

# ESTIMATION OF ASO TITER AS AN INDICATOR OF STREPTOCOCCAL INFECTION PRECIPITATING ACUTE ADENOLYMPHANGITIS IN BRUGIAN LYMPHATIC FILARIASIS

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**Abstract.** Recurrent episodes of acute adenolymphangitis (ADL) are important clinical manifestations of lymphatic filariasis which contribute significantly to the progression of lymphedema. It is increasingly being recognized that secondary bacterial infections play an important role in the etiology of ADL. We examined the role of streptococcal infection as a precipitating factor of ADL in brugian filariasis, by determining the anti-streptolysin O (ASO) titers and by isolating the causative organism wherever possible. The study population consisted of 30 patients with filariasis related ADL (Group A), 30 patients with chronic filarial edema (Group B) and 60 age and sex matched healthy adults (Group C). ASO titer was estimated by the latex agglutination method at the time of entry into the study, at the 15th day and at 3,6 and 12 months. ASO titers were persistently elevated in 90% of patients in Group A and a portal of entry for bacterial infection was detected in all of these patients. In Group B only six patients had persistently elevated ASO titers. These patients had grade III lymphedema and three of them had monilial infections in the affected limb. In the control group none had persistently elevated ASO titers. The elevated ASO titers and the detection of a site of entry for bacteria in patients with ADL supports a streptococcal etiology for this condition.

## INTRODUCTION

Lymphatic filariasis is a major health problem with over 1 billion people living in areas endemic for filariasis and nearly 120 million with various forms of the disease. India, with over 22 million microfilaria carriers and 16 million clinical cases (of whom 6 million suffer from acute manifestations), has the largest number of cases in the world. Even though bancroftian filariasis is the most common form of the disease in India, *Brugia malayi* infection is common in parts of Kerala. In Alappuzha district one such pocket of *Brugia malayi* exists in Sherthallai taluk where the estimated prevalence of lymphatic filariasis was 9.9% of the general population (Sabesan *et al*, 1992).

It has been observed that the chronic forms of lymphatic filariasis are more common in the lower socio-economic groups of the population (Pani *et al*, 1989; Shenoy *et al*, 1995). A large proportion of these patients suffer from repeated and often inca-

pacitating attacks of acute adenolymphangitis (ADL), characterized by high fever and painful, tender and reddish swelling of the affected region and/or the draining lymph nodes.

Attacks of adenolymphangitis are an important feature of lymphatic filariasis. Although the disease produces gross deformities it is often only these episodes of ADL that force patients with lymphatic filariasis to seek medical attention. Recurrent attacks of ADL also hasten the progression of filarial edema resulting in elephantiasis. Surveys conducted in Pondicherry and Sherthallai estimated a frequency of 4.47 ADL episodes per year for bancroftian filariasis and 2.2 episodes for brugian filariasis. On an average, such attacks last for four days, which result in absence from work and thus economic loss (Pani *et al*, 1990). In Sherthallai, the economic loss due to brugian filariasis was estimated to be 1.6 lakhs mandays/year (Sabesan *et al*, 1992). It is therefore important to prevent and promptly treat these ADL attacks in patients with lymphatic filariasis.

It is increasingly being recognized that secondary bacterial infections play an important role in the etiology of acute attacks. We have previously

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shown that ADL attacks in patients with lymphatic filariasis are precipitated by secondary bacterial infection which gain entry through broken skin (Shenoy *et al*, 1995).

There is growing evidence to suggest that streptococci are the commonest organisms involved in the causation of these attacks. Studies in experimental animals (Ewert *et al*, 1980) and in patients with lymphatic filariasis (Olszewski and Jamal, 1994; Pani *et al*, 1995; Olszewski *et al*, 1997) have demonstrated these organisms in the skin and lymphatic fluid of patients with lymphedema. We have previously shown that 28 out of 65 patients with lymphatic filariasis had elevated ASO titers (> 200 IU) and that elevated titers (> 200 IU) were seen in 40% of patients in whom a portal of entry for bacteria could be demonstrated (Shenoy *et al*, 1995). Other pieces of indirect evidence also point to an important role for streptococci in the causation of ADL attacks. For example patients with ADL often receive oral or parenteral penicillin, the drug of choice for the management of streptococcal infections, for the treatment of their ADL attacks.

In the present study we examined the role of streptococcal infection as a precipitating factor of acute adenolymphangitis in brugian filariasis, by determining the ASO titers and by isolating the causative organism, wherever possible.

## MATERIALS AND METHODS

This study was carried out in the Filariasis Chemotherapy Unit, TD Medical College Hospital, Alappuzha.

### Patient population

The study population consisted of 30 patients with acute filarial adenolymphangitis, 30 patients with chronic filarial edema and 60 age and sex matched normal healthy adults. Thus there were the following three groups:

**Group A:** The 30 patients in this group had filariasis related acute adenolymphangitis (ADL) at the time of entry into this study. A diagnosis of filariasis related ADL was made: 1) When an attack of acute adenolymphangitis was associated with underlying filarial edema or 2) When there was a history of at least 2 ADL attacks in the past year, in the absence

of filarial edema.

**Group B:** The 30 patients in this group had chronic lymphatic filariasis without any ADL episode during the previous six months.

**Group C:** 60 age and sex matched normal healthy adults.

### Study design

All subjects enrolled in the study underwent a detailed clinical examination. In patients belonging to groups A and B, the lymphedema was graded as follows:

**Grade I.** Mostly pitting edema reversible on elevation of the affected limb.

**Grade II:** Mostly non-pitting edema, not reversible on elevation of the affected limb.

**Grade III:** Mostly non-pitting edema with dermatosclerosis and warty excrescences.

In these patients a diligent search was made for any evidence of discontinuity in the skin either in the form of trivial injuries, fungal infections or any similar cause. Laboratory investigations such as complete hemogram, urinalysis and serum chemistry were carried out in all the subjects enrolled in the study. Anti-streptolysin O (ASO) titers were estimated by latex agglutination method. All samples were tested using the ASO (Latex) test kits supplied by Avon laboratories. These kits were used to estimate ASO levels in a semiquantitative fashion using a suspension of polystyrene latex particles coated with stabilized Streptolysin O. Positive and negative controls were used in each batch. In patients belonging to group A swabs from sites of infection for isolation of streptococci were taken wherever indicated.

All patients were asked to report to the clinic whenever they had an acute attack and these were confirmed by the physician in charge of the study. Each attack was treated using a combination of antibiotics and anti-inflammatory agents.

All study subjects were followed up on the 15th day, 3rd month, 6th month and at the end of one year. ASO titers were estimated in all patients at the time of the admission to the study and at all the follow up points. Cultures from the sites of infection were repeated where indicated.

## RESULTS

The age and sex distribution along with the grades of edema in the three groups are given in Table 1.

Table 1

Age, sex distribution of edema grades.

Groups	A(30)	B(30)	C(60)
Mean Age	39	40	38
Males	20	17	37
Females	10	13	23
Edema Gr I	5	1	0
Edema Gr II	4	17	0
Edema Gr III	21	12	0

There were 20 males and 10 females in group A. The age group ranged from 20-56 years (mean 39 years). In 21 patients there was grade III edema, whereas in 4 it was grade II and grade I in the remaining 5 patients.

The ASO titer was positive throughout the study in 27 out of 30 patients belonging to Group A and in all of them there was a local source of infection in the affected limb in the form of monilial infection in the interdigital webs in 14 and minor injuries in 14, out of whom two patients had both monilial infection and minor injury in the affected limb. One other patient had infected eczema.

Even though swabs for bacteriology were taken from all patients who had a local source of infection, only in 8 the culture was positive. Streptococci were grown in 2, *Staphylococcus aureus* in 4 while there was mixed growth in two specimens.

In group B patients there were 17 males and 13 females and their ages ranged from 18-59 years (mean 40 years). The lymphedema was grade I in one patient, grade II in 17 and Grade III in remaining 12 patients.

In this group of 30 patients with chronic filarial edema ASO was negative in 20 patients. While six had ASO titers raised throughout the study period, there was only a transient rise in the remaining four. The six patients with persistently elevated ASO titers had grade III edema and three of them had

monilial infection in the affected limb. Among the 60 age and sex matched controls in group C, the ASO was negative in 57 and was only transiently elevated in three.

## DISCUSSION

Even though ADL attacks in lymphatic filariasis have been extensively studied, their pathogenesis is not well understood. Earlier postulates favored parasitic causes such as mechanical irritation caused by the movement of the adult parasite inside the lymphatics, inflammation induced by moulting fluid or toxic products from the dead parasite (Ottesen, 1980). There is now mounting evidence to suggest that secondary bacterial infection is the most important factor in the pathogenesis of ADL in filariasis. It has been observed that injuries (often trivial), fungal infections and loss of epithelium due to similar causes predispose to bacterial infections, especially by streptococci, resulting in ADL. Local dissemination of streptococcal infection is through lymphatics producing rapidly spreading lymphangitis and lymph node enlargement. Recurrent ADL occurs commonly in higher grades of lymphedema and elevated ASO titers are also seen more frequently in this group (Shenoy *et al*, 1995).

Streptococci are known to produce different exoenzymes, one of which is Streptolysin O. Antibodies to this exoenzyme (ASO) are positive in 80-85% of patients infected with streptococci (Klein, 1980). Measurement of ASO antibodies in the human sera has been proved to be exceedingly useful as an indicator of recent streptococcal infection and are easily measured by quantitative laboratory tests (Gray *et al*, 1994). The latex agglutination assay used in the present study has been shown to have a sensitivity of 91%, specificity of 86%, positive predictive value of 83% and negative predictive value of 92% (Gerber *et al*, 1990). Rise in ASO titer begins about one week after the infection and peaks 2-4 weeks later. In the absence of complications or reinfection ASO titers return to normal levels in 6 to 12 months.

In the present study an attempt was made to assess the relation between streptococcal infection and the ADL episodes of lymphatic filariasis. During the one year study period persistently elevated ASO titers were noticed in 27 out of 30 patients (90%) with ADL in group A who also experienced

recurrent ADL attacks during the study period. It is also significant that in all the 27 patients with elevated ASO titers in the ADL group, a portal of entry for secondary infection was present in the affected limb.

In group B consisting of patients with chronic filarial edema without any recent ADL, persistent rise in ASO titer was seen only in 20% and in the control group C, none of the subjects had persistent elevation of ASO titers. A local source was also seen in 50% of patients with persistent elevation of ASO titers in the chronic lymphedema group.

Even though persistent rise in ASO titers was seen in all grades of edema in the ADL group A, significantly it was observed only in cases with grade III edema in the chronic lymphedema group. While moniliasis is the predisposing cause in higher grades of edema, minor injuries top the list in grades I and II edema. In limbs with elephantiasis the toes are closely apposed and presence of moisture in the interdigital webs predisposes to fungal infections. The resulting injury to the skin favors entry of secondary pathogens.

We have previously shown that in lymphedematous limbs injuries, often trivial, fungal infections or loss of epithelium due to any cause predispose to ADL (Shenoy *et al*, 1995). It is this local loss of integrity of the skin that aids the entry of pathogens into the lymphatics. In the present study we have demonstrated a positive relation between the presence of a portal of entry for bacteria in the affected limb and elevated ASO titers. Since elevated ASO titers are associated with recent streptococcal infection, the persistent elevation noticed in 90% of cases with ADL in group A indirectly suggests that streptococcal infection was a precipitating factor for these ADL episodes.

An attempt was made in this study to isolate the pathogens responsible for the ADL attacks by taking swabs from any wet lesion present in the affected limb from patients in group A. In 8 out of the 30 patients bacteria were isolated; streptococci in 2, staphylococci in 4 and mixed growth in 2. The delay between collection of the swabs and delivery at the laboratory necessitated by the distance involved might be responsible for the low positivity.

The persistently elevated ASO titers in patients with recurrent ADL favor a streptococcal etiology for the ADL of lymphatic filariasis. These ob-

servations are important in the management of filarial edema, prevention of further ADL episodes and thus arresting the progression of the disease. This study like other recent ones (Shenoy *et al*, 1995; Olszewski *et al*, 1997), also convincingly demonstrates additional proof of the role of bacterial infection in the causation of ADL. These findings have important implications for the prevention and treatment of acute ADL episodes. Local care of the lymphedematous limb combined with local application of antibiotic or antifungal creams and long term antibiotic therapy assume significance in the light of the findings of this study. Lastly we believe that this study, by demonstrating that streptococci are the main organisms involved provides useful information on the choice of antibiotics in the management of ADL attacks. Controlled clinical trials which explore these issues are clearly required to define the prevention and management of ADL attacks associated with lymphatic filariasis.

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