Synopsis

Toll Like Receptor Polymorphisms In Relation To Malaria In The Indian Population

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Malaria is an important public health concern in countries where transmission occurs regularly. It is a complex disease that varies widely in epidemiology and clinical manifestations in different parts of the world. The variability is the result of factors such as the species of malaria parasites that occur in a given area, their susceptibility to commonly used or available anti-malarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and the behaviour and level of immunity of the exposed human population.

Till present no successful vaccine, or chemotherapeutic treatment has been made available to the malaria exposed population and loads of efforts are being made in order to develop new strategies. Moreover, in order to have an effective treatment, the genetic makeup of the parasite, *Plasmodium*, the vector, *Anopheles* and the human host plays an important role. The genetic makeup of the malaria exposed population plays a vital role, as it predisposes the individual's immune response, mounted against the invading pathogen. Thereby, polymorphism studies of various immune genes are being carried out to understand their role in disease in particular. One such molecule that has gained importance in the recent years is Toll like Receptors (TLRs).

Toll like Receptors (TLRs), an integral part of the innate immune system, was first described in drosophila and later in mammals, playing an important role in recognizing the invading pathogen including malaria parasite *Plasmodium*. In the recent past the role of TLRs in various infectious diseases has been elucidated and with the identification of non conventional ligands like glycosphatidylinsitol (GPI) of *Plasmodium falciparum* for TLR2, hemozoin bound host fibrinogen for TLR4 and parasite histone-DNA complex acts as an
immunostimulatory component for TLR9, the role of TLRs in malaria caught the limelight. The mechanism of counteraction is very complex, and various factors including the genetic makeup of the exposed population can be of utmost importance for its predisposition to malaria.

A number of polymorphism studies have been carried on TLR2, TLR4, TLR9 and TIRAP (Toll-interleukin-1 receptor (TIR) domain containing adaptor protein) also known as Mal (MyD88 adaptor-like) in various infectious diseases showing the association of the disease with the polymorphism be it beneficial or deleterious. Thus, studies regarding the allelic prevalence of TLRs and TIRAP genes in various parts of the world, where malaria is an endemic disease, may provide substantial data for developing therapeutics in treatment of malaria.

An introduction to the malaria disease, its historical background, its various control measures and the counter-attack of the immune system to the infection has been described in **CHAPTER I**.

India even though being one of the malaria endemic countries of the world, research on the allelic prevalence of TLR 2, 4, 9 and TIRAP in the gene pool of different malaria endemic populations has not yet been dealt with, thus making this kind of study on TLR gene polymorphism a potential area of research in malaria.

Therefore, considering it to be a prospective area of research on the Indian population residing in malaria endemic regions, this study was carried out with the following objectives in mind.

- To study the allelic prevalence of Toll like receptor (TLR) 2 (Arg677Trp, Arg753Gln), 4 (Asp299Gly, Thr399Ile), 9 (-T1237C, -T1486C) and TIRAP (Ser180Leu) in the Indian population from malaria endemic regions.
• To investigate whether these SNPs of TLR2, 4, 9 and TIRAP show any association with malaria in these malaria endemic populations of India and are under any selection pressure.

• To study the effect of these polymorphisms on the innate immune response against exposure to *Plasmodium falciparum*.

A review of the current literature on the innate immune system and Toll like Receptors has been presented in **CHAPTER II**.

Materials and Methods employed in this study have been described in **CHAPTER III** where malaria patients from seven different eco-epidemiological regions of India were genotyped for TLR2, TLR4, TLR9 and TIRAP polymorphisms using DNA sequencing methods. Functional studies were carried out to see the effect of these polymorphisms on the immune response upon *Plasmodium falciparum* infection where blood was taken from pre-checked volunteers for polymorphisms in TLR4, TLR9 and TIRAP and thereafter subjected to whole blood stimulation assay and PBMC stimulation assay.

In **CHAPTER IV** the results obtained both from genotypic studies as well as functional studies have been presented. In genotypic studies no variation was observed at residue positions 677 and 753 in TLR2 whereas residue positions 299 and 399 in TLR4, nucleotide positions -1486 and -1237 in TLR9 and residue position 180 in TIRAP were highly polymorphic. In TLR4 the mutant allele G for residue position 299, had the lowest frequency in Nicobar (0.0454) and highest in Vizianagaram (0.2). On the other hand, the lowest frequency of the mutant allele T for residue position 399 was observed in East Singhbhum (0.060) and highest in North Goa (0.190). Four haplotypes, AC (Asp/Thr), AT (Asp/Ile), GC (Gly/Thr) and GT (Gly/Ile) were observed in all populations except in Kolkata, Dakshin Kannada and Nicobar district where only three haplotypes AC (Asp/Thr), AT (Asp/Ile) and GT (Gly/Ile) were observed and
GC haplotype was completely absent. Upon comparison between malaria patients and healthy subjects no significant difference was observed (P > 0.05) at both residue positions Asp299Gly and Thr399Ile of TLR 4 except in samples from Dakshin Kannada at Thr399Ile where it was found to be statistically significant \( p = 0.003 \). On the other hand, though the distribution of the two polymorphisms in TLR9 was heterogeneous amongst the malaria patients and the healthy subjects, no significant association was observed between malaria and TLR9 promoter polymorphism. However, upon comparison between malaria patients and healthy subjects the population of East Singhbhum showed a highly statistically significant \( p = 0.0001 \) difference between malaria patients and healthy subjects at position -1237. In TLR9, the C allele for locus -1237 was not observed in Nicobar district. In contrast, the C allele for locus -1486 was observed in all populations at more or less similar frequencies. In TLR9, four haplotypes (-1486/-1237) were observed in malaria patients TT, CT, TC and CC except in the population of Nicobar district where only two haplotypes were observed TT and CT, suggesting the complete absence of polymorphism at -1237 nucleotide position. In TIRAP, the mutant T allele for residue 180, had lowest frequency in Nicobar district (0.045) and highest in Kolkata (0.207). In case of TIRAP the heterozygous genotype (Ser180Leu) was observed highest in Kolkata and Sundergarh districts where the mutant genotype was completely absent. All polymorphisms were in Hardy Weinberg equilibrium.

In functional studies it was observed that in TLR4 the wild type genotype (Asp299/Thr399) gave a pronounced proinflammatory response than the double heterozygous (Asp299Gly/Thr399Ile) genotype upon *Plasmodium falciparum* infection as well against conventional ligand LPS. Moreover, in TLR9 the wild type genotype (T-1486/T-1237) and the double heterozygous genotype (T-1486C/T-1237C) expressed high levels of proinflammatory cytokines against *Plasmodium falciparum* infection as
well as against commercially available ligand ODN2006 as compared to the single heterozygous genotypes (T-1486/T-1237C and T-1486C/T-1237) for either position. In TIRAP, all the three genotypes including wild type (Ser180), heterozygous genotype (Ser180Leu) and mutant genotype (180Leu) expressed moderate levels of cytokines except in IL-12 by the heterozygous genotype upon *Plasmodium falciparum* infection.

The results of the present study have been discussed in the light of previous reports in **CHAPTER V** where implications of present study have been discussed. Here it was suggested that combined with the genotypic and functional results TLR2 polymorphism was absent in the Indian population and an overall heterogeneous pattern of TLR4, TLR9 and TIRAP polymorphism can be attributed to genetic drift. The presence of mutation at 399 in TLR4 may have a deleterious effect on the proinflammatory phenotype of the 299 SNP when present alone and it can be inferred that GC haplotypes confers protection from mortality and *Plasmodium falciparum* predominance may be one of the factors responsible for its ongoing selection in malaria endemic regions in India. Conversely, presence of mutations at both positions -1237 and -1486 in TLR9 may have an additive effect on the proinflammatory phenotype of the mutant allele C at -1237 even though at the genetic level no association with malaria was observed. Finally in TIRAP, at the genetic level the mutant genotype may be undergoing negative selection pressure for its reduction from the malaria endemic populations of Kolkata and Sundergarh and at the functional level it has been observed that Ser180Leu heterozygote does produce an early proinflammatory response thereby protecting the individual from disease severity.