REVIEW
OF
LITERATURE
2. REVIEW OF LITERATURE

Filaria is an important disease of mankind in the tropical and subtropical regions of Africa, Asia and America. Filaria was known as early as 600-250 BC (Lawrence, 1967). It is a group of human and animal infectious disease caused by nematode parasites belonging to the order filaridea. Parasites known to cause human infections belong to the genera Wuchereria, Brugia, Onchocerca, Dipetalonema, Mansonella, Loa and Dirofilaria. However only two genera Wuchereria and Brugia are responsible for human lymphatic filariasis (Sasa, 1976).

2.1 Life cycle of lymphatic filarial parasite

Lymphatic filarial parasite requires two hosts the definitive host-man or vertebrate animal (monkey, cat, rat and jirds) and the intermediate host- mosquito to complete its life cycle (Fig.1). The adult worms live in the lymphatic system and produce mf, which is an embryonic or prelarval stage produced viviparously. The microfilariae are ingested during the blood meal of the vector mosquito. Mf taken up by the mosquito exsheath in the gut and within an hour penetrate the midgut, migrate to the thoracic muscles. In the thoracic muscles the parasite becomes thicker and shorter as compared to the mf and is called the first stage larva (L1). At about 5th day the L1 moults to become the second stage larva (L2) which is slightly more active than the L1. By 9th - 10th day the L2 moults to become the infective stage (L3). This is a very active stage, which at maturity migrates mainly to the proboscis, but can also be seen in other parts of the mosquito. When the infected mosquito feeds on the human host, L3 larvae are deposited on the skin surface, during the process of probing and prior to the puncture of the host skin with the proboscis. After withdrawal of the proboscis the L3 migrates, gets into the wound and travels to the efferent lymphatics and subcapsular sinus. Approximately 9-10 days after entry the L3 moults to become the fourth stage larva (L4). The final moults occurs at approximately 35-40 days after the entry of L3 (Edeson and Laing, 1959). The L4 larva attains sexual maturity after 25-50 days.
Fig. 1 LIFE CYCLE OF LYMPHATIC FILARIAL PARASITE

A- adult
B- development of microfilaria
C- microfilaria
D- mosquito biting man and ingesting blood with microfilaria
E- L1 stage (sausage stage)
F- L2 stage
G- Female mosquitoes liberating L3 larva

d- Pupa

e- emerging adult

PARASITE IN MOSQUITO (INTERMEDIATE HOST)

PARASITE IN MAN (DEFINITE HOST)
2.2 Clinical signs

Inflammation of lymph nodes (adenolymphangitis) and lymphatic vessels particularly of the extremities is the main clinical feature of brugian filariasis. Pitting oedema and finally leading to elephantiasis are the clinical hallmarks of the chronic stage (WHO, 1992). Other manifestations of the disease include the asymptomatic microfilaraemia, obstructive consequences like lymphoedema, hydrocoele, occult infection called TPE and acute episodes of adenolymphangitis (ADL) (Ottesen and Nutman, 1992).

2.3 Experimental filariasis

Efforts to combat the widespread parasitic diseases have benefitted from the availability of the animal models. The ideal animal model for human parasitosis was aptly described as the one faithfully recapitulating the parasitic, immunologic and pathologic attributes of the disease, making available large quantities of parasitic material and leading to the detailed immunological analysis (Philip et al., 1988).

The only filarial parasite naturally occurring in man and easily transmissible to animals is the subperiodic form of B. malayi. The use of Brugia species in experimental studies are of special significance since they are the same as or closely related to those found naturally infecting in man (Sasa, 1976). Edeson et al. (1960) succeeded in transmitting B. pahangi from cats to cats and other animals. He reported that the prepatent period was 59 days in cats and 57 to 84 days in jirds (Meriones unguiculatus). Among the rodents, male Meriones unguiculatus and male M. natalensis were susceptible to B. malayi infection. Zaini et al. (1962) reported worms in the heart of hamsters infected with B. pahangi. Ramachandran and Pacheco (1965) found that in the early developmental stages B. pahangi worms were mainly in the skin, subcutaneous tissues and the caracass of cotton rats, whereas after maturity they were only in the heart and pulmonary artery. Ahmed (1967) reported B. malayi and B. pahangi worms mainly from lymph glands and testes of spleenectomized rats and cotton rats. Dissanaike and Paramananthan (1961) reported that B. buckleyi inhabits the heart and pulmonary arteries of the Ceylon Hare. Vincent et al. (1976) studied the
development of *B. malayi*, *B.pahangi* and *B. patei* in *Meriones unguiculatus* and they showed a characteristic tendency to localize within the heart and pulmonary arteries.

Ash and Riley (1970a,b) succeeded in transmitting both *B. pahangi* and *B. malayi* in the Mongolian jird from cats and dogs. The adult worms were found in heart, lungs and testes and more numbers were present in the latter than the former. The mode of distribution of mature *B. malayi* was investigated in cats by Ewert, 1971 and in rhesus monkeys and jirds by ElBihari and Ewert (1971). He found that the majority of worms remained in the lymph vessels distal to the popliteal node for approximately 6 weeks and thereafter, they moved to the heart and lungs.

McCall *et al.* (1973) reported that intraperitoneal infection of *Meriones unguiculatus* with *B. pahangi* lead to a high percent recovery of developing larvae or adult filariae and about 90-100% of the filariae that was recovered were located in the peritoneal cavity. Patranyi *et al.* (1975) found that in *M. natalensis* infected by *B. malayi*, 47% of female worms were found in lymphatic system and the prepatent period was 128.8 days. Whereas Sanger *et al.* (1981) had observed a prepatent period of 107 days and recovered majority of adult worms from heart and lungs and less number from the testes and lymphatics. Murthy *et al.* (1997) showed that the peritoneal environment of *M. natalensis* is not conducive to the development of *B. malayi*, which is due to its high macrophage activity in the peritoneum compared to that in the jirds, where large number of worms were recovered through intra peritoneal infection (Mak *et al.*, 1994). Tyagi *et al.* (1998) reported that *B. malayi* in immunosuppressed (cortisone) *M. coucha* showed prepatent period of 90.7 days and the adult worm recovery was also higher as compared to the controls.

2.4 Changes in host physiology in relation to parasitic infection

The last few decades have seen great advances in the parasite response and host defence, especially on the free radical and the antioxidant defence mechanisms. Macrophages, neutrophils and eosinophils release superoxide radicals as a host defense mechanism to kill the invading parasites (Bannister and Bannister, 1985). Electron microscopic and phase contrast microscopic studies have shown that eosinophils damaged
the tegument by the release of their granules on the surface of the parasite (Ackerman et al., 1981). Initially it was believed that the membrane basic proteins were the most powerful toxic component of eosinophils responsible for the killing of the parasite. But later, it was shown that the eosinophil cationic protein is more toxic than membrane basic proteins \textit{in vitro}. The ability of eosinophils to kill the parasite \textit{O. volvulus} (Green et al., 1981) and \textit{B. malayi} (Sim et al., 1982) \textit{in vitro} had been well demonstrated.

Batra et al. (1989) studied the status of superoxide dismutase, catalase, xanthine oxidase and lipid peroxidation in liver, lungs and spleen of \textit{M. natalensis} during \textit{D. viteae} infection. Xanthine oxidase and lipid peroxidation exhibited stimulation, while these antioxidant enzymes showed depression in liver and spleen. On the other hand in lungs, the antioxidant enzymes were elevated and this lowered the lipid peroxidation. But no parallel study had so far been reported regarding \textit{B. malayi} infection induced changes in \textit{M. natalensis}, especially antioxidant enzymes and its relation to DEC treatment, membrane bound enzymes and their damage, testicular damage and the histopathological changes.

2.5 Chemotherapy

The microfilaricidal efficacy of DEC was detected by Hewitt et al. (1947) in \textit{L. carinii} infected cotton rats and \textit{D. immitis} infected dogs and also for the treatment of human bancroftian filariasis (Santiago and Stevenson, 1947). Hawking et al. (1950) demonstrated that the drug had no direct action \textit{in vitro} on mf and adults of \textit{L. carinii}. But when administered to cotton rats infected with the parasite, mf rapidly decreased from circulating blood and were trapped in the sinusoids of liver, where they were destroyed by phagocytosis, such an action of DEC was interpreted as 'Opsonin-like". Subsequent studies on DEC showed strong activity against microfilaria of \textit{O. volvulus}, \textit{Loa loa}, \textit{W. bancrofti} and \textit{B. malayi} in man (Zahner and Schaus, 1993). Significant lethal action against adults of \textit{B. malayi} and \textit{B. pahangi} in cats (Edeson and Buckley, 1959) and against \textit{Wuchereria} and \textit{Brugia} in man (Chen, 1964) was reported. Kume et al. (1964) and Tulloch et al. (1970) reported that daily administration of DEC prevented maturation of \textit{Dirofilaria immitis} in dogs. Aubrey and Copeman (1972) reported that \textit{Dirofilaria immitis} does not mature in dogs when adequate doses of DEC was given for 3 successive days every 2 months. Ewert and
Emerson (1975) showed that DEC treatment of cats infected with *B. malayi* resulted in a reduction of living larvae at 2-10 mg/kg body weight. Ewert *et al.* (1983) reported that weekly administration of DEC was the most effective in *B. malayi* infected cats.

DEC has direct physiological effects on eosinophils (Mackenzie, 1980) and after DEC treatment eosinophils can be found degranulating on the surface of microfilaria (Gibson *et al*., 1976; Kephart, 1984; Racz, 1982). The mechanism by which filarial parasites are killed by DEC is not clearly understood. Most of the clearance occurs in the liver, spleen and lungs in association with a mixed inflammatory cell reaction with large number of eosinophils surrounding the dying mf (Woodruff, 1951). Ackerman *et al.* (1981) had demonstrated eosinophilia and elevated levels of membrane basic proteins in bancroftian filariasis after treatment with DEC. Lammler *et al.* (1975) reported that *M. natalensis* infected with *L. carinii* developed anaemia, an increased sedimentation rate and leucopenia. After treatment with DEC and a compound HOE 258V, there was a transient change towards normal in the peripheral blood values. Morikov *et al.* (1991) reported that the antioxidant properties of drugs including DEC citrate in various microsomal lipid peroxidation models: NADPH, ascorbate and CCl4 dependent. The most strong antioxidant of direct action turned out to be DEC citrate and dipyridamole. The dose most generally for treating bancroftian filariasis is 6 mg of DEC per kg body weight per day orally for 12 days. For brugian filariasis, the recommended doses range from 3 mg to 6 mg per kg of body weight per day upto a total dose of 36-72 mg of DEC per kg of body weight (WHO, 1992).

The anthelminthic agents such as macrocyclic lactone ivermectin, the amoscanate derivative CGP 6140 and the benzothiazole derivative CGP 20376 were investigated for their invitro modulatory effects on eosinophilic effector cells by Tischendorf *et al.* (1993). The results indicated that the reactive oxygen metabolites were produced at an increased rate at low doses of ivermectin and CGP 6140. The toxic potential of eosinophils includes the secretion of stored granular cationic proteins and the de novo generation of oxygen intermediates. The effect of the macrofilaricidal agent of 2, 2'-dicarbomethoxylamine -5,5'-dibenzimidazolyl ketone on the metabolism of reactive oxygen species in *A. vitaeae* and *M. natalensis* was studied by Batra *et al.* (1992). The host tissues i.e., subcutaneous and the adjoining muscle tissues exhibited elevated levels of antioxidants and GSH. It is shown that
the compound kills the filarial worm by paralysing the $\text{H}_2\text{O}_2$ detoxifying capacity without altering reactive oxygen species metabolism of the host.

2.6 Histopathological studies

The pathological changes associated with larvae and adults of $B. \text{pahangi}$ in cat and dog included hyperplasia of lymph follicles and reticular cells of the nodal stroma (Schacher and Sahyon, 1967). Mak, (1983) had observed inflammatory reactions with granuloma formation with the death and disintegration of the $W. \text{bancrofti}$ either due to treatment or other causes. The dead parasites were engulfed in cellular mass consisting of large number of eosinophils, lymphocytes and other mononuclear cells. He had also observed that, during heavy microfilaraemia, there was destruction of mf in the spleen, giving rise to acute and chronic inflammatory reactions. Lesions consisting of granulomatous reactions to dead and disintegrating larvae had been observed in $B. \text{malayi}$ infected jirds (Mak, 1983). The development of $B. \text{malayi}$, $B. \text{pahangi}$ and $B. \text{palei}$ in the pulmonary arteries of Meriones unguiculatus and the associated pathological changes were reported by Vincent et al. (1976). The major pathological changes included granulomas induced by larvae and adults, obstructive endarteritis and chronic interstitial inflammation. Malone et al. (1976) reported cellular infiltration of plasma cells and eosinophils in organs of hamsters infected with $B. \text{pahangi}$. Live and dead worms with eosinophils and mononuclear infiltration near the area were observed in testis. Haemosiderosis and gaint cells were observed in lungs. Crandell et al. (1982) reported lesions in organs of ferrets infected with $B. \text{malayi}$ and $B. \text{pahangi}$. Case et al. (1991) reported fragments of worms in kidney, spleen, liver, lungs, pulmonary blood vessel and lymphatics of ferrets infected with $B. \text{malayi}$. Eosinophilic abcesses, epithelioid and giant cell granulomas, perivascular cellular infiltration and pigmentation in organs were also noticed.