2

Parasite Biology

2.1 Studies on genetic structure of human malaria parasites

2.1.1 *Plasmodium falciparum*

Plasmodium falciparum, the deadly parasite species is showing an increasing trend in India. Therefore, a study has been taken up to understand the population structure of the field isolates. Isolates from different geographical regions were analysed for surface protein markers—MSP-1 and MSP-2 for length as well as sequence variations. Analyses revealed highly polymorphic nature of Indian *P. falciparum* isolates on the basis of length as well

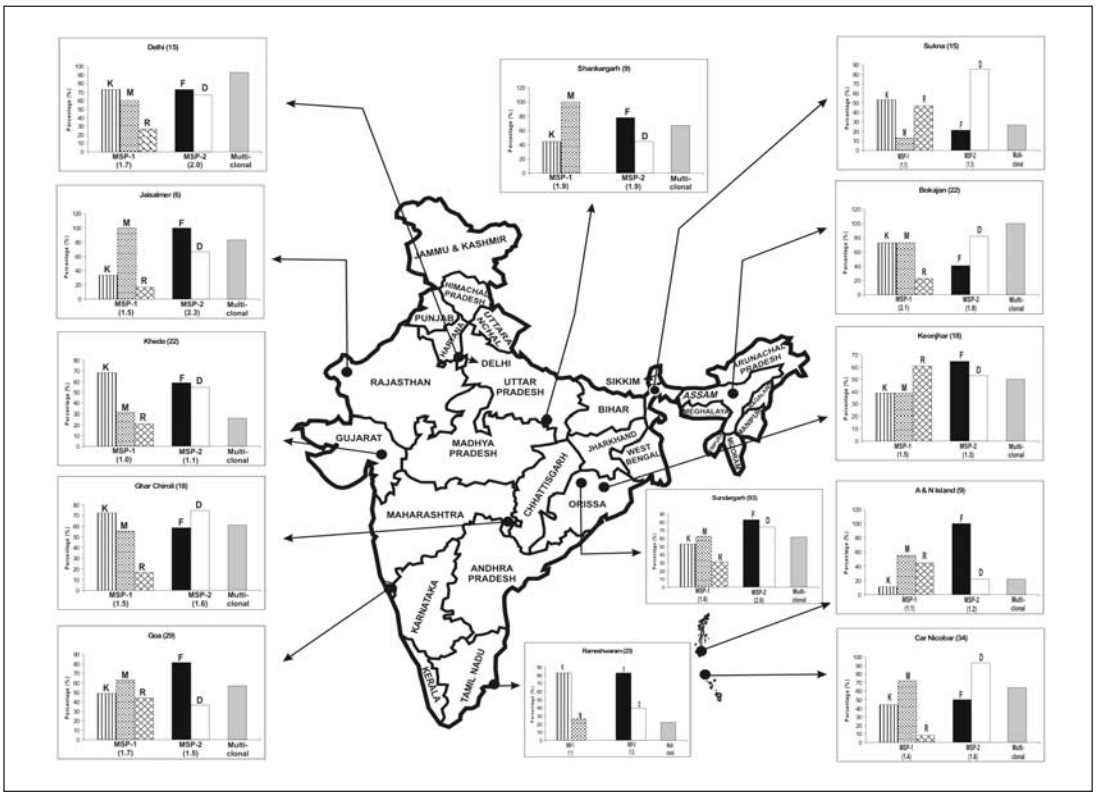

Fig. 2.1: *P. falciparum*: distribution of MSP-1 & 2 families among field isolates in India

as sequence data at these two loci. Three families of MSP-1 (K1, MAD20 & RO33) are prevalent in majority of the study sites. K1 and MAD20 families have shown allelic polymorphism while RO33 family was observed to be monomorphic with a single allele of 160 bp length in all the study areas.

In MSP-2, two families FC27 and 3D7 were observed in all the study sites and both the families have shown length variations. Fig. 2.1 shows the proportional prevalence of various families of MSP-1 and MSP-2. Sequence analysis of various alleles of MSP-1 and MSP-2 families revealed that Indian *P. falciparum* isolates present a mixed allelic composition representing different global regions. Sequences obtained have been submitted to Genbank.

Drug resistant markers

Field isolates from Rameswaram (Tamil Nadu), Panna (Madhya Pradesh), Sonapur (Assam), Sundargarh (Orissa) and Udaipur (Rajasthan) have been analysed for mutations in *pfcrt* (*P. falciparum* chloroquine resistance transporter) genes which are known to be associated with chloroquine resistance. Analysis revealed a high frequency of CQ resistant associated mutation (K76T) in *pfcrt* gene among the Indian field isolates. Fig. 2.2 shows the observed proportion of isolates showing K76T mutation in *pfcrt* gene among Indian *P. falciparum* isolates.

2.1.2 *Plasmodium vivax*

P. vivax, the most virulent species of malaria parasite in India that contributes about 55% of the total malaria positive cases in the country is responsible for huge burden of morbidity. Therefore, with an aim to understand extent of genetic diversity existing among the species, field isolates collected from different regions of the country with different eco-epidemiological conditions were analysed for polymorphism in genomic sequence by PCR, RFLP and sequencing. Following marker genes were used for the study.

GAM-1 (Gene coding for transmission blocking antigen)

Field isolates analysed from different geographical regions of the country such as Delhi, Tamil Nadu, Goa, Car Nicobar, Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar, Maharashtra and Orissa revealed dimorphic nature of GAM-1 among Indian isolates. Both Belem and Chession type alleles were present with prevalence of Belem type allele (above 70%) in all the sites studied except in Orissa. In Orissa isolates Chession allele was predominant (about 80%) which is similar to Thailand isolates, while isolates of other areas showed resemblance to Korean isolates. On the other hand, Indian isolates showed a totally different picture from Sri Lankan

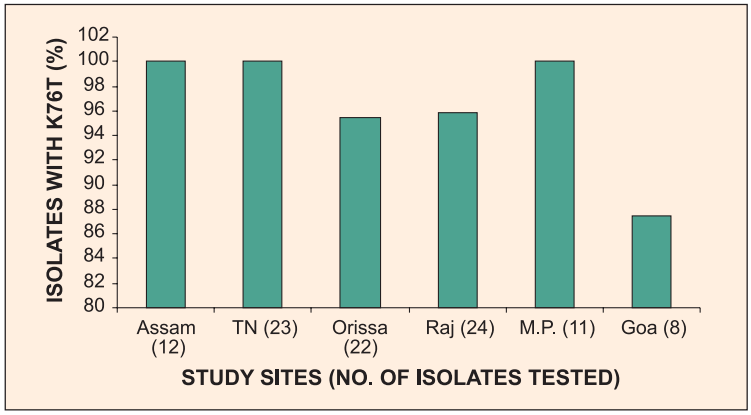

Fig. 2.2: Proportional prevalence of *pfcrt* K76T mutation among Indian *P. falciparum* field isolates

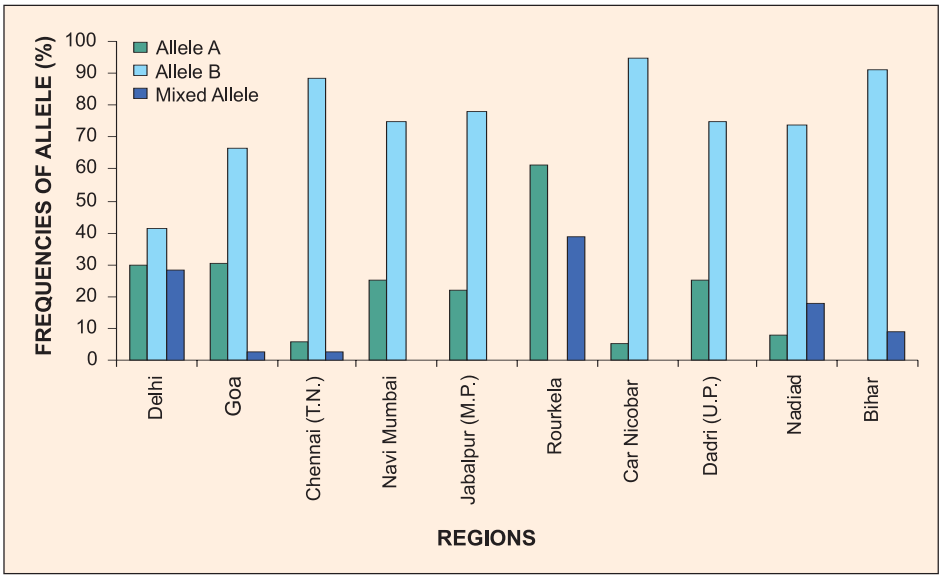

Fig. 2.3: Distribution of *P. vivax* GAM-1 alleles in different parts of India. Allele A–Chession type and Allele B–Belem type. Mix allele – presence of both Belem and Chession type together in the isolates

isolates which were high polymorphic. Fig. 2.3 shows the proportional prevalence of two alleles (exclusively or on combination) among the Indian isolates.

MSP-3α

PCR revealed three different fragment sizes (length variants) with predominance of 1.8 kb fragment (above 75%) in isolates studied from different regions such as Delhi, Tamil Nadu, Goa, Car Nicobar, Gujarat, Madhya Pradesh, Maharashtra and Orissa. RFLP with Alu I and Hha I revealed a total of 19 different alleles among Indian isolates compared to 13 isolates reported from Papua New Guinea (PNG) and Thailand. Sequence analysis of the PCR amplified fragments (all 3 variants 1.8, 1.4 & 1.2 kb) revealed that 1.8 kb variant resembles closely to Korean and Thailand data while 1.4 kb variant resembles more to Venezuela data and 1.2 kb variant is like Chession strain. Study further revealed block-I region of MSP-3α was deleted in 1.2 and 1.4 kb fragments, in addition 1.2 kb variant α-helix was also deleted thus creating a high degree of diversity among the isolates. Fig. 2.4 gives the distribution of three size variants of MSP-3α among Indian isolates.

Pv SSUrRNA – Two sub-types

It is believed that *P. vivax* from the new world should be considered a separate subspecies to that present in the old world. In order to confirm/reject the hypothesis, we analysed the 18S SSUrRNA S-type gene. Isolates from different regions of India were analysed and the results revealed the presence of both old and new world type S-gene of SSUrRNA among the isolates with an even split of 60% old world and 40% new world type. Similarly, isolates from PNG and Brazil also showed presence of both the types. In PNG isolates predominance of old world type (90%) was observed while among Brazil isolates predominance of new world type (70%) was observed. Above observation suggested the presence of both the S-type allele frequencies worldwide clearly demonstrating the global distribution of the two alleles and no geographical subdivision. Fig. 2.5 shows the prevalence of old and new world S-type allele of SSUrRNA among isolates of India and other global regions.

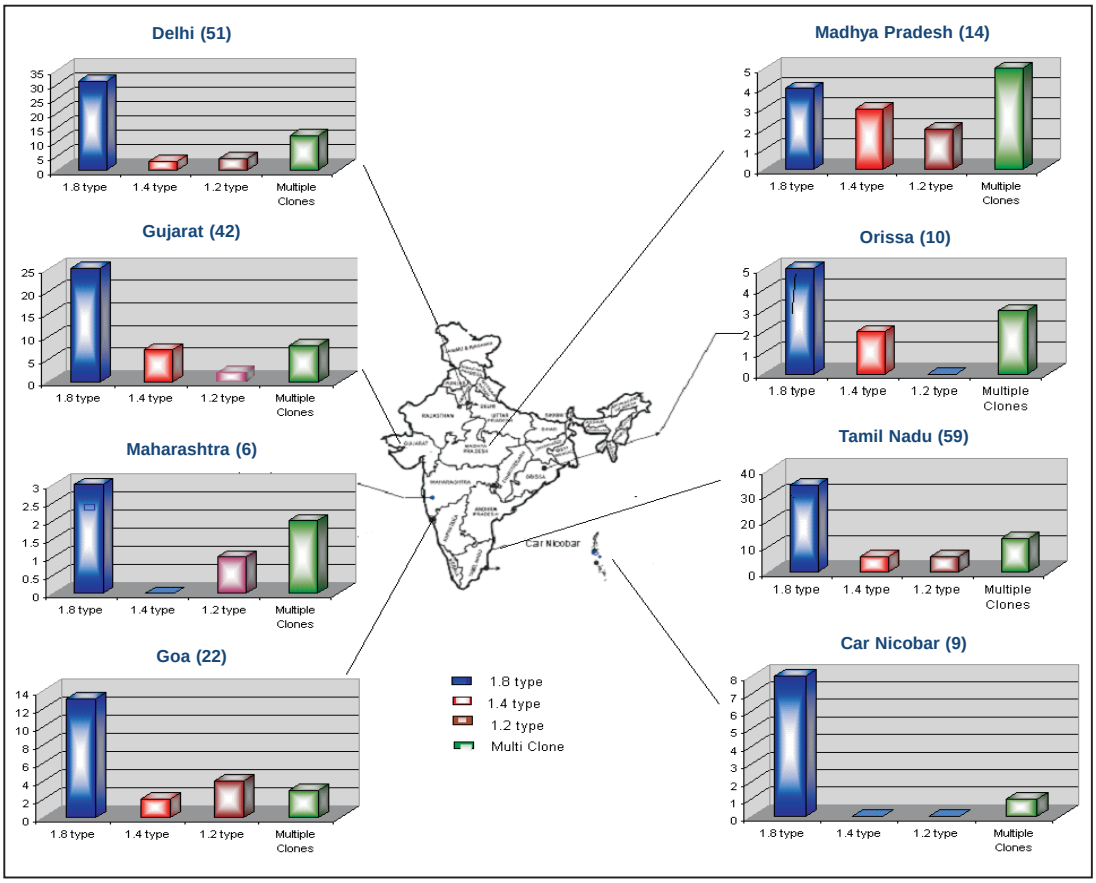

Fig. 2.4: Allelic distribution of MSP-3α in India

2.2 Development of nuclear DNA markers for evolutionary studies in *P. falciparum*

Estimation of genetic diversity in species populations and inferring evolutionary dynamics of different genes are important in biomedical research, especially in finding new drug target genes and developing new effective drugs and vaccines. Recent researches in evolutionary genetics have revealed that estimation of genetic diversity is strongly dependant on the genetic markers used, thus making appropriate evolutionary inference both at species and gene level, difficult. Considering these facts, we have developed nuclear DNA markers in human malaria parasite—*P. falciparum* using the published whole genome sequence information. Specifically we follow the approach described recently in *Drosophila* to analyse genetic fragments that are located in introns (non-coding sequences) to infer the demographic history of populations. Such fragments are amplified by PCR technique by designing primers in the flaking exons (coding DNA sequences). Following this approach that is commonly known as EPIC (exon priming intron crossing) fragments, we developed a total of 170 putatively neutral fragments in the whole genome of *P. falciparum* (Fig. 2.6). Likewise, in order to detect endemic mutations in different alleles in the *pfcrt* gene and measure genetic diversity

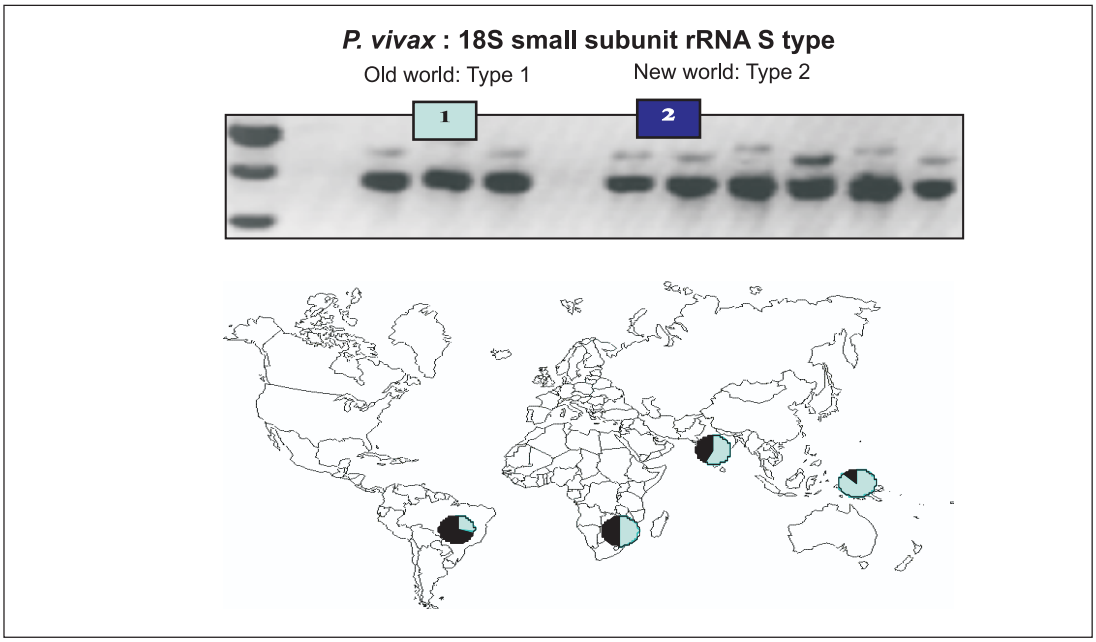

Fig. 2.5: Global distribution of old and new world S-type allele of 18S SSU rRNA

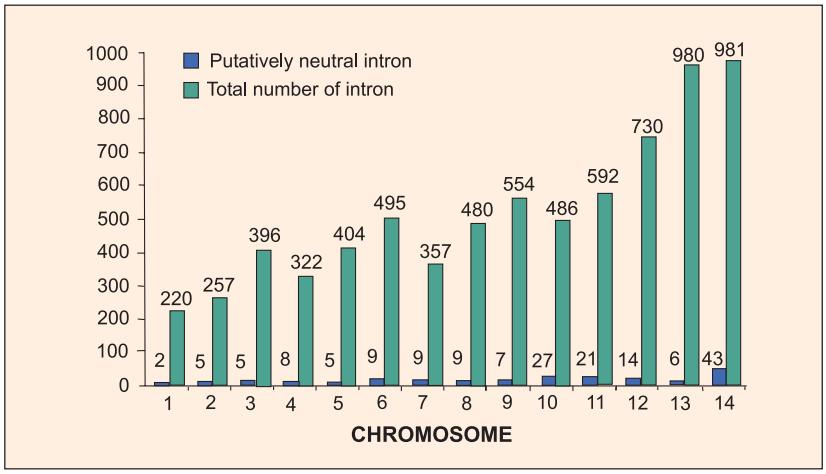

Fig. 2.6: Putative neutral introns in *P. falciparum*

in both the resistant and sensitive varieties in isolates collected in different geographical populations in India, we intend to sequence the whole *pfcrt* gene, instead of looking at the specific local mutations. We designed three primary primer pairs for amplifying the whole *pfcrt* gene and five nested primer pairs for sequencing the whole gene in short stretches (Fig. 2.7). Considering the drug pressure in the field

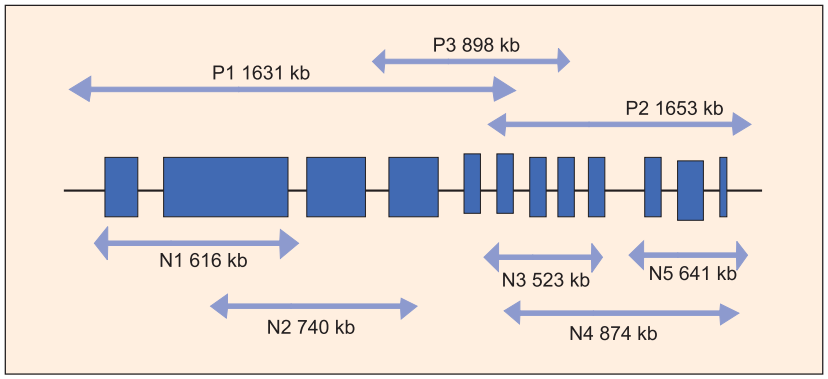

Fig. 2.7: Primary primers for amplifying *pfcrt* gene and whole gene in *P. falciparum*

and reported sweeping of the resistant allele of the *pfcrt* gene by natural selection, we are interested to know the detailed evolutionary history of this gene in Indian *P. falciparum*. The development of nuclear DNA marker would thus help in understanding the precise roles of demography and natural selection in evolution of *P. falciparum* in India.

2.3 Evolution of introns in the genome: inference from the whole genome scanning of the human malaria parasite *P. falciparum*

The *P. falciparum* genome is unique in several aspects. The long proteins compared to homologous in other organisms, high AT rich genome (80.6%), etc. are some of the important observations from different studies. In this context, a genome-wide survey and study of *P. falciparum* for understanding of genome organisation might point towards the unique characteristics of the species. The comprehensive data sets available in public databases such as PlasmoDB and NCBI combined with the availability of substantial sequence tracts from *P. falciparum* has made it possible to embrace this genome wide study (Fig. 2.8). It has become clear that introns have been gained and lost in different lineages at various rates. In this context it is of particular interest to estimate

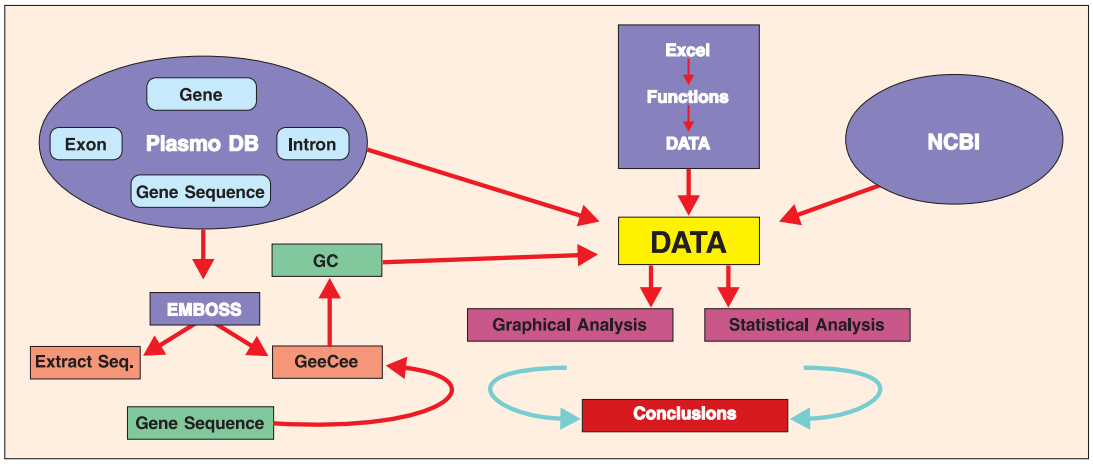

Fig. 2.8: Schematic representation of data collection and processing

the intron densities in lower eukaryotes, such as *Plasmodium*.

We followed a defined method (Fig. 2.8) for data retrieval from the web data source (PlasmoDB and EMBL) and collected according to our analytical need. We found most genes were small but the hypothetical genes were comparatively larger than the genomic average. However, functional genes were small in general and clustered in the sub-telomeric regions of the chromosome. Introns in the functional genes were found to be smaller than the average genomic length and small genes were considerably rich in GC content. The number of genes on each chromosome was marginally correlated to chromosome size. A statistically significant positive correlation (Pearson's $r = 0.92$, $p < 0.001$) was detected between the number of introns and chromosome size. A statistically significant positive correlation ($r = 0.93$, $p < 0.001$) between the number of introns and number of genes along each chromosome was also observed. Generally, the number of introns was found to increase in the increasing order of the chromosome size. However, this pattern was found to be distorted for the two largest chromosomes, chromosome 13 and 14 (Fig. 2.9). As evident in the figure, very large sized introns were found in chromosome 14, the largest of all chromosomes.

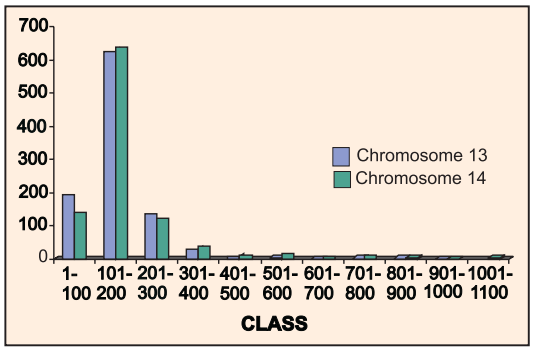

Fig. 2.9: Intron frequency table based on size in chromosomes 13 and 14

The other observed features in the present study, like conservation of GT-AG splice site, presence of polypyramidine tail at 3′ end, etc. could throw light to the theories suggesting the invasion of introns in eukaryotic genome after the separation from other lineages as suggested by intron late theory. The highly AT rich introns detected in this study seems to contribute much to the overall AT based nature of *P. falciparum* genome. Most genes are intron-less and fall in the size range of 100-200 bp in length.

2.4 Identifying single clonal infections from the archived sample

Blood spots collected on Whatman 3 mm filter paper strips from microscopically positive *P. falciparum* subjects and those available in NIMR archive were analysed for the presence of diverse MSP-1&2 and GLURP alleles for identification of isolates having single clone of *P. falciparum*. Ninety single clone isolates have been identified from Orissa, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Rajasthan and Gujarat.

2.5 Development of website on the genetic and biological diversity of Indian *Plasmodium*

Workers in the field have already documented enormous genetic and biological diversity in malaria parasites in India and more studies are pouring in reporting wide diversities in both the characteristics of the parasites. However, there is a strict lack of comprehensive information on the biological and genetic diversity of Indian species of *Plasmodium*. In view of this, a webpage containing comprehensive information on the biological and genetic diversity of Indian Plasmodia has been developed. This webpage would also contain information on the workers actively involved in this field. The website can be accessed from www.plasmodiversity.org.in. The beta version of the website is already been released and the screenshot of the website is depicted in Fig. 2.10.

2.6 Expression of T-helper cell epitopic regions of circumsporozoite protein (CSP) of *P. falciparum* in *Escherichia coli*

Importance of T-cells in malaria immunity has been appreciated for long. Two T-helper cell epitopes have been located at the C-terminal end flanking, the

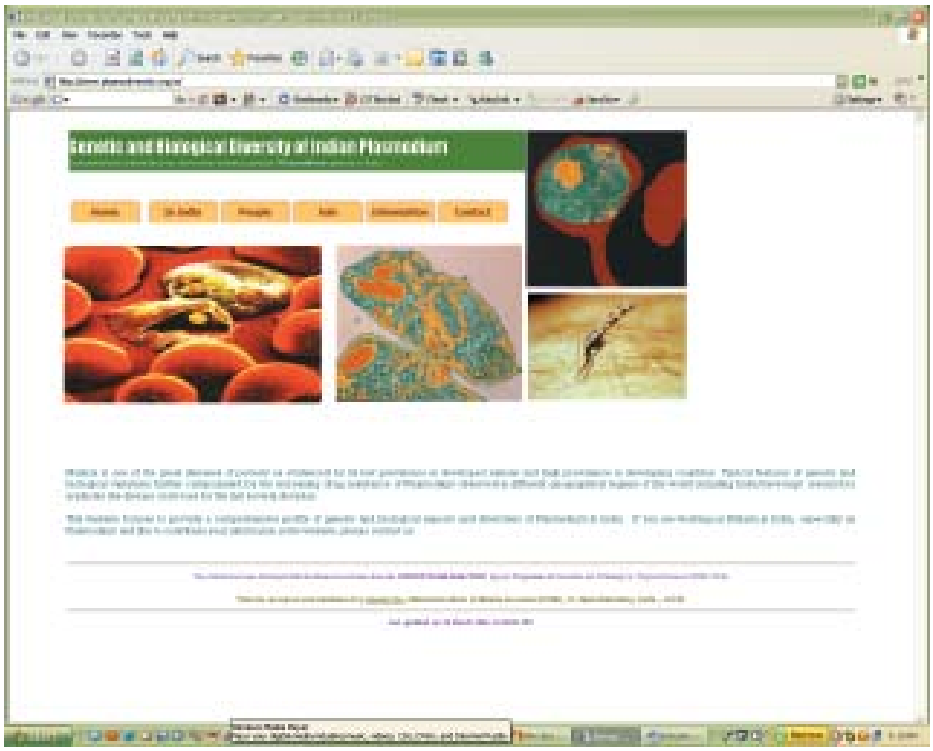

Fig. 2.10: Website on genetic and biological diversity of Indian *Plasmodium*

highly conserved region RII of CSP. Th-epitopes show variation. Even then if the variations are restricted and can be grouped, then the prototype variants from the group could be included into subunit polyvalent vaccine against sporozoites. The genetic variation in Th-epitopes of 148 *P. falciparum* isolates collected from different epidemic and endemic regions of India have been studied. Variations have been found to be regionally unbiased in the sense that similar type of variation has been found in different regions. The allelic variants could be categorised into 4 groups. Since the number of variants is small, then the prototype variants could be included into a subunit polyvalent vaccine against sporozoites.

The prototype variants have been cloned into Pqe-40 expression vector and expressed it as a fusion protein fused to DHFR (Dihydrofolate reductase) gene tagged to 6 His residue. Fusion protein has been purified and the study on the immunogenicity of fusion protein is in progress.

2.7 Relatively simple genotype of *P. falciparum* isolates from India as determined by anchored primer amplification of DNA

Genotyping of *P. falciparum* is important for therapeutic efficacy and other purposes. Various methods have been developed for genotyping of malaria parasite especially for *P. falciparum*, but they have their limitations. The poly A and poly T using oligonucleotides anchored at the 32 end of the primer, the genetic differences in the AT rich DNA sequences can be displayed. Genomic DNA of *P. falciparum* isolates was prepared from the blood samples collected from both endemic and epidemic areas of India. This genomic DNA was PCR ampli-

fied using single anchored poly A or poly T primers. With dinucleotide anchored primer in most of the cases, smear was obtained, even at increased concentrations of $MgCl_2$ whereas with tri, tetra and penta nucleotide anchored primers, though different patterns of bands were observed, they were less complex compared to the bands seen in the study. Thus, it can be concluded that the genotype of Indian *P. falciparum* is more simple than that of the isolates from other geographical regions of the world.

2.8 Association of chloroquine resistant molecular markers in *P. falciparum* isolates from India

Chloroquine (CQ) is the most widely used drug for the treatment of malaria, but development of CQ-resistant parasites has made both treatment and control of malaria complicated. Mutational changes in three genes, *pfmdr1*, *pfcrt* and *cg2* have been attributed to chloroquine resistance. However, alleles of a single gene could not be accounted for CQ-resistance. An association of alleles of these genes is believed to be responsible for CQ-resistance. Twenty CQ-resistant and 20 CQ-sensitive *P. falciparum* isolates from different geographical regions of India have been studied to determine the association of CQ-resistant molecular markers. Genomic DNA was prepared from 20 CQ-resistant and 20 CQ-sensitive isolates. The portion of the gene, harbouring mutational site was PCR amplified using primers flanking the mutational sites. PCR amplified products were purified and sequenced. It has been found that some of the resistant strains contain the *pfmdr1* mutation N86Y, while others did not. Same is the situation with *pfcrt* K76T. Thus, it appears that mutation in any single gene is not responsible for CQ-resistance, but together they might confer CQ-resistance. However, the role of cg2 gene is ambiguous. Therefore, it can be concluded that, association of mutation in *pfmdr1* and *pfcrt* might be responsible for CQ-resistance.

2.9 Serum and immunoglobulin mediated inhibition of intraerythrocytic growth of *P. falciparum in vitro*

A study on malaria parasite profile and acquired immune response to *P. falciparum* stage specific proteins was conducted in subjects reported with fever in eight different areas of the country, where malaria is seasonal. Both *P. vivax* and *P. falciparum* infections may occur in population during natural course of infection of malaria. This study was undertaken with the following objectives: (i) to measure the level of antibodies developed during natural course of malaria infection in individuals of various age groups of the study areas of different endemic situations, (ii) to analyse the effect of immune pressure exerted by antibody on *P. falciparum* parasites by sera and immunoglobulins mediated growth inhibition *in vitro*, and (iii) to determine the quantitative measurement of parasite invasion with variable level of antimalarial antibodies as a result of selecting subpopulation of parasites *in vivo* for maintaining parasite infection below threshold.

One hundred thirty-eight patients of eight study areas were included in the study. These 138 patients were categorised into five epidemiological age groups, such as: 1–<5; 5–<10; 10–<15; 15–<25 and >25 yr. *P. vivax* was detected in 49 (35.5%) and *P. falciparum* in 33.3% patients. Finger prick blood samples were collected by cross-sectional surveys during July to November of the years 2000–04, from all 138 patients; those were reported with malarial symptoms, especially fever. Some patients had earlier history of malaria but found malaria parasite negative at the time of blood collection. Thick and thin smears were examined after staining with JSB stain. Parasite density was estimated by counting the number of parasites per 200 leucocytes and was converted to number of parasites/µl blood taking 8,000 leucocytes/µl as standard. After collection of blood samples, patients were treated with antimalarial drug as per national treatment policy for malaria in India.

All the sera were tested for antimalarial antibody by ELISA against defined peptides and crude erythrocytic antigens of *P. falciparum*. Sera of these patients demonstrated low, moderate and high levels of antibodies against Pf-stage specific antigens—MSP-1, AMA1, RAP1, EBA175 and infected erythrocyte lysate (*Pf* crude antigen). The effect of antibody positive pooled serum and immunoglobulin fractions

with high ELISA O.D. values was assessed in *P. falciparum* culture by determining the merozoite invasion inhibition (MII) and parasite total growth inhibition (PGI). The sera of responder group containing antibodies showed variable effect in parasite growth of two *P. falciparum* isolates (Figs. 2.11 and 2.12). A positive correlation between MII and PGI of two isolates has been observed. Sera containing high titre of antibodies reacted efficiently in immunofluorescence assay. Reactivity was more pronounced with late trophozoite and schizont stages. Similarly, pooled antibody positive sera showed low to high intensity of colour development in dot blot assay.

Malaria morbidity and mortality in human populations vary greatly under different circum-

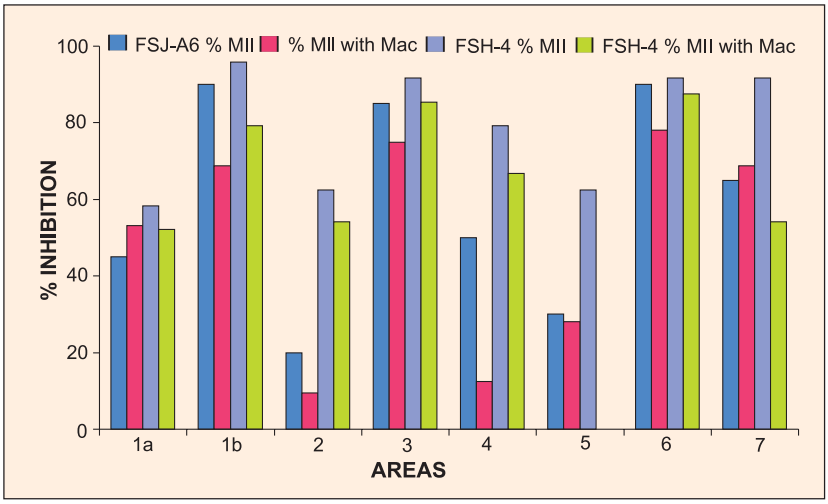

Fig. 2.11: Effect of serum in *P. falciparum* growth *in vitro*

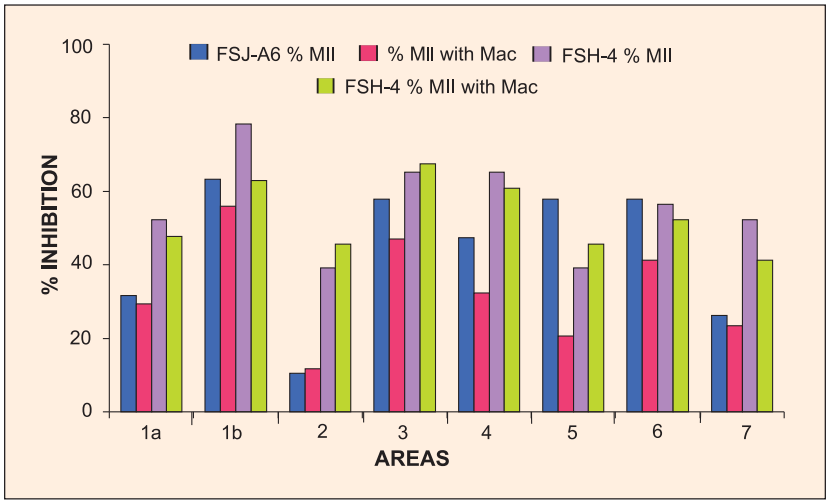

Fig. 2.12: Effect of immunoglobulin in *P. falciparum* growth *in vitro*

stances. With the attainment of age and repeated exposures to malaria infection, immunity builds up and it results in decline of malaria incidence and also clinical tolerance in adults. Inhabitants of malaria endemic areas in general possess antimalarial antibodies in circulation. In endemic areas, there are still some healthy populations who remain free from malaria due to their innate immunity against malaria.

Human serum varies considerably in its ability to support parasite growth. The specific antibodies may play an important role in mediating protective immunity and it is probable that these antibodies require the cooperation of sensitised effector cells to achieve parasite destruction *in vivo*. Human beings, who are semi-immune because of chronic and repeated infections with *P. falciparum*, may also possess cross-reactive immunity to some strains of *P. falciparum*.

The sera from various endemic zones are able to select parasite subpopulation based on their specific neutralising activities. Since inhibition of parasite growth by antibody-mediated merozoite agglutination *in vitro* may reflect an important mechanism of protective immunity, thus characterisation of *P. falciparum* for antigenic diversity within and in between geographic areas using those sera might be helpful in selecting strains for inclusion in malaria vaccine strategy.

2.10 Raising antibodies against synthetic peptides of PfHRP-2 and pLDH using microsphere delivery: Development of diagnostic reagents

Antigen tests are promising tools for the diagnosis of malaria. Two such antigens are *P. falciparum* histidine rich protein (PfHRP-2) and lactate dehydrogenase (pLDH). PfHRP-2 is water soluble protein released from parasitised erythrocytes into *in vitro* culture supernatants; pLDH, a glycolytic pathway enzyme of the malarial parasite is produced by sexual and asexual stages and can be detected in culture supernatant and plasma of infected patients. The present study was aimed to develop indigenous, rapid and sensitive immunodiagnostic method based on the detection of PfHRP-2 and pLDH antigens in the blood. Unique peptide sequences of PfHRP-2 (two regions) and pLDH (three regions) antigens were synthesised by solid phase technique and purified to homogeneity. The antibodies against these sequences were raised in mice as well as rabbit using microspheres (PLGA)

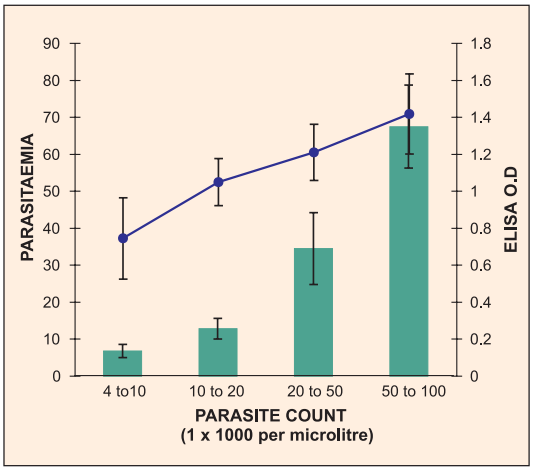

Fig. 2.13: Relationship between mean parasitaemia (parasites/µl) (■) and mean HRP-2 antigen concentration (-●-) in patients of *P. falciparum* malaria

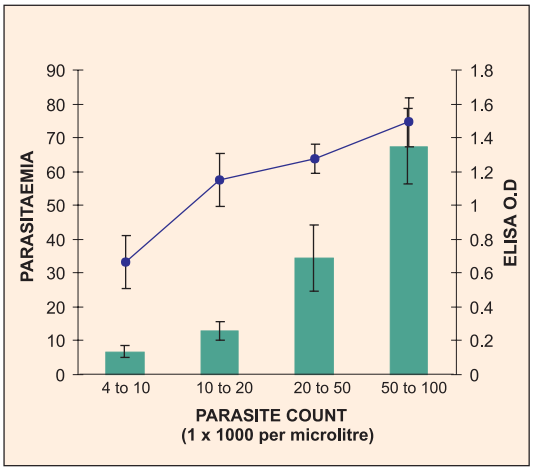

Fig. 2.14: Relationship between mean parasitaemia (parasites/µl) (■) and mean LDH antigen concentration (-●-) in patients of *P. falciparum* malaria

to generate high titre and affinity antibodies. The peptide specific peak titres varied from 25,000–50,000 and affinity of the antibodies produced was found to be in order of 0.73–5.3 nM. The antibodies generated using microspheres were able to detect the PfHRP-2 and pLDH antigens in the culture supernatant and parasitised RBC lysate of *P. falciparum* respectively by sandwich ELISA up to 0.002% parasitaemia level (Figs. 2.13 and 2.14). The assay allowed the detection of parasite infections of 0.08–2.68% parasitae-mia with a sensitivity of 100% in the whole blood of *P. falciparum* positive patients. No cross-reactivity was observed with *P. vivax* infected patients.

2.11 Detection of circulating antigen, antibody and immune complexes in individuals of endemic area as immunometric parameters in malaria

Human blood samples collected from local malaria clinics, hospitals and by cross-sectional surveys in malaria endemic areas were tested by enzyme immunoassay for circulating malarial antigens, antimalarial antibodies and antigen-specific circulating immune complexes. The assays were done in serum and finger-prick blood absorbed on filter paper. A total of 117 patients were screened for malarial parasite by microscopy; 54 were found positive and rest 63 were negative at the time of blood collection but they suffered from malaria in the recent past. The ELISA tests for the detection of antimalarial antibody, circulating antigen and circulating immune complexes were done in 117 sera and FP eluates as a pilot study. The results of sera and FP eluates were comparable. Out of 54 malaria positive samples, 51 and 52 were detected positive for FC-Ag in sera and FP eluates, respectively. It shows that the test has high diagnostic efficiency. Only 25–28% samples of negative group were detected FC-Ag positive due to very recent infection in these individuals. Antibody was detected in > 90% malaria patients whereas in blood smear negative group about 86% showed sero-positivity for antimalarial antibody. The bound antigens in the form of CICs were detected more in malaria positive cases than those with smear negative. The test parameters were applied in 240 FP eluates collected from different age groups (43, 60, 56 and 81 aged 1–<5, 5–<10, 10–<15 and >15 years, respectively). The average antibody titre $-\log_2$ varied from 6–10. Both TC-Ag and CIC were found at lower level in younger age groups (Fig. 2.15). Although microscopic demonstration of malarial parasites in blood films is the method of choice to

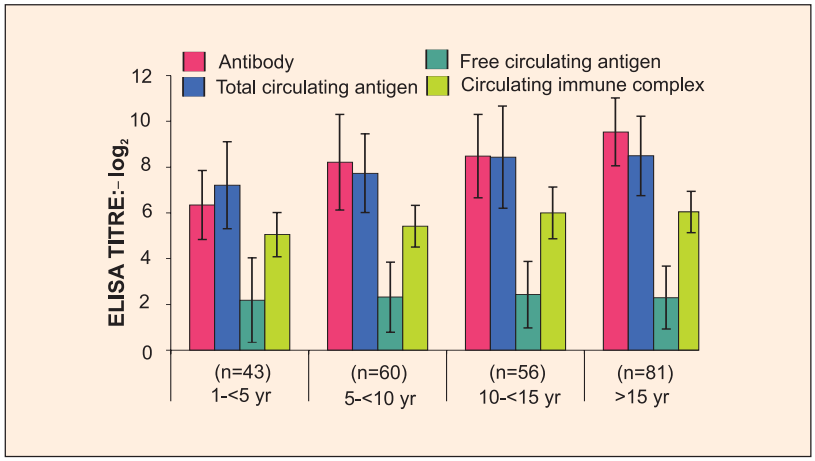

Fig. 2.15: Antibody, total circulating antigen, free circulating antigen and circulating immune complex profile in the study subjects (n = 240). The bar diagram is plotted with mean ELISA -$\log_2$ titre of each group

diagnose acute malaria, detection of circulating malarial antigens, antimalarial antibodies and immune complexes may help as supplementary tool for immunodiagnosis. When applied in a community-based study, these tests would be able to monitor infection dynamics.

2.12 Immune responses to *P. falciparum* antigens and disease susceptibility among inhabitants seasonally exposed to malaria

In malaria endemic areas, the acquisition of antimalarial immunity is progressive as observed by age wise gradual decrease in morbidity and mortality to malarial infection. This study was aimed to assess the relationship between *in vitro* immune responses and susceptibility to malaria based on a protective longitudinal study for one year.

In the present study, two groups of children, younger (1–<5 yr) and older (5–<15 yr) were enrolled in two areas (A-1 and A-2) of northern India where malaria is though seasonal but the epidemiology is different. The frequency of humoral and cellular reactivity to six synthetic peptides (CSP, MSP-1_{19}, AMA1, RAP1, EBA175 and PfG27) and crude blood stage antigens of *P. falciparum* were measured in the study subjects.

Individuals of A-1 showed very less number of malarial episodes compared to A-2 during one year. Clinical protection in children of A-1 was related to elevated levels of antibodies detected against CSP, MSP-1_{19}, AMA1 and RAP1. However, anti-EBA175 antibody level was almost alike in two groups. Higher lymphocyte proliferation responses to MSP-1_{19}, AMA1 and RAP1 peptides were observed in the study subjects of A-1 than A-2. The differences in T-cell reactivity as overall higher response rate of IL-4 and IFN-γ but lower IL-10 and TNF-α to MSP-1_{19}, AMA1 and RAP1 were observed in individuals of two areas. The immune responses among individuals of two ecotypes highlight the immunogenicity of the molecules, namely MSP-1_{19}, AMA1 and RAP1 and their relation to clinical protection.

2.13 Molecular characterisation of aspartic protease gene from *P. vivax*

Aspartic proteases from *P. falciparum*, known as plasmepsins have been characterised and are believed to have a promising potential in antimalarial chemotherapy. Aspartic proteases from *P. vivax* have been characterised. Characterisation at the biochemical and molecular level will be helpful in designing new drugs against *P. vivax* and *P. falciparum*. As an extension of the previous work, a study was planned to characterise Aspartic protease gene in *P. vivax* with the following objectives: (i) N-terminal sequencing to confirm the homology to known Aspartic protease of *P. falciparum;* (ii) to find out the difference among the samples collected from different geographical regions (different forms of plasmepsins namely I, II, IV, etc.); (iii) to characterise the Aspartic protease genes from *P. vivax;* (iv) to clone and express the gene of Aspartic protease from *P. vivax* and compare with that of *P. falciparum;* (v) to confirm activity of recombinant protein equivalent to native protein using conformational/ biochemical analysis; and (vi) use of recombinant enzymes for kinetic analysis of inhibitors/drug designing for drug target.

This study will be helpful to elucidate the biochemical properties and biological role of malarial proteases and will foster the development of protease inhibitors as a new antimalarial drug. This rational drug design will help in targetting common susceptible sites of both *P. falciparum* and *P. vivax* and may prove to be a boon for the antimalarial chemotherapy.

2.14 Glutathione S-transferase (GST) activity: diagnostic and protective role in *vivax* malaria

Glutathione S-transferase is the most abundant intracellular antioxidant with complex biological functions and a well-established metabolic regulator. In malarial infection, host produces oxidative stress and maintains it as a defense mechanism. The Glutathione S-transferases play a critical role in malarial diagnosis and pathogenesis. The malarial parasite—*P. vivax* is known to be sensitive to oxidative stress,

Table 2.1. Comparison of serum and plasma GST activity in healthy and *P. vivax* infected patients

Subjects	GST (IU/L)		
	Minimum	Maximum	Mean ± SD
Serum			
Control	7.25	16.65	11.65 ± 2.95
P. vivax	2.6	15.6	6.43 ± 3.29
Plasma			
Control	6.15	13.23	10.09 ± 2.68
P. vivax	2.37	13.56	5.65 ± 3.15

and thus the antioxidant enzyme—GST has attracted interest in the field of diagnosis and monitoring in view of malarial complications.

With this objective in mind, we have collected 39 (32 Male/7 Female) clinical isolates of malaria patients infected with *P. vivax* and 9 (Male) healthy controls from clinic and analysed serum and plasma GST activity in *P. vivax* patients and healthy control essentially following the procedure described by Habig *et al** (Table 2.1).

Serum and plasma GST levels of *P. vivax* infected patients had less GST activity (6.43 ± 3.29 and 5.65 ± 3.15 IU/L) as compared to healthy controls (11.65 ± 2.95 and 10.09 ± 2.68 IU/L), ($p < 0.0004$) and ($p < 0.0007$). A significant correlation in the GST activity of control subjects and *P. vivax* patients (Fig. 2.16) was observed ($r = 0.95$). Further studies are in progress.

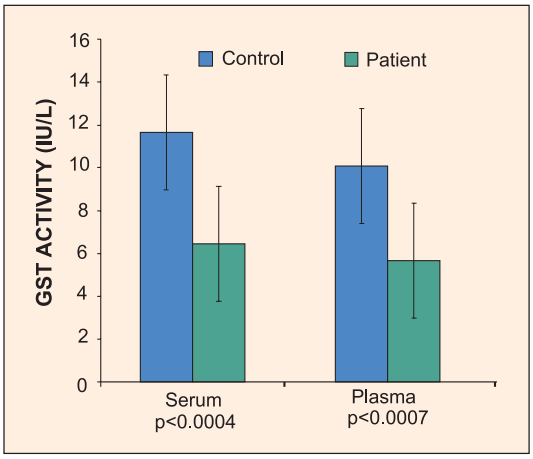

Fig. 2.16: Comparative glutathione S-transferases (GST) activity in plasma and serum in *vivax* patients and healthy controls

*Habig *et al* (1974). *J Biol Chem* **249**(22): 7130–7139.

2.15 Malaria Parasite Bank

Parasite Bank is supporting a large number of organisations working on various aspects of malaria. Biological material including non-human and human plasmodia preserved/maintained at the Malaria Parasite Bank were supplied to various research organisations. During 2004–05, 16 *P. falciparum* isolates adapted to *in vitro* culture conditions and characterised for drug susceptibility to CQ have been supplied to the Department of Biotechnology, AIIMS, New Delhi and another 30 *P. falciparum* isolates were supplied to Division of Molecular Biology, NIMR as part of collaborative studies. Twenty *P. vivax* isolates were given to ICGEB, New Delhi on payment. Human and/non-human malarial parasites were given to PGIMER, Chandigarh, JNU, New Delhi and University of Hyderabad, Hyderabad. As a part of resource generation we have already started charging for the biological materials supplied from the Parasite Bank and also for screening of medicinal plants for their antimalarial activity. During 2005–06, 14 *P. falciparum* and 5 *P. vivax* isolates from Mangalore, one *P. vivax* from Bangalore, 3 *P. falciparum* and 3 *P. vivax* isolates from Chennai were collected.

2.16 Screening for antimalarial activity of the synthetic compounds in *P. falciparum* culture lines

A number of compounds were synthesised by novel methods to study their antimalarial activity *in vitro* in *P. falciparum*. Some novel derivatives as six new Baylis-Hillman adducts were synthesised based on substituted 2-chloronicotinaldehydes and five 1-aryl-4,6-diamino-1,2-dihydrotriazines were synthesised using neat technology under microwaves. These compounds were tested *in vitro* against chloroquine and pyrimethamine sensitive and resistant *P. falciparum* strains.

2.17 Screening of medicinal plant extracts/ fractions for antiplasmodial activities

Screening of medicinal plant extracts/fractions for their antiplasmodial activity against chloroquine sensitive and resistant *P. falciparum* isolates is a routine activity of the Malaria Parasite Bank.

In the new collaborative project entitled, "Discovering antimalarials from marine organisms" (multi-institutional collaborative project with ICGEB and University of Kerala, funded by DBT) it was proposed to collect flora and fauna from marine and estuarine conditions for the preparation of extracts which is being done in University of Kerala. These extracts are being tested in ICGEB and NIMR for their antiplasmodial activity. We have already screened 32 marine samples for their antiplasmodial properties *in vitro* against CQ sensitive and resistant isolates of *P. falciparum* and few of them are showing very good anti-plasmodial activity. Screening of other samples are in progress.

Due to the resistance of parasites to almost all antimalarials available, efforts are being made to explore the possibility of new antimalarials from medicinal plants available indigenously (either in the crude form or molecules). Keeping this in mind, about 25 medicinal plant extracts were tested and some of them were showing good antiplasmodial activity *in vitro* against *P. falciparum*. Three extracts were tested *in vivo* also. These extracts showed up to 75% inhibition with 50 mg/kg body weight. Further work is in progress for the purification of compounds from these extracts.

Fifteen samples (medicinal plant extracts and pure compounds) from DRDE, Gwalior were tested for their antiplasmodial activity and few samples are being processed. Twenty-two marine samples were screened for antimalarial activity.

The collaborative project entitled, "Primary screening of the medicinal plants from Northeastern states of India for their antiplasmodial activity" is an ongoing project. Under this project 25 crude extracts and 8 fractions were tested *in vitro* till now. Out of these one crude extract tested *in vivo* showed about 60% inhibition with 100 mg/kg body weight. Further studies are in progress.

As a part of manpower development, parasite bank is actively involved in imparting training to research fellows/students in *in vitro* cultivation of *P. falciparum* and drug sensitivity testing. Also, as per request from University of Khartoum, Sudan, a workshop was conducted on identification and *in vitro* cultivation of malaria parasites, screening of antimalarials, establishment of malaria parasite bank, etc., in Sudan during 28 May to 12 June 2005. ❑