Pathology of lymphatic filariasis

Joon-Wah Mak

Abstract: Developing and adult worms of the human lymphatic filarial parasites (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*) are located mainly in the lymphatic system and occasionally in aberrant sites like subcutaneous and conjunctival cysts. Lymphatic pathology ranging from dilatation of lymphatic channels and lymphangiectasia are detected on ultrasonography in apparently healthy, amicrofilaraemic, but filarial antigen positive individuals in endemic areas. Microfilariae are distributed in various organs and may be associated with immune mediated pathology at these sites; tropical pulmonary eosinophilia is characterized by intense immune mediated destruction of microfilariae in the lung parenchyma. In the spleen and other sites, nodular granulomatous lesions can occur where microfilariae are trapped and destroyed. The finding of *Wolbachia* endosymbionts in all stages of lymphatic filarial parasites has provided new insight on the adverse reactions associated with anti-filarial chemotherapy. Inflammatory molecules mainly lipopolysaccharide (LPS)-like molecules released from endosymbionts on death of the parasites are largely responsible for the adverse reactions encountered during anti-filarial chemotherapy. Prenatal tolerance or sensitization to parasite derived molecules can immune-modulate and contribute to both pathology and susceptibility/resistance to infection. Pathological responses thus depend not only on exposure to filarial antigens/infection, but also on host-parasite-endosymbiont factors and to intervention with anti-filarial treatment. Treatment induced or host mediated death of parasites are associated with various grades of inflammatory response, in which eosinophils and LPS from endosymbionts play prominent roles, leading to death of the parasite, granulomatous formation, organization and fibrosis.

The non-human primate (*Presbytis* spp.) model of *Brugia malayi* developed for the tertiary screening of anti-filarial compounds has provided unique opportunities for the longitudinal study of the pathology associated with lymphatic filariasis. The pathology in this non-human primate model closely follows that seen in human lymphatic filarial infections and correlates with clinical evidence of lymphatic pathology as detected with ultrasonography. These studies also show that successful treatment as detected by loss of motility and calcification of worms on ultrasonography is associated with reversal of early dilatations of lymphatic channels.

IeJSME 2012: 6 (Suppl 1): S80-S86

Keywords: lymphatic filariasis, pathology, immunopathology, Brugia, Wuchereria

Introduction

A successful lymphatic filarial infection is the result of the dynamic interaction between the environment, parasite and host. It is intuitively expected that the interaction between the parasite and the host has a profound effect on the outcome of the infection. Like most helminthic infections, filariasis usually presents as a relatively prolonged or chronic infection. This is unlike protozoan infections where the process can take a fulminant course as in severe malaria leading to fatal cerebral complications, without benefiting the pathogen. The host-parasite relationship is presumably dynamic; the host defence systems, mainly immunological, constantly produces both cellular and secretary molecules in an attempt to kill, expel, or at least contain the parasite, whilst the parasite mounts evasive mechanisms to continue its survival and production of the appropriate stage for transmission. This dynamic host-parasitic interaction in human lymphatic filariasis may result in chronic infection associated with a wide clinical spectrum, unless this relationship is terminated through successful therapeutic intervention or host defence including immunological responses. An interesting concept in relation to this is that the host immune response against parasites must be achieved without loss of effective responses to other invading pathogens.[1] Invading pathogens may include those transported within the parasites and in this context all lymphatic filarial parasites carry *Wolbachia* endosymbionts.

For Correspondence:

Professor Mak Joon Wah, Division of Pathology, Faculty of Medicine and Health & School of Postgraduate Studies and Research, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, MALAYSIA

Email: joonwah_mak@imu.edu.my

Conceptually most helminthic infections that depend on a particular developmental stage in the host for transmission and propagation would not cause death of the host, at least until the particular stage of the parasite involved has developed to appropriate numbers for transmission. In contrast, the host would constantly try to kill and rid itself of the parasite or if this is unsuccessful, to minimise the resultant pathology. Thus in any helminthic infection, pathogenesis is not only directly due to the parasite but could result from host responses to the infection. Although it has been shown that T_H2-type immune responses are involved in expulsion of *Heligmosomoides polygyrus* in mice and that control of pathological inflammation in *Schistosoma mansoni* infection is through down regulation of T_H1-type response[1], it is unsure whether these play similar roles in lymphatic filariasis.

Not all successful filarial infections result in patent microfilaraemia. Filarial prevalence studies based on night blood surveys for microfilaraemia may not detect cryptic infections, and as high as 14.5% of those without microfilaraemia were positive by circulating antigen assay.[2] A review of published epidemiological studies using antigen and ultrasound examination now show that in endemic areas of filariasis, as high as a third of the infection was first acquired before age 5 years but the clinical presentation seen in adults was mostly presented after puberty.[3]

Pathogenesis

The lymph nodes and the lymphatic vessels are the primary sites where the developing and adult worms of *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* locate. Thus these are the sites where pathology and the pathological processes associated with the developing and adult worms are expected to be most intense. Recently, based on evidence from *in vitro* and animal models, it has been postulated that lymphagiectasia and remodelling of the lymphatics mediated by secretary and excretory products of these lymphatic filarial parasites may precede lymphedema.[4] This is also seen in lymph node biopsies of bancroftian filariasis patients (Fig. 1). Live worms and even those that are producing microfilariae do not evoke any obvious inflammatory response in the infected. Although dilatation of the lymphatic channels and lymphangiectasia are present early in the infection, the parasite, endosymbiont or host factors involved in the process have yet to be defined.

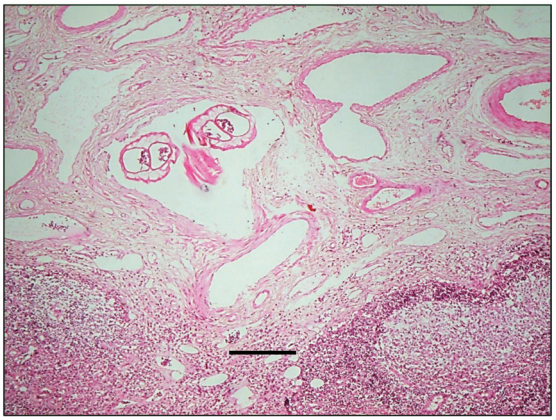

Fig. 1: Female *Wuchereria bancrofti* worm section in dilated lymphatic channels showing lymphangiectasia. Minimal to no inflammatory response seen in the peri-lymphatic or intralymphatic sites or against the worm; note the presence of a lymph thrombus (T). H&E stain; Mag. 40x; Bar: 200 μm.

A similar situation is seen in experimental infection of the primate model *Presbytis cristata* with subperiodic *Brugia malayi*. This experimental model of lymphatic filariasis developed for the tertiary screening of potential anti-filarial compounds under funding from the WHO/UNDP/TDR Special Programme for Research and Training in Tropical Diseases, has been extremely valuable in providing insight on pathological changes in the early stages of infection and that associated with anti-filarial treatment.[5] Infective larvae when inoculated subcutaneously into these primates were found mainly in the lymphatic system, especially in the lymph nodes and associated lymphatics. Developing and adult worms were found in dilated afferent lymphatics but without any evidence of inflammatory response in the

peri-lymphatic and intra-lymphatic tissues, or against the developing worms (Fig.2).

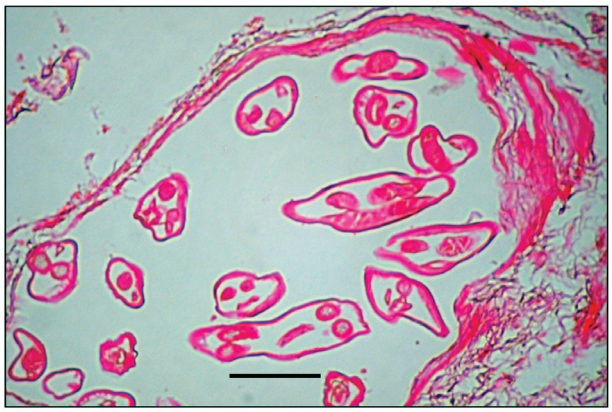

Fig. 2: Sections of subperiodic *Brugia malayi* developing worms (59 days old) in the afferent lymphatic of inguinal lymph node in an experimentally infected *Presbytis cristata* (No. 973); H&E stain; Mag. 199x; Bar: 100 μm.

Scrotal ultrasound examination has been shown to be useful in detecting the presence of motile live worms, dilated lymphatics, hydroceles and subclinical hydroceles in those with asymptomatic microfilaraemia, clinical filariasis, and those with positive antigenaemia.[6] Successful treatment with diethylcarbamazine was associated with loss of motility in worms and new scrotal calcifications.

Other Pathological Lesions

The pathological findings are mainly associated with the lymphatic system where the adult and developing parasites are normally found, as well as in the extra-lymphatic tissues where these are occasionally seen. Microfilariae are distributed more extensively as they are found in circulation and pathological changes can be seen in sites where they aggregate, are trapped, or destroyed due to various host responses. Splenic lesions in experimental infections in non-human primate range from mild to severe nodular enlargement[5] associated with granulomatous reactions (Figs. 3-5).

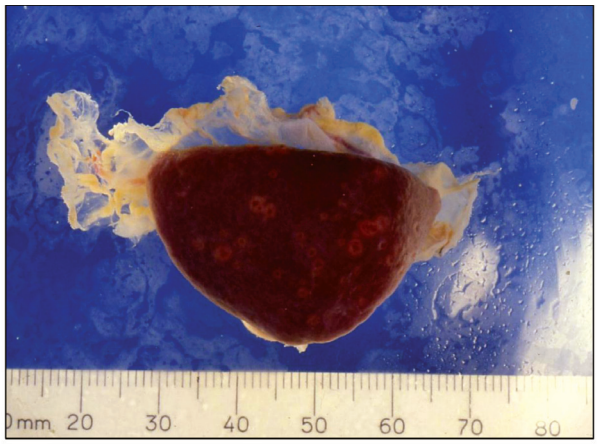

Fig. 3: Spleen showing whitish plaque-like raised lesions in enlarged spleen of *Presbytis melalophos* (No. 863) experimentally infected with *Brugia malayi*.

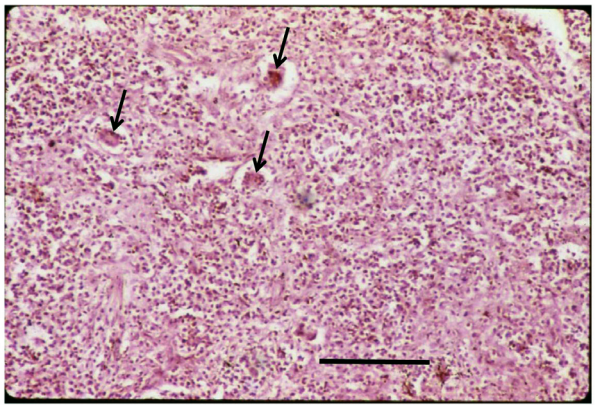

Fig. 4: Granulomatous lesions with giant cells (arrows), round cells and fibrosis in spleen of *Presbytis melalophos* (No. 863) experimentally infected with *Brugia malayi*. H&E Stain; Mag. 100x; Bar: 100 μm.

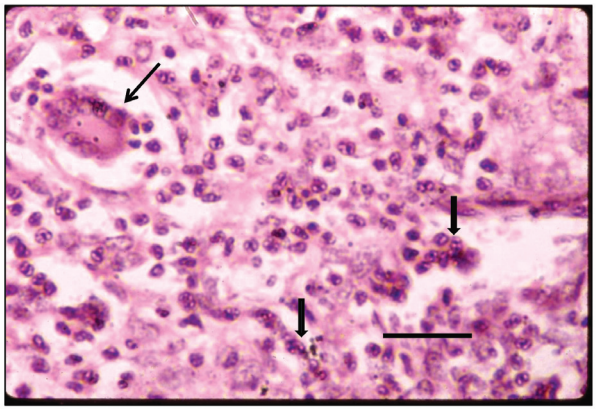

Fig. 5: Higher magnification of granulomatous lesion in spleen of *Presbytis melalophos* (No. 863) experimentally infected with *Brugia malayi*. Foreign body giant cells (thin arrow) and microfilariae remnants (thick arrows) seen. H&E Stain; Mag. 400x; Bar: 20 μm.

Host-Parasite Interactions

Older reviews of lymphatic filariasis reported that females in their reproductive years had lower microfilaria rates and densities compared to males of similar age groups[7] and that the observed higher antibody levels against adult worm antigens in them may reflect increased resistance to infection.

Genetic associations with filarial disease have been studied in various populations. The frequency of HLA-B15 was found to be significantly increased in those with elephantiasis compared to controls amongst Sri Lankans and Southern Indians.[8] However, another study amongst a Papua New Guinea population in a bancroftian filariasis endemic area showed no association of infection status (microfilaraemia or antigenaemia), lymphedema (limbs) or hydrocele with chitotrioxsidae genotype (*CHIT1*), toll-like receptor-2 (TLR2), and toll-like receptor-4 genotypes.[9] This is in contrast to the findings in Southern Indians where an association between the homozygous variant of the *CHIT1* genotype and susceptibility to infection (microfilaraemia and lymphatic dysfunction) was reported.[10] Obviously, the genetic association between infection and pathology in lymphatic filariasis needs further studies.

It has been shown that in endemic areas of filariasis, acquired immune tolerance in utero increased by about 13 times the risk of infection in children followed up to age 7 years compared to those sensitized infants. When compared to unexposed infants there was about 5-fold increased risk of infection.[11] Thus prenatal exposure to filarial antigens can affect infection outcomes and this may have implications in immune mediated pathology. However, as pointed out in a recent review some helminths regulate the functional immunity of the host through regulation of the immune system and suppression of parasite-specific immunity to ensure survival; this benefits the host by limiting immunopathology.[12]

Immunopathology

Other less known heterogeneous pathological lesions are associated with bancroftian filariasis.[13] These lesions are associated with microfilariae and other stages, parasite products, or immune complexes. These extra-lymphatic lesions may be explained by direct damage of microfilariae or their products or deposition of immune complexes (arthritis and renal damage). Pulmonary dysfunction and interstitial tissue damage associated with filarial tropical pulmonary eosinophilia (TPE) are believed to be due to pathological hyper-responsiveness to microfilariae. While the lung lesions can result in dramatic clinical presentations, similar immune mediated lesions in the spleen and other organs may be asymptomatic and easily missed.

Therapeutic Interactions and Consequences

Adverse reactions during anti-filarial treatment with diethylcarbamazine citrate (DEC) or ivermectin in those with high microfilaraemia have been attributed to the release of the *Wolbachia* endosymbionts on death of the parasite (microfilariae and adult worms) and this is supported by the detection of *Wolbachia* DNA by PCR and immunogold labelled bacteria by electron microscopy in the plasma of patients 4-48 h after DEC

treatment.[14] The inflammatory response elicited is attributed to the lipopolysaccharide-like molecules from the *Wolbachia* and the associated interleukin 6 and 10 and tumour-necrosis factor (TNF) α responses.

The importance of *Wolbachia* products contributing to pathology is elegantly shown in the study by Debrah *et al.* (2006) in which they found that treatment with the antibiotic doxycycline at 200 mg daily for six weeks in bancroftian filariasis patients effectively reduced the plasma levels of lymphangiogenic vascular endothelial growth factor-c (VEGF-C) and the associated soluble endothelial growth factor receptor-3 (sVEGFR-3) and that this decline preceded reduction in dilatation of supratesticular lymphatic vessels and lymphedema.[15]

It has been shown that rifampicin alone or in combination with doxycycline given over 14 days is more effective than doxycycline alone in inhibiting worm development, worm viability, worm load and embryogenesis in oral treatment of the murine filarial worm *Litomosoides sigmodont*.[16] Similarly, treatment with doxycycline for 21 days followed by a single DEC treatment reduced *Wolbachia* in bancroftian filariasis by 95%, and reduced the dilatation of scrotal lymph vessels.[17]

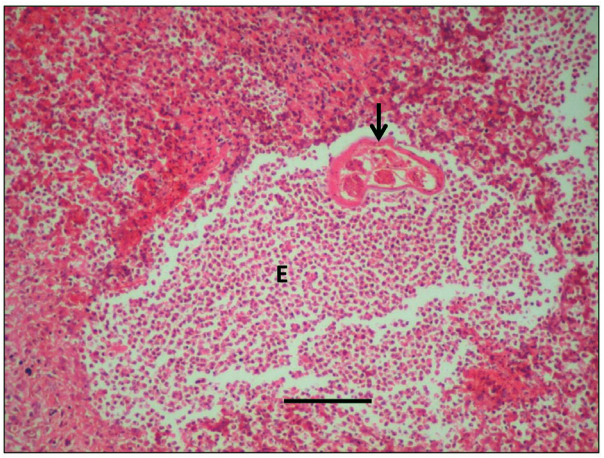

Fig. 6: *Wuchereria bancrofti* adult worm section (arrow) in lymph node surrounded by pools of eosinophils (E) and other inflammatory cells in early stages of host reaction against parasite. H&E stain; Mag. 100x; Bar: 100 μm.

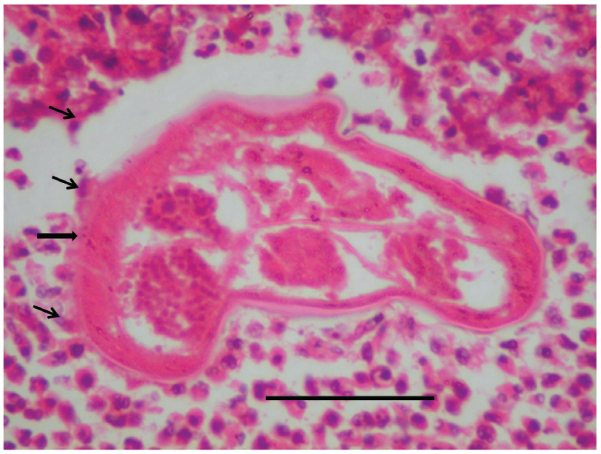

Fig. 7: Higher magnification of Fig. 6 showing eosinophils (thin arrows) attached to cuticle of *Wuchereria bancrofti* worm section. Note that the cuticle where eosinophils are attached has lost its integrity (thick arrow) and is covered by a layer of eosinophilic material. H&E stain; Mag. 400x; Bar: 50 μm.

The clinical presentation to adverse drug reactions is commonly seen within a few days of treatment with DEC especially in those with high microfilaraemia. These reactions include fever, body aches, myalgia, itchiness, and athralgia within the first 24-48 hours in brugian filariasis patients. This may be followed within about a week after start of treatment, with lymph adenitis, inflammatory nodules in the lymphatic vessels, followed by abscess formation. In endemic communities scarring due to healing of these suppurating lesions resulting from treatment or immune mediated death of worms, is commonly seen. Histological sections of these inflammatory nodules show inflammatory cells surrounding the parasites with a preponderance of eosinophils in the early stages. Eosinophils are seen surrounding (Fig. 6) and attached to the worm cuticle (Fig. 7); there is in the later stages marshalling of chronic inflammatory cells and giant cells surrounding the parasite remnants in eosinophilic necrotic material (Figs. 8 & 9), followed by fibrosis (Fig. 10). It is now known that these responses are cellular and immune mediated and directed towards both parasite antigens as

well as the LPS released from *Wolbachia* endosymbionts on death of the worms.

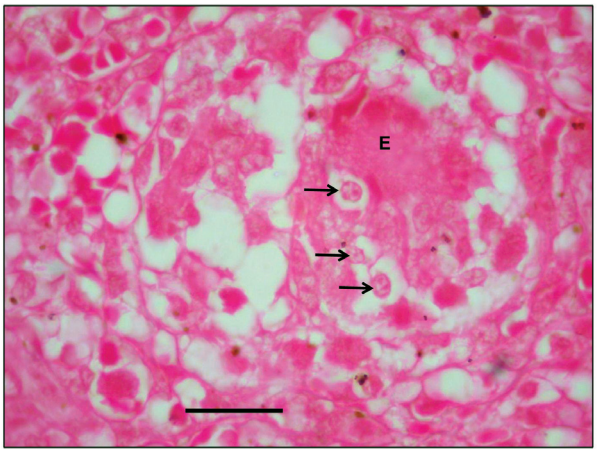

Fig. 8: Section of splenic granuloma in *Presbytis cristata* (No. 245) experimentally infected with *Brugia malayi*. Microfilariae cross sections (arrows) enmeshed in eosinophic material (E). H & E stain; Mag: 1000x; Bar: 10 μm.

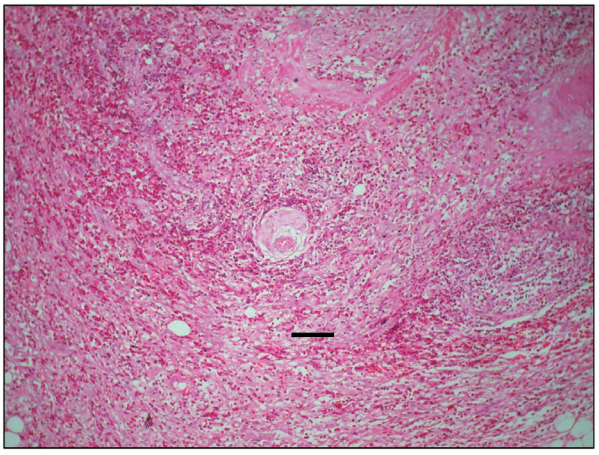

Fig. 9: *Wuchereria bancrofti* cross section in lymph node surrounded by chronic inflammatory cells, epitheloid cells and eosinophils. Fibrosis is evident and organisation of the dead worm is beginning. H&E stain; Mag. 100x; Bar: 100 μm.

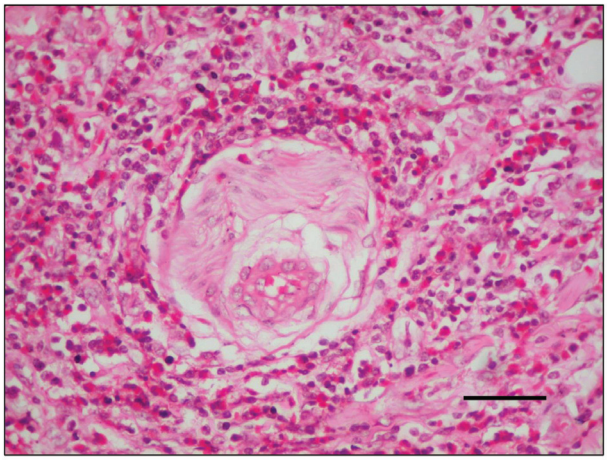

Fig. 10: Higher magnification of section shown in Fig. 8 showing fibrosis of dead *Wuchereria bancrofti* worm. H&E stain; Mag. 400x; Scale: 50 μm.

Conclusion

It is important to note that the pathological presentation of lymphatic filariasis is very much dependent on the duration of the infection, particularly the stage of infection, and whether treatment has been given. Current knowledge on the role of *Wolbachia* endosymbionts and their inflammatory molecules that are released especially when they are destroyed, have helped us to understand the pathological lesions encountered in the clinical spectrum of lymphatic filariasis. The pathological changes seen in the primate model of brugian filariasis is similar to that in human bancroftian and brugian filariasis. Experimental infection in this model, and ultrasound examination of the lymphatic system in filariasis patients show that in the early stages of the disease when the developing or mature worm is intact and healthy, the only pathology is dilatation of the lymphatics and lymphangiectasia. These lymphatic changes are believed to be due to parasite factors and products. The recurrent inflammatory responses in some patients may be due to inflammatory molecules derived from *Wolbachia* endosymbionts, especially

when these are released when filarial parasites die from host immune responses or from anti-filarial therapy. It is important to note that as the developing and adult parasites are mainly located in the lymphatic system, pathology associated with these stages are seen in these sites. Pathological changes may be due to circulating microfilariae and inflammatory lesions are seen in tissues where they are trapped and destroyed, as is seen in the pulmonary lesions of tropical pulmonary eosinophilia and in nodular lesions in the spleen.

Acknowledgements

Studies on experimental infection of *Presbytis* with *Brugia malayi* were funded by UNDP/World Bank/ TDR Special Programme for Research and Training in Tropical Diseases. Specimens of bancroftian filariasis were generously supplied by Dr Dreyer G of Brazil.

REFERENCES

1. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infections. Nature Reviews 2007;7:975-987.
2. Das D, Kumar S, Sahoo PK, Dash AP. A survey of bancroftian filariasis for microfilariae and circulating antigenaemia in two villages of Madhya Pradesh. Indian J Med Res 2005; 121: 771-775.
3. Witt C, Ottesen EA. Lymphatic filariasis: an infection of childhood. Trop Med Hlth 2001; 6(8): 582-606.
4. Benuru Sasisekhar, Nutman Thomas B. Lymphatics in human lymphatic filariasis: *in vitro* models of parasite-induced lymphatic remodelling. Lymphatic Res and Biology 2009; 7(4): 215-219.
5. Mak JW, Sim BKL, Yen PKF. Experimental infection of the leaf-monkey, *Presbytis melalophos* with subperiodic *Brugia malayi*. Trop Biomed 1984; 1: 21-27.
6. Faris R, Hussain O, El Sethouhy M, Ramey RMR, Weil GJ. Bancroftian filariasis in Egypt: visualization of adult worms and subclinical lymphatic pathology by scrotal ultrasound. Am J Trop Med Hyg 1998; 59: 864-967.
7. Brabin L. Sex differentials in susceptibility to lymphatic filariasis and implications for maternal child immunity. Epidemiol Infect 1990; 105: 335-353.
8. Chan SH, Dissanayake S, Mak JW, Ismail MM, Wee GB, Srinivasan N, Soo BH, Zaman V. HLA and filariasis in Sri Lankans and Indians. Southeast Asian J Trop Med Hyg 1984; 15: 281-286.
9. Hise AG, Hazlett FE, Bockarie MJ, Zimmerman PA, Tisch DJ, Kazura JW. Polymorphisms of innate immunity genes and susceptibility to lymphatic filariasis. Genes and Immunity 2003; 4: 524-527.
10. Choi EH, Zimmerman PA, Forster CB, Zhu S, Kumaraswami V, Nutman TB, Chanock SJ. Genetic polymorphisms in molecules of innate immunity and susceptibility to infection with *Wuchereria bancrofti* in South India. Genes and Immunity 2001; 2: 248-253.
11. Malhotra I, Mungal PL, Wamachi AN, Tisch D, Kioko JM, Ouma JH, Muchiri E, Kazura JW, King CL. Prenatal T cell immunity to *Wuchereria bancrofti* and its effect on filarial immunity and infection susceptibility during childhood. J Inf Dis 2006; 193: 1005-1013.
12. Yazdanbakhsh M, Sacks DL. Why does immunity to parasites take so long to develop? Nature Reviews (Immunology) 2010; 10: 80-81.
13. Dreyer G, Dreyer P, Piessens WF. Extralymphatic disease due to bancroftian filariasis. Brazilian J Med Biol Res 1999; 32: 1467-1472.
14. Cross HF, Haarbrink M, Egerton G, Yazdanbakhsh, Taylor MJ. Severe reactions to filarial chemotherapy and release of *Wolbachia* endosymbionts into blood. Lancet 2001; 358: 1873-1875.
15. Debrah AY, Mand S, Specht S, Marfo-Debrekyei Y, Batsa L, Pfarr K, Larbi J, Lawson B, Taylor M, Adjei O, Hoerauf A. Doxycycline reduces plasma VEGF-C/sVEGRR-3 and improves pathology in lymphatic filariasis. PLoS Pathogens 2006; 2: 0829-0843.
16. Volkmann L, Fischer K, Taylor M, Hoerauf A. Antibiotic therapy in murine filariasis (*Litomosides sigmodontis*): comparative effects of doxycycline and rifampicin on *Wolbachia* and filarial viability, Trop Med Intl Hlth 2003; 8: 392-401.
17. Mand S, Pfarr K, Sahoo PK, Satapathy AK, Specht S, Klarmann U, Debrah AY, Ravindran B, Hoerauf U. Macrofilaricidal activity and amelioration of lymphatic pathology in bancroftian filariasis after 3 weeks of doxycycline followed by single–dose diethylcarbamazine. Am J Trop Med Hyg 2009; 81: 702-711.