ABSTRACT

Lymphatic filariasis causes physical and social disability that affects over 100 million people in 83 countries in the developing world. The feasibility to eliminate lymphatic filariasis is postulated to be mainly on diagnosis to identify endemic areas followed by repeated cycles of mass drug administration (MDA) to reduce both infection prevalence and transmission rates to levels below those required for sustained transmission. Treatment for lymphatic filariasis depends almost exclusively on drugs: DEC, ivermectin and albendazole.

Vaccines and new drugs are needed, certainly because drug resistance in human helminth parasites would present a major problem for current treatment and control strategies. Without the knowledge of parasite population genetic structure, it’s hard to establish new drugs or vaccines and it is also necessary to look for the existence and distribution of drug resistant alleles in the population. Therefore the present study focuses mainly on the distribution and conservation of new emerging drug targets and vaccine candidates. For polymorphic studies, two antioxidant genes; Glutathione-s-transferase (GST) and thioredoxin(trx) which are very crucial for the parasite survival by overcoming the oxidative stress environment of the host and abundant larval transcript-2 (alt-2), a promising vaccine candidate were investigated.
Indian parasite population was chosen for the polymorphic studies. Samples from four different geographical region viz., Tamil Nadu, Puducherry, Maharashtra and Bhubaneswar, were collected as microfilaria positive (mf+ve) blood samples or blood smear stained mf+ve microscopic slides. Mf was isolated and genomic DNA was extracted from it. The selected genes were amplified using gene specific primers and the PCR products were cloned directly into sequencing vector to avoid missing and mixing of alleles. Randomly, 25 clones from each region were sequenced and the sequences were analyzed for variation using BLAST tool. Depending on the presence or absence of variation and new findings from the genetic structure, the genes were characterized further.

The polymorphic studies identified intron allelism in \textit{Wb-alt-2} gene. 29bp tandem repeats observed in \textit{Wb-alt-2} intron-2 was similar to \textit{Bm-alt-2} , 27bp tandem repeats but the 46bp repeat which was already reported in intron-3 of Bm-alt-2 was completely absent in \textit{Wb-alt-2}. This evolutionarily modified tandem repeat was exploited in developing genetic marker that differentiated bancroftian infection from brugian. \textit{alt-2} IR3 PCR based method, discriminated the major lymphatic filarial species \textit{Brugia malayi} and \textit{Wuchereria bancrofti} depending on the amplicon size generated. This direct PCR assay was sensitive and highly specific on field patient samples. In addition it also worked on artificially mixed filarial DNA differentiating both lymphatic filarial species by single step PCR. The study concluded that IR3 based PCR assay can be a best tool for PCR based diagnostics for screening large number of samples in both endemic and co-endemic area for lymphatic filariasis, at species specific level.
Evolutionarily conserved alt-2 intron-2 repeats were further studied using reporter gene assay to analyse the functional importance on regulated expression of alt-2 gene. In-vitro DNA-protein binding studies identified the 27/29bp repeat as nuclear protein binding region. Reporter gene constructs were made with known promoter, upstream of the luciferase gene. The alt-2 intron-2 was placed between the promoter and reporter gene to study the regulation of the 27/29bp repeat region on promoter driven luciferase expression. The study concluded that only the 27/29bp repeat regions in alt-2 intron-2 contribute to the suppression of luciferase expression. Nematode expression system is further needed to confirm this preliminary analysis.

Compared to alt-2, which was conserved in the exonic region, Wb-GST was found to be highly polymorphic in natural population. Three variant alleles designated as Wb-GST2a, Wb-GST2b, Wb-GST2c were found in the parasite population in India. These alleles were cloned and expressed in prokaryotic expression system and the purified recombinant protein was further characterized. Enzyme kinetics of the three variant Wb-GST proteins was compared with the wild-type by its interaction with different substrates and inhibitors. Compared to the Wb-GST WT, the variants had a lower affinity for substrates and inhibitors. Structural analysis will further provide evidence on the effect of mutation on the interaction of Wb-GST with substrates / inhibitors. Wb-trx was found to be conserved in the parasite population and proves to be very good drug target. The polymorphic studies has provided spotlights for the better understanding of the parasite genes importance in relation with the parasite survival, the genetic distribution in natural population and a support for the evaluation of new drug targets and vaccine candidates.