1 On Going Studies

1	Immunochemical characterization of filarial glutathione S-transferase and its protective potential in experimental filariasis.
2	Human Bancroftian Filariasis: Identification of Immunological markers of morbidity in Hydrocele and Elephantiasis
3	Post–DEC reactions in Human Bancroftian filariasis: An Immunobiological study in Orissa, India
4	Genetic polymorphism of host molecules involved in immunity and immunoregulation in human filariasis.
5	Development and evaluation of community development and partnership strategies for drug delivery for the control of lymphatic filariasis in urban areas of Orissa, India
6	Efficacy and Tolerability of single dose DEC of 100mg, 200mg & 300mg strength in filariasis endemic community in Orissa.
7	Studies on prevalence of 76Tcrt / 86Ymdr1 Plasmodium falciparum isolates in severe malaria cases of Orissa and its biological advantage.
8	Development of potent mosquitocidal agents from natural sources
9	Molecular Characterization of *Anopheles annularis* Complex Development of Species Specific Diagnostic Markers and Micro satellite Markers
10	Malariogenic stratification of Anugul district of Orissa using sibling species prevalence of malaria vectors.
11	A study on immunoregulation and genotyping for cytokine polymorphism in human cerebral malaria.
12	The effect of Choloroquine Chemoprophylaxis during pregnancy among the compliant and non - compliant mothers of Orissa.
13	Molecular analysis of drug resistance genes and prediction of treatment outcome in P.falciparum infections in Orissa.
14	A randomised clinical trial with Chloroquine and alternate drug regimens to study the comparative efficacy, in treatment of uncomplicated P.falciparum malaria in two endemic districts of Orissa.
15	Epidemiological characterisation of bacterial enteropathogens among the children suffering from acute diarrhoea-A hospital based study.
16	A 6-year's Prospective study of the risks of death by cause from tobacco and alcohol use among 2million Indian men and women, A multicentric study.
17	Epidemiology of viral hepatitis in tribal populations of Orissa, Madhya Pradesh/ Chhatisgarh and Jharkhand, India –a multicentric study

On Going Studies

Principal Investigator:
Dr. M. K. Beuria
Co- Investigators:
Dr. M.K. Das and Dr. M.S. Bal
Funding : Extramural (DST)
Starting Date : March 2005
Closing Date : February 2008

1. Immunochemical characterization of filarial glutathione S-transferase and its protective potential in experimental filariasis.

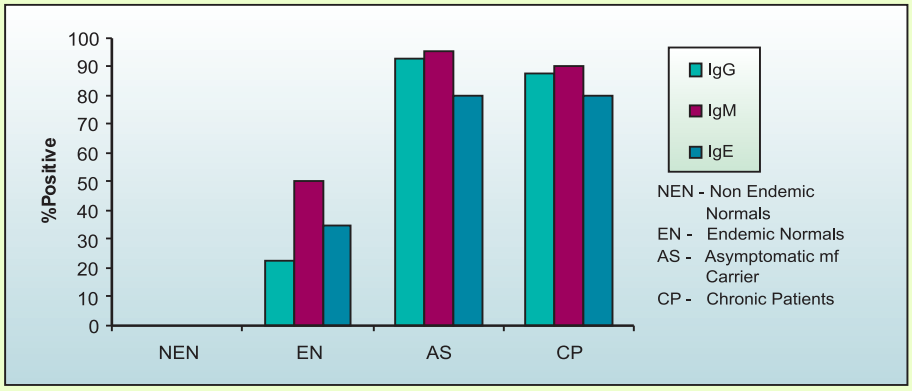
Objectives:

1. To determine recognition pattern of anti-Glutathione-S- transferase (GST) antibodies (SDS-PAGE and immunoblotting) in filarial sera.
2. To determine the cytokine responses specific to GST in filariasis.
3. To evaluate the protective potential of GST to clear microfilariae in experimental infected animal.

Work Progress:

Glutathione-s-transferases, which help in parasite survival against host-induced damages, are detoxification enzymes. These enzymes have been used as component of anti-parasitic vaccine in Schistosomiasis, Fascioliasis and in Chaga's disease. In this study we have purified Glutathione-s-transferase from cattle filarial parasite Setaria digitata to evaluate it's role in human filariasis. We have earlier determined the IgG and IgM antibodies to Glutathione-s- transferase in individuals living in areas endemic for Wuchereria bancrofti infection. Majority (90%) of infected individuals (asymptomatic microfilaraemic and chronic patients) were seropositive for both the antibodies compared to normal individuals (20%) of endemic regions. IgE antibodies were determined in different group of filarial sera. Seropositivity of 80%, 60%and 35% were observed in chronic patients, asymptomatic microfilaraemics and in endemic normal respectively. None of the sera collected from non-filarial endemic regions were found positive for any antibody isotype. The effect of filarial serum on enzymatic activity of GST was studied. Inhibition was noticed in all categories of filarial sera. Incubation of sera from non-filarial region could not inhibit the enzymatic activity.

Fig1: Prevalence of antibody isotype to GST in different group of filariasis



Principal Investigator:
Dr A.K.Satapathy,
Co-Investigators:
Dr A.S. Kerketta, Dr P.K. Sahoo, and Dr. B.Ravindran
Funding: Intramural
Starting Date : March 2006
Closing Date : February 2009

2. Human Bancroftian Filariasis: Identification of Immunological markers of morbidity in Hydrocele and Elephantiasis

Objectives:

1. To evaluate filarial specific as well as mitogen induced T-cell proliferative responses in hydrocele and lymhoedema patients
2. To quantify inflammatory cytokines and chemokine levels in patients with hydrocele and lymhoedema and correlating with severity of chronic manifestation.

3. To type TNF receptors, Type I & Type-II genetic polymorphism in hydrocele and lymphedema patients.

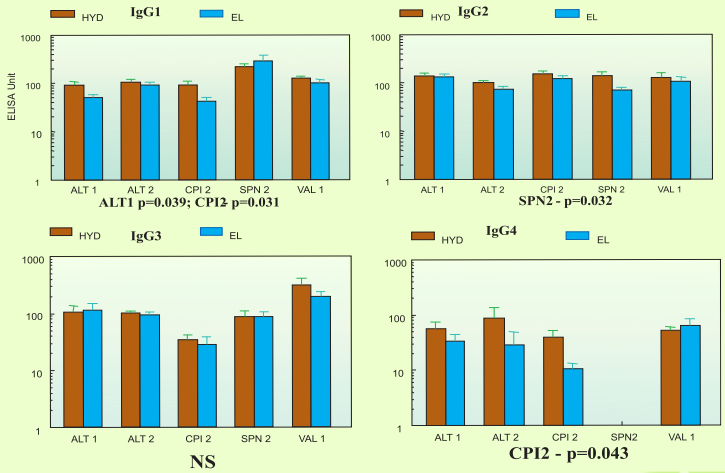
Background

Hydrocele and elephantiasis are two major clinical manifestations associated with chronic Bancroftian filariasis. Testicular hydrocele is a frequent clinical finding of lymphatic filariasis of men, yet genital involvement in women is rarely reported. In contrast elephantiasis occurs with equal frequency or more commonly in women than men. The more important question still remains as to why some individuals develop one form of pathology, where as others develop another form. Although hydrocele and lymphedema / elephantiasis are two diverse form of chronic manifestation, Separate immunological data distinguishing between patients with elephantiasis or hydrocele have not been readily available. The studies conducted so far on the immune response in chronic disease manifestation are taking both hydrocele and lymphedema as single group. Altered expression of cytokines as well as chemokine receptors is associated with the development of disease in some parasitic infections such as Schistosomiasis and Chaga's disease. In addition, chemokine receptors have been implicated in the pathogenesis of Rheumatoid arthritis, multiple sclerosis, atopic dermatitis and allergic asthma. However such immunological markers differentiating the two diverse forms of chronic disease manifestations for filariasis are not known. An attempt has been made to address these issues.

Work progress

Antibodies to the following recombinant proteins were quantified in hydrocele and lymphedema patients. Abundant Larval Transcript- 1) ALT-1; 2) ALT-2; 3) Serpin –2 (SPN-2); 4) Cystein Protease inhibitor –2 (CPI-2) and VAL. The first two molecules produced by infective larval stages while SPN-2 is synthesized only by microfilarial stages and CPI-2 is present on the surface of adult filarial worms. So far 22 cases of Hydrocele and 21 Lymphedema cases were studied with varing severity. The responses to ALT-1, ALT-2 and VAL were similar in both the groups. Isotype analysis (Fig-1) shows IgG1 antibody to ALT-1 and CPI-2, IgG2 antibody to SPN-2 and IgG4 antibody to CPI-2 were significantly high in hydrocele than lymphedema indicating that humoral response of the two diverse forms of chronic disease appear to be different from each other.

IgG Isotypes to 5 different recombinant filarial proteins in Hydrocele and Lymphoedema cases



On Going Studies

Principal Investigator :
Dr. B.Ravindran,
Co-Investigators :
Dr S.K.Kar, and Dr A. K. Satapathy
Collaborator :
Dr. Achim Hoerauf, Bernhard Nocht Institute of Tropical Medicine, Hamburg, Germany.
Funding : Extramural (Indo-German)
Starting Date : March 2003
Closing Date : December 2006

3. Post–DEC reactions in Human Bancroftian filariasis: An Immunobiological study in Orissa, India

Objectives:

1. To study the role of endosymbionts Wolbachia in mediating reactions after administration of DEC in infected human subjects.
2. To study the role of endosymbionts Wolbachia in mediation of inflammatory responses in human filariasis during acute disease episodes

Background

The project addressed the issue of side reactions observed in population after administration of single dose of Diethylcarbamazine citrate, the anti-filarial drug being currently used as MDA for control of lymphatic filariasis. It is generally believed to be associated with microfilarial density in the subject although empirical data for this is not available. Since post-DEC reactions often appear similar to LPS mediated inflammation and an endobacteria such as Wolbachia are known to reside in Mf, the current study was undertaken to investigate the association between Wolbachia density and post-DEC reactions. The underlying principle is that Wolbachia are susceptible to tetracyclines / doxycyclines and DEC mediated reactions should be preventable in Mf carriers by pre-treatment with the above antibiotics.

Work Progress

Two strategies were followed; first, to treat Mf carriers with doxycycline for different duration and then administer DEC to monitor reactions by both clinical observation as well as by observing indicators of host inflammatory response through measurement of inflammatory molecules viz. TNF-á, IL-6 and RANTES as well as Wolbachia density to analyze association between them; second, to treat cohorts of subjects, (with and without patent infection) with DEC and analyze the association as described above. The first approach is being pursued independent of the second approach; it is being done in three phases- in each phase 4 groups of Mf carriers are being used, one placebo and three treated with doxycycline for different durations (5, 10 and 21 days) and subsequently treated with DEC to monitor reactions. The following is the summary of results for the second approach: 1) Pre-treatment TNF-á levels were significantly more in Mf carriers (AS) and patients with chronic filarial disease (CH) (free of detectable infection) in comparison to subjects with cryptic infection (CR) (amicrofilaraemic with filarial antigenemia only), 2) Post–DEC reactions were significantly more in AS and CH cases as compared to CR cases and prevalence was comparable in the two (AS and CH) groups, 3) post DEC reactions were associated with significant elevation of TNF-á only in AS cases and not in CH cases, 4) conversely, significantly elevated levels of RANTES was observed only in CH cases and not in AS cases after administration of DEC, 5) plasma IL-6 levels were found to be significantly elevated in AS cases in comparison to CR and CH categories (pre drug administration) and after DEC administration, the levels of IL-6 decreased significantly in CR and CH cases and not in Mf carriers, and 6) plasma Wolbachia levels (as shown by real-time PCR) significantly decreased within 24 hrs after DEC consumption in CR and CH groups and not in the AS group.

Cumulative clinical scoring of post –DEC reactions in each case was monitored as shown in Table 1. Subjective reactions such as headache, body ache and nausea were not taken into account since they could be results of re-call bias.

Table 1

Clinical Scoring Criteria:	Scoring	24 hrs	48 hrs	72 hrs	Total
Fever — <102	1+				
Fever — >102	2+				
Vomitting	1+				
Lymphangitis	2+				
Orchitis- Tender/nontender	2+				
Adenitis- Tender	2+				
Adenitis- NonTender	1+				
* Scoring rate : 2 + and above is taken as Reactor					

Three cohorts of subjects' viz., a) Mf carriers b) people with cryptic infection and c) patients with chronic filarial disease were treated with 6 mg/Kg of DEC and post-DEC reactions were monitored as mentioned above. The results shown in Table 2 indicate that presence of Mf is needed to mediate reactions. There were no significant reactions encountered in CR and CH cases while 45% of Mf carriers clinically reacted on administration of DEC. More significantly, the reactions are dependent on density of circulating Mf as shown in Fig 1. Mean higher density of Wolbachia resulted in greater frequency of development of reactions following DEC administration.

Table 2: Post DEC Reaction

AS (Mf+), n=18		CR (CFA+), n= 18		CH (CFA-), n= 15	
Reactors No (%)	Non-Reactors No (%)	Reactors No (%)	Non-Reactors No (%)	Reactors No (%)	Non-Reactors No (%)
8(44.44)	10(55.56)	1(5.550	17(94.45)	2(13.33)	13(86.67)
Stat: *AS Vs. CR: p= 0.00178, AS Vs. CR/CH pool: p = 0.0096					

Fig 1

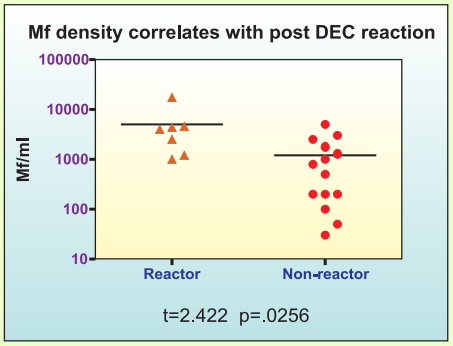


Fig 2

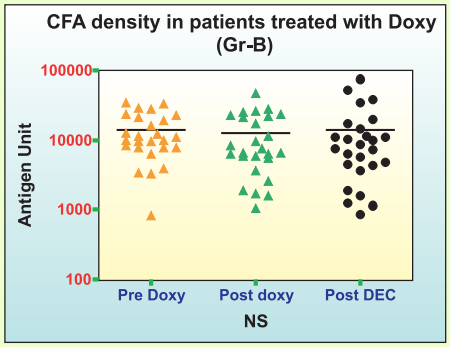


Fig 3

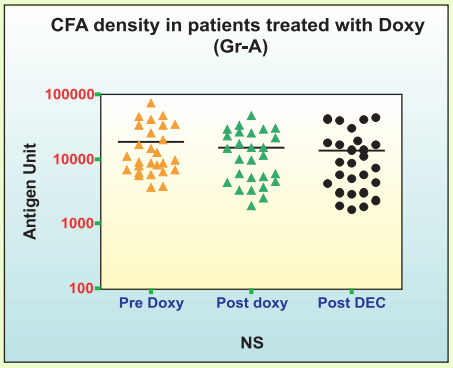


Fig 4

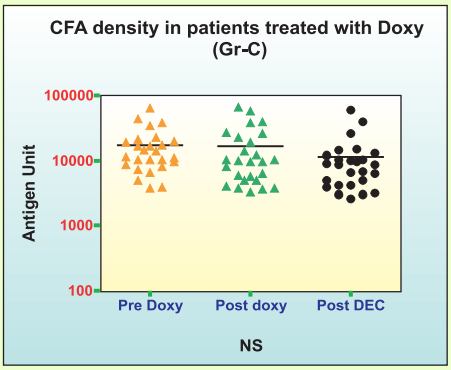


Fig 5

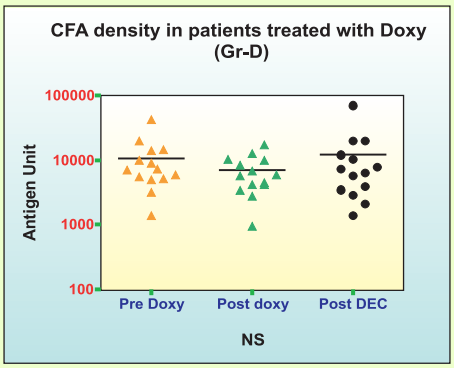
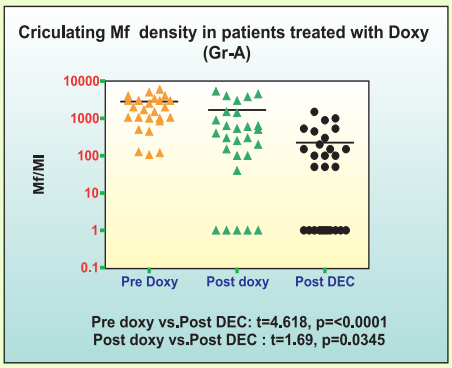


Fig 6



As mentioned earlier that 4 groups of Mf carriers were treated with Doxycycline for varying periods of duration – there was no significant change in circulating filarial antigen levels was observed among different groups as shown in Figs 2-5. The Mf density decreased significantly (Figs. 6-9) following Doxycycline treatment for 21 and 10 days. There was a significant decrease in Wolbachia levels in Mf as shown by real-time PCR (Figs 10-13). Treatment for 21 days and 10 days were effective while 5 days treatment did not significantly eliminate intra-cellular Wolbachia in Mf. The post-DEC reaction was associated with Wolbachia density (Fig 14) further emphasizing the importance of Wolbachia endosymbionts in mediating reactions.

Fig 7

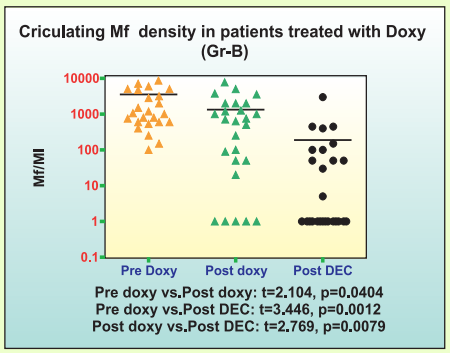


Fig 8

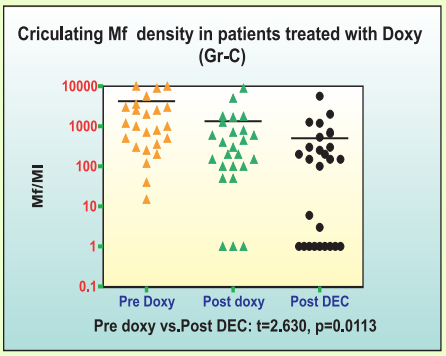


Fig 9

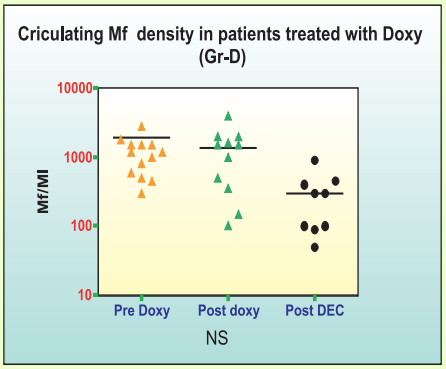


Fig 10

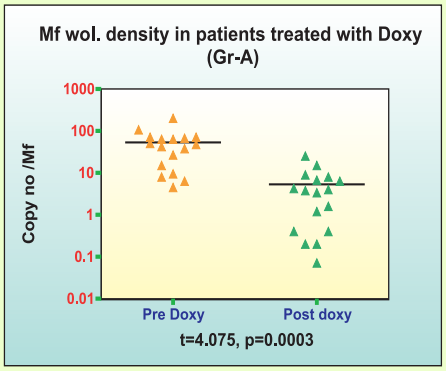


Fig 11

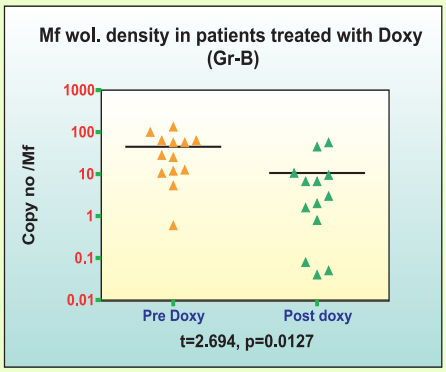


Fig 12

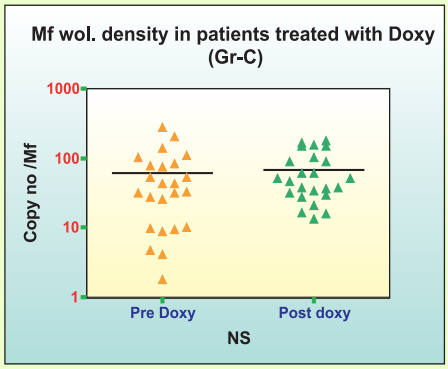


Fig 13

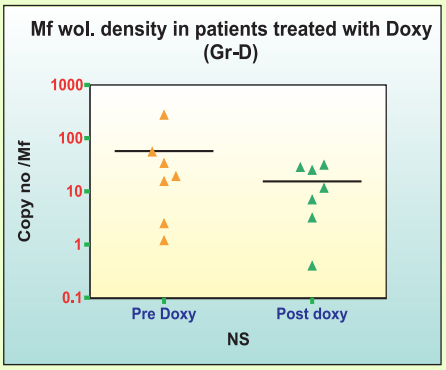
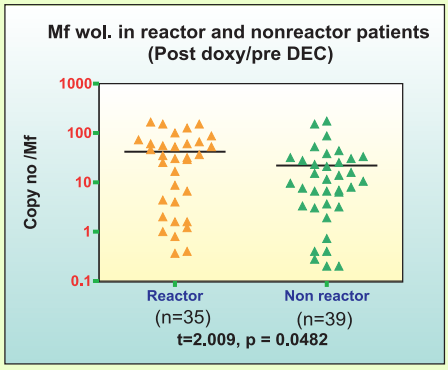


Fig 14



On Going Studies

The Toll Like Receptor (TLR) family is a group of pattern recognition receptors. TLR-4 is found on surface of mammalian monocytes, macrophages and neutrophils. It recognizes endotoxin (lipopolysaccharide present in cell wall of gram negative bacteria) as its ligand and results in induction of inflammatory molecules. Two known mutations in TLR4 gene (Asp299Gly, Thr399Ile) have been known. Filarial parasites contain an endosymbiont Wolbachia with LPS like molecules. Since post DEC reaction exhibited significant association with higher levels of wolbachia in mf, we assumed that TLR-4 mutation could potentially influence the post DEC reaction in human filariasis. To check this possibility of host genetic factors playing a role in post DEC reaction, the prevalence of TLR 4 genotypes were assessed in all the Doxy/DEC treated cases to correlate with side reactions (Table -3). TLR 4 mutation frequency was significantly more in non-reactors (37.70%) in comparison to reactors (17,07%). This indicates that TLR4 mutation could protect human hosts from side reactions associated with DEC treatment.

Table-3: Frequency of TLR4 mutation in Reactor and Non-reactor (21 days Doxy + single dose DEC)

	Reactor (n=41)	Non reactor (n=61)
TLR4 (299)	4 (9.75%)	15 (24.59%)
TLR4 (399)	6 (14.63%)	14 (22.95%)
TLR4	7 (17.07%) *	23 (37.70%) *

* Reactor Vs Non-Reactor P = 0.0283

Principal Investigator:
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Co-Investigator:
Dr A.K.Satapathy
Collaborator:
Dr S. Sharma, TIFR, Mumbai
Funding: Extramural (ICMR)
Starting date: March 2005
Closing date: August 2006

4. Genetic polymorphism of host molecules involved in immunity and immunoregulation in human filariasis

Objectives:

1. To type polymorphism of human genes that play a role in innate and/or adaptive immunity in human filariasis
2. To correlate genetic polymorphism with clinical and parasitological status in human filariasis

Work progress:

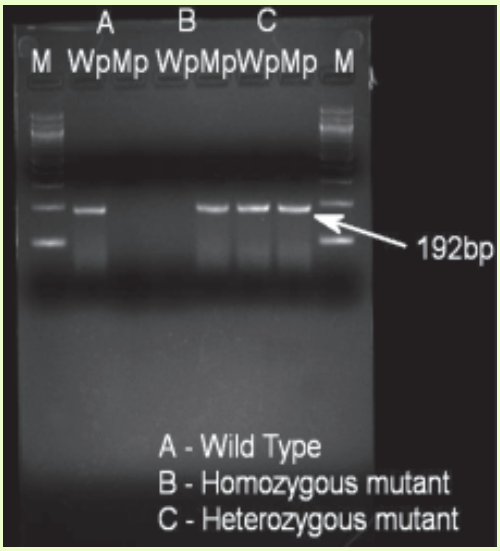
A spectrum of clinical manifestations can be observed in human communities with in lymphatic filariasis endemic areas. A large proportion of infected subjects are free of overt disease manifestations and most of the patients with chronic disease are free of current patent infection. Unlike several other infectious diseases, there is no clear consensus that patent infection would necessarily lead to development of chronic disease in human lymphatic filariasis. Longitudinal epidemiological studies indicated that development of chronic disease need not necessarily be a definitive consequence of patent infection in a given host conversely evidence exists for development of chronic disease with out prior

experience of patent filarial infection. These observations point towards the possibility of host genetic factors playing a significant role in the clinical as well as parasitological outcome in the exposed population. Experimental studies conducted in genetically well-defined strains of mice have clearly indicated a role for genetic factors both against infective larvae and microfilarial stages of filarial parasites.

The Toll Like Receptor: The TLR family is a group of pattern recognition receptors. TLR-4 is found on surface of mammalian monocytes, macrophages and neutrophils. It recognizes endotoxin (lipopolysaccharide present in cell wall of gram negative bacteria) as its ligand and results in induction of inflammatory molecules, which play a critical role in innate immunity against such bacterial infections. Two known mutations in TLR4 gene (Asp299Gly, Thr399Ile) have been known. Mutations of TLR-4 gene in human population results in quantitatively decreased LPS mediated signaling leading to enhance susceptibility to some of bacterial infections. Increased LPS mediated signaling could also be a contributing factor in some of the inflammatory conditions. Since development of overt chronic manifestation in filariasis has been associated with involvement of inflammatory reaction, and filarial parasites contain an endosymbiont Wolbachia with LPS like molecules, we assumed that TLR-4 mutation could potentially influence clinical as well as parasitological status in human filariasis and studied TLR-4 polymorphism in different clinical groups of human Bancroftian filariasis.

The TLR4 Asp299Gly mutation is caused by substitution of A to G in the coding region at position 896. We typed the mutation by PCR assays using the (wild type) Forward primer-5'-CTTAGACTACTACCTCGATGA-3', and (mutant) Forward primer 5'-CTTAGACTACTACCTCGATGG-3', (allele specific interaction at the 3' end) with a common anti-sense Reverse Primer- 5'-TAAGCCTTTTGAGAGATTTGA-3'. PCR assay was performed as follows: 12 cycles of 10 seconds at 950 C followed by 60 seconds at 65 0C, followed by 17 cycle of 10 seconds at 95 0C, 50 seconds at 60 0C and 30 seconds at 720 C. The final extension was 7 min at 720C. The final amplicon of PCR product is 192 bp. The amplified products were analyzed by electrophoresis on 2.5% low melting agarose gels stained with ethidium bromide. DNA from normal subjects will get amplified with 'wild type' primers - heterozygous mutations will be characterized by amplification with both 'wild type' as well as 'mutation' dependent primers. DNA from individuals homozygous for TLR4 mutations will get amplified only with 'mutation' dependent primers as shown in Fig 1.

Fig 1.



To check the possibility of host genetic factors playing a role in the clinical as well as parasitological outcome in the exposed population, the prevalence of

On Going Studies

TLR-4 Asp299Gly genotypes was assessed in about 450 subjects of filariasis with different clinical manifestations. The frequency of Asp299Gly TLR4 mutation did not significantly differ in individuals with various categories in comparison to endemic controls. Mutation frequency in male and females were analyzed separately. Patients with hydrocele were found to display lower frequencies than endemic controls; however the percentage of mutation was not statistically significant between different categories (Fig-2 and 3). These

Fig 2.

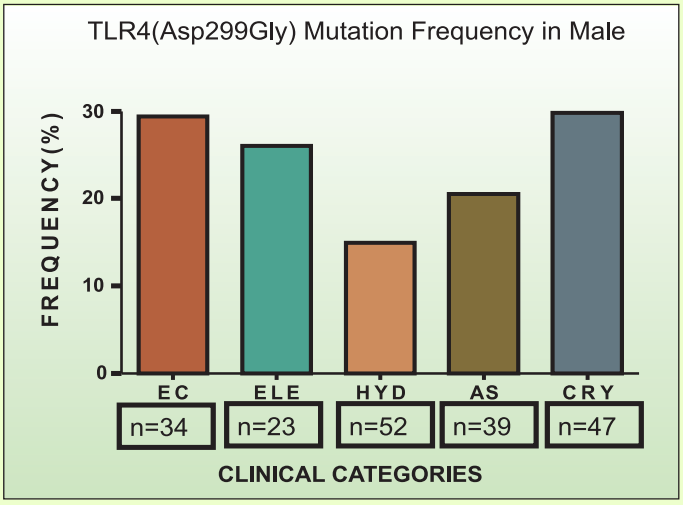
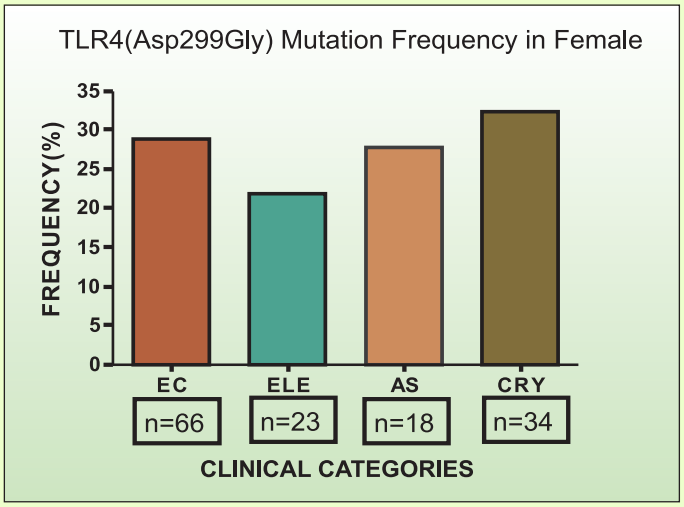


Fig 3.

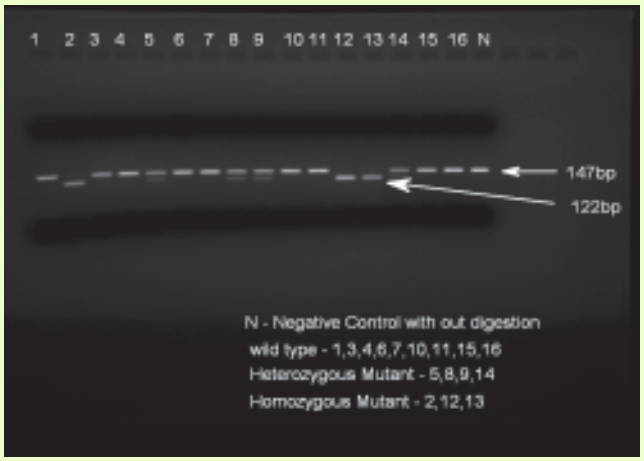


finding suggest that TLR4 mutation Asp299Gly in endemic population does not play a major role in determining the clinical or parasitological outcome in human lymphatic filariasis.

The second major TLR4 (Thr399Ile) mutation is caused by a C to T substitution at position 1196 in the coding region. This mutation was detected by PCR amplification of involved region with sense primer 5'-GCTGTTTTCAAAGTGATTTTGGGAGAA-3'

that contains a mismatch at position –3 from the 3' end of the primer (G instead of C) to create a Hinf I cleavage site when the mutant 1196T allele is amplified. Antisense primer was 5'-CACTCATTTGTTTCAAATTGGAATG-3'. Isolated DNA was amplified with these primers using a thermal cycler. The parameters were an initial denaturation at 95°C for 7 min, followed by 35 cycles: denaturation at 95°C for 30 s, annealing at 62°C for 30 s, and elongation at 72°C for 32 s. The final elongation was at 72°C for 5 min followed for a cooling to 4°C. The elongation product 147-bp fragments were digested at 37°C with Hinf I for 4 hrs resulting in fragments that either were cut into two fragments of 122-bp and 25-bp (allele T) or were not restricted (C allele) (Fig-4). These fragments were analyzed by electrophoresis on 2.5% agarose gels stained with ethidium bromide.

Fig 4.



We assessed the association between TLR4 (Thr399Ile) polymorphism and parasitological outcome/ chronic manifestations of filariasis in a large population base from filarial endemic areas. Fig-5 shows the frequency of TLR 4 (399) mutation in different clinical categories of filariasis in males. TLR-4 (399) mutation frequency was significantly more in endemic controls (28%) in comparison to patients with chronic disease (7.1%) in males. Similarly, the frequency mutation of TLR 4(399) in patients with active infection was significantly high in comparison to patients with chronic manifestations. This indicates that TLR-4 mutation (Thr399Ile) could protect human hosts from developing chronic filarial disease in males. Such an association of TLR-4 (399) mutation was not observed in females (Fig-6). Hydrocele, the most common chronic manifestation in filariasis is restricted to only males and the

Fig 5.

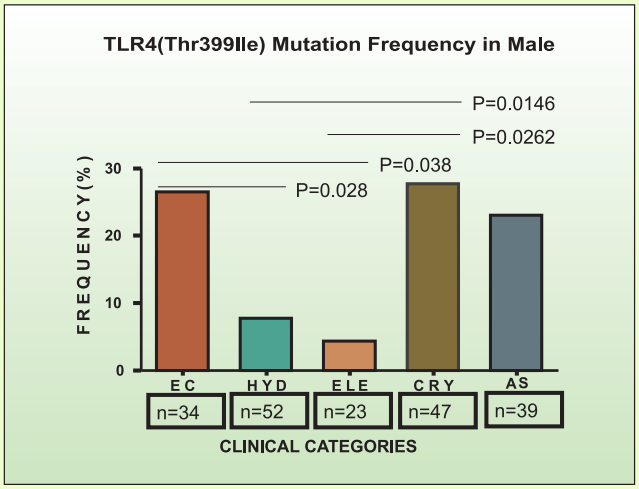
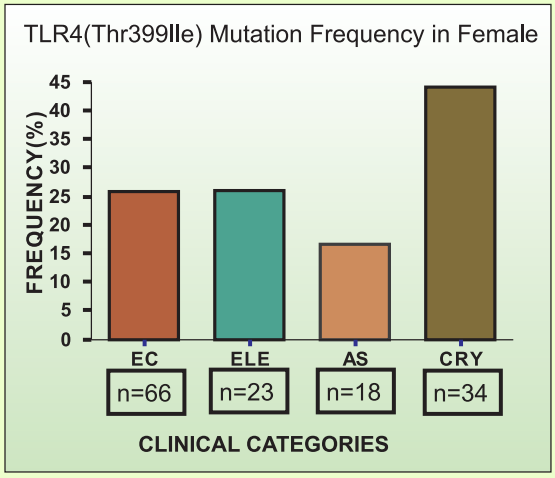


Fig 6.

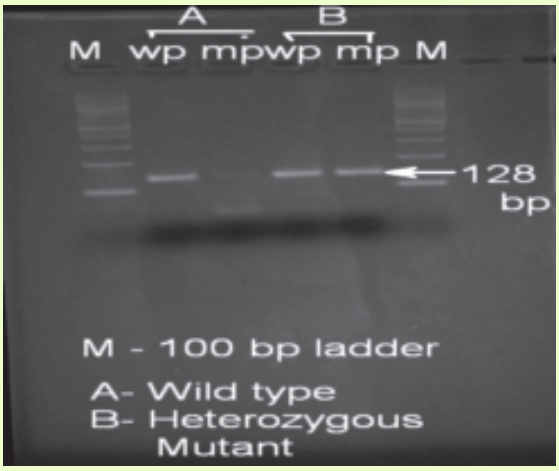


observed difference in elephantiasis (observed in both males and females) does suggest that this mutation plays a role only in males and not in females.

The Mannose-binding lectin: MBL is a C type collectin that participates in pathogen recognition, opsonization, phagocytosis and complement activation. A common set of structural polymorphism, known as the B, C and D alleles (together known as the O alleles) within a 15-bp span in exon 1 disrupt the function of MBL. The structural change in exon 1 results in a disruption of the binding domain. Structural variants have been associated with a wide range of infectious and autoimmune disorders. Low MBL levels are associated with susceptibility to certain infection in contrast high MBL levels might exacerbate the renal complications of diabetes. Since the development of chronic manifestation in filariasis has been associated with involvement of inflammatory reaction, we assumed that MBL mutation might have an association in the clinical as well as parasitological status in human filariasis.

Allelic specific PCR was performed to genotype codon 52(D) of MBL2 gene. Two sense primers, one for mutant 5'- TCTCCCTTGGTGCCCATGACG-3' and another for wild 5'- TCTCCCTTGGTGCCCATGACG-3' was chosen. To maintain specificity one non-complimentary nucleotide was placed at forth position from 3' end. The common antisense primer was 5'-GCAGCGTCTTACTCAGAAACTGTG-3'. The PCR was initiated by 12 cycle of 950C for 10 seconds and 620C for 10 second, followed by 27 cycle of 950 C for 30 seconds, 620 C for 30 second, 720C for 30 seconds and the final extension was at 720 C for 7 minutes. PCR products were resolved by electrophoresis in 3% agarose with ethidium bromide and visualized under UV light. The final elongation product is 128 bp (Fig-7).

Fig 7.



We analyzed the distribution of genetic polymorphism of **MBL 52 D** gene against parasitological and clinical outcomes following exposure to filarial parasites in a W. bancrofti area. About 400 individuals with different clinical