Chapter 4

DISCUSSION
4. Discussion

4.1- Present status in world and history of L.F

The world health organization has targeted lymphatic filariasis for elimination as a public health problem by the year 2020\textsuperscript{131,132}. India, which has the highest burden of L.F, has set a target for national elimination of L.F by the year 2015\textsuperscript{133}. Although annual mass administration of a single dose anti-filarial drugs to entire endemic communities for the control of transmission of lymphatic filariasis is being implementation many countries, including India, under which approximately 50 million cities are currently being covered annually\textsuperscript{134} morbidity arrangement\textsuperscript{135} aimed at the alleviation of the suffering of the individual patient has, in many countries only recently begun to be addressed.

\textit{Wuchereria bancrofti} or Filaria bancrofti was known from old times and its effect on producing elephantiasis was described by Persian scientists, Avicenna (981 - 1037 C.E.) And Ar-Razi (864 C.E.). In 1878 Patrick Manson found the role of Culex fatigans in the transmission of the infection. The infection with the parasite or filariasis bancrofti is prevalent in the Far East in China and southern Japan, Vietnam, Thailand, Malaysia, Philippines, Borneo, Celebes, New Guinea, Sumatra, Flores, Soemba, and Timor; In Asia in Burma and India; in Malagasy and Mauritius; in Yemen and Saudi Arabia; in West Africa and tropical East Africa including Egypt; in Europe, it was formerly found in Barcelona, Hungary, Yugoslavia, and Turkey; and along the coast in northern South America including northeast Brazil, the Guiana and Venezuela and in parts of Central America. The periodic form of \textit{W}.bancrofti infection has disappeared from the mainland of Australia, from North America. and from some of the islands in the Caribbean (WHO, 1967).
4.2 Screening and Diagnostic test

In Amravati only knotts test is used for screening of lymphatic filariasis, so the present study also included knotts test or thick smear test. It is cheap method and that why easily can be done in laboratory. But in some cases the persons who do not have lymphoedema but having symptoms of filariasis and that person if visited during day time to NFCU, Amravati, at that time then we have choose provocation test for them whether he is positive or not for infection.

4.3 DEC provocation test

The administration of DEC (Diethyl Carbamazine) in the daytime in areas where microfilaria (mf) of *Wuchereria bancrofti* exhibits nocturnal periodicity provokes mf to enter the peripheral blood. Specimens is taken is emphasized. The main practical uses of this provocation test are as a routine diagnosis tool, in estimating prevalence and density of mf in a community, as a preliminary screening method for assessing potential filaricides and in evaluating the parasitological response to mass chemotherapy.

4.4 People counseling

Lymphatic filariasis occurs in individuals of all ages and both sexes but prevails in those of low socioeconomic status\(^{136}\). As the chronic manifestations of lymphatic filariasis appear most frequently in life, clinical and pathological investigation have focused on the adult population. Our present study indicates that the prevalence of chronic forms of lymphatic filariasis is age dependent in both sexes, but generally higher in males than in females. This observation is in keeping with similar studies of chronic filariasis condition from South India\(^{137,138}\) and Varanasi, North India\(^{139,140}\). Elephantiasis of the leg was the most frequent clinical sign, while hydrocoel was comparatively rare.
In present study, around 60% of the interviewed people know the fact that the spread of this disease is caused by mosquito bite. Ramaiah et al (1996) observed that only 20% of the population know that the spread of the disease is the mosquito bite. In their study, the literacy was very low compared to the present study.

The present study observed a statistically significant association between the education level, knowledge regarding the spread of this disease, precaution and screening method, and treatment.

It was also noted from the observed caused that 20% believed that filariasis can be prevented while 80% uncertain about the disease whether it can be prevented or not.

In present study people were aware about the spread and prevention of the disease. This may be because of their education study conducted by Pani et al (2002) also observed were aware about the role of mosquito in the transmission of filariasis.

Both sexes infected but in male it is more than female. It has been also observed by counseling that in adult it is more prevalent than young and old age.

In lymphatic Filaria patient left leg is more affected than right leg, than both legs. Most of the people have pain in affected part while very few have no pain at all.

But though it is found more in male it means not that its prevalence is always more in male. Because its infection is not sex specific. Both sexes infected equally but as female hesitate to come front and hidden causes there is difference in observation.

In present study during survey it is found that one community ‘Bari’ was most affected than any other community and these communities people know about their travel history they said that they were previously working in dhobi (Pan tanda in Marathi’s) which are the breeding place for mosquito. So it may be possible that people infected from there. Only few people found to use the mosquito nets.
4.5 ELISA test

The study was conducted in Sri Lanka during the period 1982 to 1998. There were two components, firstly a cross-sectional study and then a longitudinal study. Microfilaraemia was determined by microscopic examination of night blood films. Microfilaraemia associated anti-filarial antibodies were determined by ELISA. Enzyme-linked immunosorbent assay (ELISA) detects filaria-specific immunoglobulin G4 antibodies in unconcentrated urine. The ELISA was positive in 87 of 91 (95.6%) urine samples collected from people with Wuchereria bancrofti microfilariae, antigen, or both. Of 298 urine samples collected in Thailand, Lao People's Democratic Republic, and Japan, where no human filariasis is known, 295 (99.0%) were negative by ELISA. Various intestinal nematode and fluke infections did not interfere with the ELISA. Urine samples with sodium azide could be kept at 37 degrees C for 4 weeks, and the time of urine collection did not influence ELISA results. This ELISA can be used to identify endemic foci of filariasis.

The filter paper-based ELISA, which detects circulating *W. bancrofti* antigen, was used successfully to determine post treatment filariasis endemicity in a very remote area in Madang Province \(^{41}\).

Bancroftian filariasis in three endemic communities in northeastern Tanzania, serum antibody responses were analyzed in selected individuals in relation to the long-term observations on microfilaraemia. Comparison of responses in three categories of adults (microfilaria [mf] positive at both surveys, mf positive at first but mf negative at the second survey, and mf negative at both surveys, respectively) indicated no significant differences between the mean levels of filarial-specific IgG1, IgG2, IgG3, IgG4, or IgE measured by ELISA. \(^{42,43}\)

The study includes the use of CFA estimation by ELISA being a sensitive and specific diagnostic tool for the evaluation of the true prevalence of the disease. The
high CFA prevalence in the study area necessitates early intervention measures to check its transmission.

4.6. Hematological analysis

A case presentation

An 11-year-old girl from Kozan a town near the city of Adna, in southern Turkey, pain had started in her both legs. The problem had started in her left ankle nine months earlier, and had extended to the upper part of the legs. As swelling progressed, the leg became warm and skin turned red. The girl had then developed an episode of fever and the severity of the symptoms in the left leg increased when this episode occurred. The same symptom arose in girl’s right leg a few months after the swelling was first noted in her left ankle.

The child lived with her family in a house with a garden, and the family raised livestock. There was no family history of filariasis she had never lived in any other region of Turkey. Physical examination revealed that body weight and height were within normal percentiles and body temperature was 36.1°C. Vital sign were normal. The skin on both legs was dry and hyperemic. Other symptoms were normal.

Laboratory investigations revealed haemoglobin 10.9 g/dl, white blood cell count 3500/mm³, total eosinophil count 900/mm³.

Examination of a peripheral blood smear showed nothing abnormal. Urinalysis and serum levels of blood urea nitrogen, creatinine, electrolytes, linear enzymes and Ig E were normal. There was nothing remarkable on echocardiography also.

In one study the patient was mildly anemic (haemoglobin, 120 g/l, with a normal erythrocyte sedimentation rate, there was lymphopenia but no eosinophilia. No microfilariae or acid-fast bacilli were detected in thick blood films taken at midnight or in a urine sample serological and intradermal test for Filaria were also negative.
In one study hematological parameter were within normal limits and there was no significant difference. 146

Hematological and parameters were within normal limits and did not change significantly between day (pre-therapy) and day post therapy in any of the three drug groups single dose albendazde, diethylcarbamazine citrate (DEC) or co administration of Wuchereria bancraefti in a symptomatic microfilaraemic volunteers in Pondicherry, south India a hospital based study 147.

In one study blood hematological temporarily rise from 44% to 50-55% in human males, all of which seems tolerable in healthy patients. (A packed cell volume $>\sim 55\%$ is the recommended clinical threshold requiring therapeutic blood letting during apparent polythemia (Robert a freitas jr , nanomedicine, volume II A; biocompatibility landes bioscience, georgetown, TX 2003)

So in present study the result within normal limit for haemoglobin concentration, total white blood cell count, differential count, absolute eosinophil count, crythrocyte sedimentation rate and packed cell volume, RBCs, MCV, RDW, TLC, MPV, MCH, WRCS, MLHC, ESR, MID, PVC found to in normal limits except few cases. It is an indication of inflammation. The ESR test is a simple test dating back to the ancient Greeks. A specific amount of diluted, unclotted blood is placed in a special narrow tube and left undisturbed for exactly one hour. The red cells settle towards the bottom of the tube, and the pale yellow liquid (plasma) rises to the top. After 60 minutes, measurements are taken of the distance the red cells traveled to settle at the bottom of the tube. Laboratories use two methods, the Westergren and the Wintrobe, each method produces slightly different results. Most laboratories use the Westergren method. Normally red cells don’t settle far toward the bottom of the tube. Many diseases make extra or abnormal proteins that cause the red cells to move close together, stack up, and form a column (rouleaux). In a group, red cells are heavier and fall faster.

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The faster they fall, the further they settle, and the higher the ESR.

In the present study the ESR found to be increased because of inflammation due to lymphatic filariasis.

4.7- Biochemical analysis

A case report

A 53-year-old Asian man with type 2 diabetes mellitus presented to the emergency department with acute onset of generalized muscle cramps. He reported a 2-month history of polydipsia, chyluria, and passing milky urine with blood lots. He had traveled widely throughout subtropical Asia. On examination he was normotensive with no lymphadenopathy, abdominal masses or oedema.

Urinalysis showed marked proteinuria, glycosuria and haematuria. The urine protein excretion rate was later confirmed to be 15.57 g/24 h (ref. internal (RI), 0.02-0.15 g/24 h). Urine triglyceride measurement and lipoprotein electrophoresis confirmed. The appearance of chylomicrons in the urine after an oral fat tolerance test.

Biochemical analysis of serum showed the following level: 121 mmol/L (RI, 134-146 mmol/L), creatine 46 ?mol/l (RI, 60-105 mol/l), glucose 91.7 mmol/l (RI, <5.5 mmol/l), total cholesterol 5.2 mmol/l (RI, <5.5 mmol/l). The patient had marked hypoproteinemia and hypoalbuminemia, with a total protein level of 39 g/l (RI, 63-86 g/l) and albumin level of 21 g/l (RI, 35-50 g/l) respectively.

Infecting Wister rats with Litomosodes carinii developed rodent model of filariasis. Liver function test, plasma protein concentration, and synthesis rates of liver-formed proteins were estimated in these rats at 63 and 90 days post infection. At 63 days post infection aspartate aminotransferase and alkaline phosphatase were significantly increased. Plasma total proteins and plasma albumin were in the normal range, however at 90 days post-infection all these parameters were affected, reflecting pro-
gressive live involvement.

Hypoalbuminemia at 90 days post infection did not appear to be decrease synthesis rate, indicating higher catabolism.

In one study biochemical analysis of serum showed the following level:

Creatine 46mmol/l (RI, 60-105?mmol/l)
Glucose 19.7mmol/l (RI, <5.5mmol/l)
Total cholesterol 5.2 mmol/l (RI, <5.5 mmol/l)

The patient had marked hypoproteinemia and hypoalbuminemia, with a total protein level 39g/l (RI, 63-80g/l) and albumin level of 21 g/l (RI, 35-50g/l) respectively. The patient was mildly anemic (haemoglobin level, 120g/l [RI, 130-170g/l], with a normal erythrocyte sedimentation rate.

Biochemical parameter were within normal limit and there was no significant difference.

Biochemical parameters were within normal limit and did not change significantly between day 0 (pre-therapy) and 7 post-therapy in any of the three drug group single dose albendazole, diethylcarbamazine citrate (DEC) or co administration of albendazole with DEC in the clearance of Wuchereria bancrofti in a symptomatic microfilaraemic volunteers in pondicherry, south India, a hospital based study.

In south canara district of Karnataka there was an individual finding since this region is endemic for filariasis. Raised erythrocyte sedimentation rate (ESR) in 104 (53.7%) raised blood urea in 21 (14.21%) and serum creatine in 14 (11.57%) case. Abnormal liver function test was observed in 28 patients, raised direct and indirect bilirubin in 28 (14.73%) and serum glutamic pyruvate (GPT) in 25 (13.51%) case.

In the present study, the mean bilirubin level was found to be 0.298182 mg/dl. This normal bilirubin value indicates that the population under study might not be
suffering from haemolytic blood disease.

However the serum urea and serum creatinine levels analysis of the LF diseased patients showed a normal range thus indicating that renal failure was a rare occurrence in the population studied.

The serum alkaline phosphatase activity measured in LF diseased patients showed considerable normal levels with a mean value of 169.2273 U/L. This indicates that the population under study may be suffering from hepatobiliary disorder. In the present study the result within normal limit for serum protein albumin and globulin.

In two patients the blood glucose found to be more than normal it make become of diabetic condition. In one of the patient the protein albumin was found to be less. It may be become of nutritional status.

Normal cholesterol level indicates that there is no effect of LF on coronary artery disease.

4.8 Phylogenetic study

Until recently, the data for most nematode phylogenies consisted of relatively few morphological characters derived primarily from light microscopy and often by individual effort of the lone taxonomist. Molecular phylogenetics, bioinformatics and digital communication technologies have substantially altered the dynamics of nematode systematics, creating conditions where collaborative strategies are much more productive than individual effort. This approach was exemplified by the analyses of Blaxter et al. (1998) based on small subunit (SSU) rDNA sequences of 53 nematode species.

Just seven years later, SSU rDNA sequences are available in public databases.
for more than 600 nematode species. In an effort to translate the implications of SSU rDNA sequences into classification, De Ley & Blaxter (2002, 2004) proposed a system based primarily on the molecular backbone of SSU phylogenies.

The molecular data published to date confirm the presence of three early nematode lineages, corresponding to the previously recognized subclasses Chromadoria, Dorylaimia and Enoplia (Lorenzen, 1981; Inglis, 1983). The exact order of appearance of these three lineages is not yet resolved. It seems likely that Enoplia appeared first, and it is even possible that Dorylaimia and/or Chromadoria could have originated within Enoplia. On the other hand, SSU data also allow for the possibility that Dorylaimia diverged first, which is an intriguing possibility because all known Dorylaimia are absent from marine habitats. A "Dorylaimia first" topology would therefore imply that the ancestor of all nematodes was perhaps a freshwater organism, and not a marine animal as more commonly assumed (De Ley & Blaxter, 2004).

Within Chromadoria, a number of clades have arisen in a series of successive bifurcations and radiations. These clades are classified as separate orders, including for example the predominantly marine Chromadorida and Desmodorida, as well as orders that have also diversified extensively in freshwater sediments (e.g., Monhysterida) and moist soils (e.g., Plectida). In addition, the chromadorian monophylum also includes the clade formerly ranked as class Secernentea, a hugely successful radiation of predominantly terrestrial nematodes. SSU phylogenies place this taxon at the crown of Chromadoria and as sister group to the order Plectida. For this reason, De Ley & Blaxter (2002, 2004) classified it instead as order Rhabditida, thereby greatly expanding the contents of this taxon compared to all previous systems.

The basic division of the nematode are between clade I, II, C & S, III, IV and V.

Clade I corresponds to the Dorylaimia (with the addition of the free living
Mononchida, insect-parasitic Mermithida and invertebrate parasitic Triplonchida) includes invertibrates, vertebrates, plant parasites and both marine (benthic) species as well as terrestrial ones. The human parasitic trichuris and trichinella are in this clade.

Clade II corresponds to the Enoplida (with the addition of the Triplonchida) and includes marine and plant parasitic species.

Clade C & S (Chromodorida and Secernentea) is a combination of the marine chromodoria and all the taxa previously grouped in the class Secernentea. Chromodorids are free living marine species, with a few terrestrial and freshwater representatives. The Secernentea can be further divided into three clades that grouped as animal parasitic, plant parasitic and free living species.

Clade III includes only animal parasites. The clade includes 4 traditional orders: The ascarides (Ascaris, Taxocora); the spirurids (filaria such as Brugia and Onchocerca); the Oxyurids (the pinworms such as Enterobius); the rhigonematids (millipede parasites).

The different orders are very close genetically. The Spirurida may be an invalid taxon.

Clade IV includes animal parasitic, plants and fungus parasitic and freeliving groups. Clade IV is much diverse genetically than clade III. (Dorris 1999).

Clade V links the free living, microbivorous Rabditida and Diplodasterida with vertebrate parasitic strongylida. *C. elegans* is a Rabditid. The nematode in this clade are very diverse genetically and analysis using SSU rDNA reveals significant structure.

In the present study total nineteen (19) 18SrRNA sequences were retrieved from GenBank. The sequences were aligned with Clustal X (reference). The multiple alignment reveals 458 number of polymorphic sites. The polymorphic site includes 41 numbers of transitional pairs and 24 numbers of transversional pairs. Similarly, equal num-
ber of indels (insertion deletions) are also found. Using Neihbor joining method (Saitou and Nci) Phylogenetic tree was constructed. To test the reliability of branched 1000 bootstrap replicates were generated. The tree shows four distinct clades I-IV (fig.3.35 pageno.130).

The Indian sequences cluster together to form a monophyletic clade, designated as clade-IV. Interestingly, two clusters have been identified within the Indian clade. It will be interesting to know how and when this cluster diverges from each other. The presence of these two diverged clusters further point towards geographical complexities. Similar divergent cluster has also been identified in other species like cattle (Baig .et al 2005). In the clade IV, the wucheria from Calicut, Wardha and Guntur region more tightly form a sister clade. The Brugia malai another close cousin nematode with known history of causing filariasis cluster with the sister clade formed by wucheria species.

Clade III includes Loa loa, which has worldwide distribution, most prominently found in western central Africa, form sister Clade with nematode Dipetalonema found in American reptile. Clade III also includes Onchocerca cervicalis, which has worldwide ditribution, found in horse, Setaria digitata found in cattle of United States and Canada and Acanthocheilonema viteae found in North American fishes.

Clade II includes Dirofilaria immittes, which is dog heartworm nematode and Onchoceridae found in horse shows close relation.

Clade I include Serratospiculum tenth causes parasitic filaria of falcons, Spirocerca found in dogs, Cyrnea found in turkeys and dogs, Physaloptera alata found in lizards, Ascarophis arctica found in fishes, and Neoascarophis found in deep sea fishes which is sister chromatid of Rhabdochona dedudata found in fishes.