 ± 0.05	Nonendemic

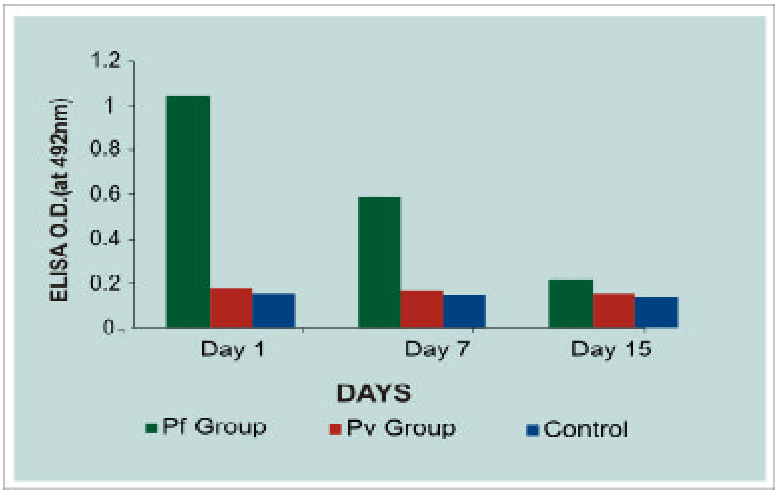

Fig. 4: HRP-2 antigen profile in three study groups

patients (36 with *P. falciparum* and 4 with *P. vivax* infection) were tested for histidine rich protein–2 (HRP-2) antigen by antigen capture assay and for parasite-specific antibodies, both IgM and IgG isotypes by indirect ELISA against HRP-2 peptide and *P. falciparum* infected erythrocyte lysate. These patients were treated with recommended doses of chloroquine and they were followed-up for antigenaemia and antibody level.

HRP-2 antigen detected in whole blood was quite high on Day 1, thereafter a significant decrease in the HRP-2 antigen level was observed on Day 7 and Day 15 in all patients. Similar trend in both anti-HRP-2 and anti-*Pf* IgM was observed. Anti-HRP-2 IgG levels increased moderately in 31 out of 36 on Day 7 and Day 15 as compared to Day 1. Immunoglobulin-G detected in patients against *Pf* lysate showed an increasing trend as compared to Day 1. Significant positive correlation was observed between HRP-2 antigenaemia and IgM in sera of individuals from *P. falciparum* group, whereas negative correlation was achieved between HRP-2 antigen and IgG levels. Blood samples of four *P. vivax* patients had no detectable amount of HRP-2 antigen and anti-HRP-2 antibody, but measurable amounts of IgM and IgG were detected against *Pf* crude antigen due to serological cross-reactivity. Five healthy normal individuals exhibited negativity in three sets of assays (Figs. 4 and 5).

This study provides information that during natural course of infection a high level of circulating free HRP-2 antigen could be detected and at least a measurable or detectable amount could be found even up to seven days after antimalarial treatment. Persistence of antigen was found to be associated with development of antigen-specific antibodies and its antibody mediated neutralization after successful response to antimalarial treatment.

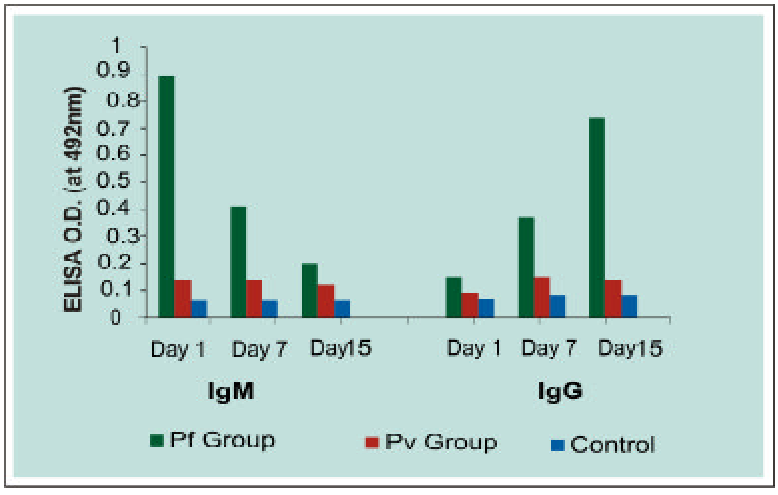

Fig. 5: Anti HRP-2 IgM and IgG profile in three study groups

Studies on Monoclonal Antibodies against *Plasmodium vivax* Erythrocytic Stages

In continuation of the earlier work, monoclonal antibodies derived from two hybridomas MAb1B3C6 and MAb4E6 were tested for their reactivity in *P. vivax* infected erythrocytes by immunofluorescence and

in *P. vivax* crude lysates transferred c nitrocellulose membrane after electr phoresis. Both the antibodies reacte with early and late trophozoites and wi schizont by IFA test (Fig. 6). By imm noblotting, MAb1B3C6 antibody reac ed with nearly 42kDa protein ar MAb4E6 antibody reacted with 30kI proteins of a pooled preparation *P.vivax* parasites collected from diffe ent geographic areas of the country. These two proteins were named as P1 and P2.

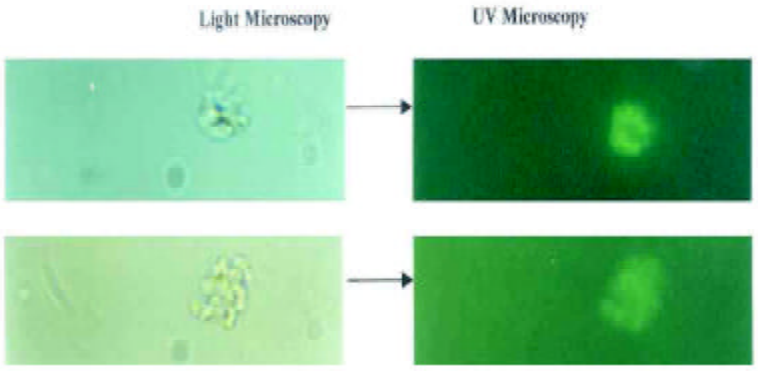

Fig. 6: Indirect immunofluoroscence of acetone fixed *P. vivax* schizont reacted with MAb1B3C6 and MAb4E6

P. vivax protein from crude parasitized erythrocyte lysate was purified by affinity chromatography using two sets of columns of 6MB-Sepharose coupled with immunoglobulin fraction of MAb1B3C6 and MAb4E6. Purified proteins (P1 and P2) were transferred on PVDF membrane and were put in the sequencer. Sequencing of the proteins, P1 and P2 showed significant alignment with erythrocyte membrane associated antigen of one *P. falciparum* clone: p*Pf* and the sequence of P2 showed significant alignment with serine protease inhibitor of *Rattus norvegicus*. Work is continued on the isolation of high affinity parasite antibodies from existing panel of clones to develop diagnostic reagents.

PCR-based Identification of Malaria Parasites

Simple PCR assay using small subunit ribosomal DNA primers was employed. In a total of 79 *P. falciparum* positive bloodspots tested, 98.7% gave amplification in PCR assay, however, in 11 microscopically *P. falciparum* negative bloodspots, none of the samples showed amplification in PCR assay. A multiplex polymerase chain reaction to differentiate *P. falciparum* and *P. vivax* in a single assay was standardized using a mixture of specific primers for each of the two species. The two species could be identified on the basis of size corresponding to 183 and 206 bp respectively for *P. falciparum* and *P. vivax* (Fig. 7).

Studies on Morphological changes in Cerebellar Purkinje cells

A study has been initiated to see the morphological changes taking place in cerebellar purkinje cells and in the surrounding parenchymal cells during cerebral malaria. The study aims to see the effect of plant extracts with antimalarial properties in cerebral malaria in the experimental animal model using Swiss albino mice and *P. berghei ANKA* strain.

Primary Screening of Herbal Products for their Antimalarial Activity

There is an urgent need for new antimalarials particularly to those parasites, which are showing resistance to the existing antimalarials. The medicinal plants or parts of the plants used for the treatment of fever in rural/tribal areas collected from different geographical regions of India were tested for their antimalarial properties. In addition to 13 medicinal plants tested *in vitro* for their antimalarial activity, two new plants collected from Kerala and Uttaranchal have been tested *in vitro* for their antiplasmodial activity. The *in vitro* test was done using both chloroquine sensitive and resistant isolates of *P. falciparum* using schizont maturation inhibition assay. The IC_{50} values were 1.5 ? g/ml and 6 ? g/ml respectively.

In vivo schizontocidal activity was tested for one of the above extracts which was showing good effect. Based on the results of the schizontocidal activity of the earlier studies a compound was prepared by mixing two 50% ethanol extracts and was given to *P. berghei* infected Swiss albino mice. Three groups of five animals each were taken. One group received no drug, which served as control. The other two received 100 mg and 50 mg/kg body weight each respectively. From the control group all the fiveanimals died by Day 9 of experiment where as from the first experimental group out of five animals three survived and in the second group only one animal survived the infection and the average survival rate of other four animals survived was 13 days.

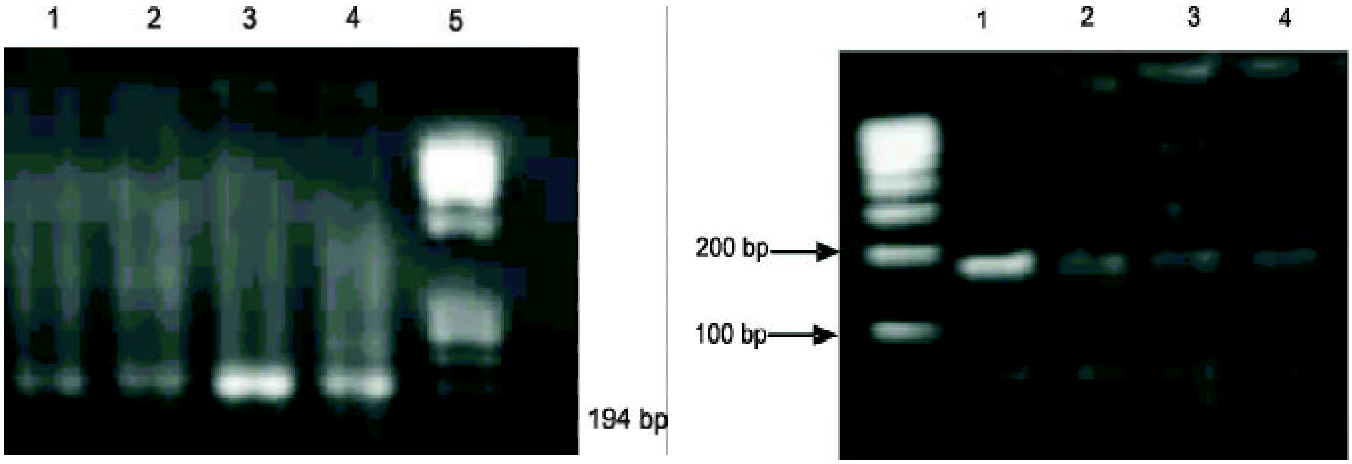

Fig. 7: Gel electrophoretograms showing differentiation of *P. falciparum* and *P. vivax*