Summary

Malaria continues to be serious health problem special in the developing countries around the tropical and sub tropical regions of the world. With around one million deaths occurring every year and 40% of world population at risk malaria has serious impact on socio economic state of the nations. India contributes about 2 million cases of malaria every year of which half of the infections are caused by *Plasmodium falciparum* the killer malaria parasite. Northeastern states hold an important position in the malaria map of India as it is a high risk zone.

Control of malaria by and large, mainly dependent on two aspects- mosquito vector control and chemotherapy against the malaria parasite. Entire malaria control programme depends on these two aspects and proper effectiveness of these two aspects is must. Development of insecticide resistance and improper implementation of control measures vector control still not fully successful. Due to this chemotherapy plays an important role in malaria management. There are mainly three types of compound available for chemotherapy against malaria parasite viz- aryl amino alcohol compound (e.g. chloroquine, quinine, mefloquine etc.); antifolate compounds (e.g. sulphadoxine-pyrimethamine) and artimisinin and its derivatives (e.g. artemether, artesunate etc.).

Resistance to antimalarial drugs, posing serious threat to the entire chemotherapy regime against the malaria parasite. *Plasmodium falciparum* the killer malaria parasite has developed resistance to all the available antimalarial drugs except artimisinin group of compounds. Spread of malaria to newer areas and reemergence of malaria in areas where malaria has been eradicated could be attributed to drug resistance. Resistance to chloroquine emerged simultaneously in Southeast Asia and Latin America in 1960s.
Similarly sulfadoxine pyrimethamine resistance and mefloquine resistance have emerged in 1980s again in Southeast Asia. After that antimalarial drug resistance has become a global problem with the passage of time. In India chloroquine resistance was first reported from Assam and then resistance to other antimalarials also reported from northeastern region as well as from other parts of India.

Epidemiological studies using molecular biology tools have given rise molecular epidemiology which helped in greater understanding of disease and become helpful while formulating disease control strategies. *Plasmodium falciparum* varies greatly in epidemiological characteristics as which may be related to the genetic makeup of the strain. High degree of diversity is seen in genes encoding surface anti gens which have very important role in immunity and in different transport proteins which have role in antimalarial drug resistance.

There are a few molecular markers the presence of which could be correlated with antimalarial drug resistance. For chloroquine one of the most widely used and effective drug, the development of resistance to it can be the attribution of two important molecular markers. *Plasmodium falciparum* chloroquine resistance transporter protein (*Pfcrt*), a food vacuole membrane transport protein play important role in chloroquine resistance. Mutation at 76th amino acid position where lysine is replaced by threonine play pivotal role in conferring resistance. This marker has been widely used and tested in different field conditions and found to associate with chloroquine resistance. Similarly another protein *pgh1* a digestive vacuole membrane protein also found to be associated with chloroquine resistance. This protein is encoded by *Plasmodium falciparum* multiple drug resistance gene. It exists in dimorphic form. In several field epidemiological studies it has been found that association of *Pfmdrl* N86Y mutation
with CQ resistance. Though its role in conferring chloroquine resistance is doubted, its association with increased level of resistance has been proved in many studies. These two molecular markers are considered to be useful in molecular survey of drug resistance.

Genetic diversity in the antigenic proteins of *Plasmodium falciparum* has been one of the major concern in development of a universal malaria vaccine. Polymorphisms in these genes occur mainly due to sexual reproduction of the parasite inside the mosquito vector. Genetic diversity studies can provide valuable information regarding transmission intensity, disease severity and protective immune response and population structure of the parasite in an area. Merozoite surface protein 1 and 2 (MSP1 and MSP2) are very important antigenic protein as far as development of vaccine. Another protein Glutamate rich protein (GLURP) also has similar kind of property of tendem repeat variation. Variation in the repeat length of these genes is characteristics feature which has been widely used for genetic diversity

Keeping these in view a study of molecular epidemiological study of drug resistance and genetic diversity was undertaken with the objectives- Drug resistance status of *P. falciparum* and its correlation with molecular markers along with genetic diversity in MSP1, MSP2 and GLURP genes and its relation with disease severity and transmission intensity.

Three malaria endemic as well as geographically isolated areas of the northeastern region viz- South Tripura District, Sonitpur District and Nagaon District have been selected for the study. In south Tripura District epidemiological survey reveal very high slide positivity rate (SPR) (25.2%). Again *Plasmodium falciparum* infection was found
very high slide falciparum rate (SFR) being 22.3%. Age wise distribution of the malaria positive cases reveal that age group 4-10 years is the worst affected SFR being 33.2% ($\chi^2 = 64.8; \text{df} = 4; \text{p}< 0.001$). Though difference observed between SPR of premonsoon and monsoon seasons yet without significance. Sex wise distribution of the cases revealed that there was no significant difference between SFR and SVR for the males (23.4% and 3.3%) and those for the females (20.2% and 2.3%) ($\chi^2 = 1.2; \text{df}=1; \text{p}> 0.05; \chi^2 = 0.77; \text{df}=1; \text{p}> 0.05$).

Among the positive cases one arm prospective study of clinical and parasitological response after in vivo administration of CQ was carried out. 63 individuals positive for P. falciparum mono infections were included in the study. In the in vivo study, treatment failure was found in 19 (30.2%) cases, whereas 44 (69.8%) cases responded adequately to the chloroquine treatment (ACPR). Among the treatment failure cases 16 (84.2%) cases were early treatment failure (ETF) and 3 (15.8%) were Late Treatment failure (LTF). Clinical response of chloroquine in different age groups revealed that treatment failure cases were highest in 0-5 year age group (47.4%). Among the treatment failure cases, ETF (n=16; 84.2%) were higher (p=0.001; $\chi^2 = 10.5; \text{OR}= 14.1$) than LTF (n=3; 15.8%). Prevalence of Pfcr1 K76T mutation and Pfmdr1 N86Y mutation revealed that Pfcr1 K76T was found in 25 cases where it was absent in 38 cases similarly pfmdr1 N86Y mutation was found was found in 28 no of samples and absent in 35 number of samples. Pfcr1 K76T mutation was recorded in all the treatment failure cases whereas, Pfmdr1 N86Y mutation was found in 52.6% cases only. Age wise distribution showed that both K76T and N86Y mutations were more prevalent among the patients below 11 years (p<0.0001). Pfcr1 K76T mutation was more sensitive and specific as compared to pfmdr1 N86Y mutation in corresponding the chloroquine drug resistance
as sensitivity and specificity of Pfcrt K76T was 1 and 0.86 as compared to 0.53 and 0.59 in Pfmdr 1 N86Y mutation. Sequence analysis of 72 to 76 pfcrt gene codons revealed the presence of CVMNT, CVIET, CVMNK and CVIEK haplotypes. Mutant CVIET haplotype was predominantly distributed (42.1%) followed by CVMNK (26.32%), CVMNT (23.68%) and CVIEK (7.89%) haplotypes irrespective of their drug susceptibility status. Further, the CVIET haplotype was more widely distributed in the treatment failure cases (63.2%) in comparison to CVMNT.

Epidemiological survey in Sonitpur District also revealed a similar picture SPR being 38.6% with 91.04% cases of P. falciparum. Along with P.falciparum, P. vivax and P.malariae cases were also recorded though less in number. P. malariae was recorded for the first time in the district. Age wise and sex wise distribution of malaria did not project any significant finding however lower age groups reported more number of cases. 19.4% patients showed high anemia and among them 57.41% cases belong to >11 years age group ($\chi^2$=21.5; p= <0.0001). Pfcrt K76T mutation was recorded in 44 (72.13%) isolates whereas Pfmdr 1 N86Y mutation could be detected in 28 (41.79%) isolates only. The total number of isolates with Pfcrt K76T mutation were significantly higher than the wild type ($\chi^2$=22.16; p= <0.0001; OR=0.149 (95% CI=0.07-0.33). On the other hand, no significant difference was observed among Pfmdr 1 N86Y mutant and wild isolates ($\chi^2$=0.52; p= 0.47; OR=1.39 (95% CI=0.68-2.83). Again 32.7% samples were found to contain both the mutations i.e. Pfcrt K76T and Pfmdr1 N86Y mutation together.

From Nagaon District of Assam samples 44 malaria positive samples were collected out of which 41 cases were found to contain P. falciparum mono infection while rest are P. falcipaum and P. vivax mixed infection. Distribution of mutant allele of the both the
genes were higher than the wild types. *Pfcrt* K76T mutation was present in 38 (92.68) numbers of samples while *Pfmdr* N86Y mutation was present in 36 (86.8) numbers of samples.

Genetic diversity study of the South Tripura District and the Sonitpur District revealed that, both the study site showed high degree of diversity in three studied loci viz- MSP1, MSP2 and GLURP. In South Tripura district higher diversity was observed in MSP2 gene but multiplicity of infection (MOI) was found higher in MSP1 gene. Frequency distribution of allele types of the genes showed that MAD20 allele of msp1 and 3D7 allele of msp2 higher frequency compared to other two. Higher MOI of MSP1 observed in case of MSP1 in the chloroquine resistant cases. In Sonitpur District diversity was found to be less in comparison to South Tripura District. However MOI of MSP2 was found higher than MSP1 and GLURP.

The study reveals that chloroquine resistance is higher in this region. Previous studies conducted in this region also support our finding. However, drug resistance should be studied at micro level and level of resistance varies greatly spatially. Again studies on drug resistance molecular marker showed that it is associated with CQ resistance but its presence in CQ susceptible cases suggests that it may not be useful singly in determining drug resistance. Moreover, their presence in high proportion indicates increasing speed of spread of CQ resistance.

Genetic diversity of *P. falciparum* in the study area showed similarity to Southeast Asian countries as well as some highly malaria endemic area of Africa. As malaria in Northeastern region in not uniform, some areas are highly endemic and some are almost malaria free so, its parasite population also show considerable diversity. Frequency of
MSP1 gene was found to be higher in all the studied population and its MAD20 allele was found most prevalent. In case of MSP2, 3D7 allele was found more prevalent. This allele was found more prevalent in severe cases.

Thus it can be stated that high diversity and high drug resistance is widely prevalent in the area. So, due attention must be paid to these issues while formulating control proramme. As variation in these parameters is a localized phenomenon, therefore a variable type of control strategy may be adopted to avoid spread and development of drug resistance.