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Diagnostic Potential of Filarial Skin Test (FST) with Brugia Malayi Larval Antigen

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Abstract: ***Introduction: Filariasis is caused by thread like helminthes placed under family Filaridae. The adult organisms have preference for lymphatics and responsible for various clinical manifestations including filarial fever, adenolymphangitis, Elephantiasis, hydrocoele, chyluria etc. Material & Method: In this study, selection of population was done on random basis including normal healthy, filarial related patients and patients with other diseases. Out of total population of 520,210 belonged to normal healthy individual, 108 patients had filarial related diseases and the rest of the individuals were having different other diseases. Result: On comparison and analysis of all the parameters, the specificity and sensitivity of the filarial skin test using B. malayi larval antigen (L3) is fairly well established by the results of the present study, which shows that FST has a fairly constant reproducibility, that is validity of the test is in the order of 90-95%. Conclusion: The above data may only represent a tip of the iceberg of the problem because the filarial survey reports are generally based on thick night blood smear and clinical manifestations which can not detect subclinical or occult filariasis. The disease is not fatal yet is responsible for considerable morbidity and social stigma and many avoidable surgical interventions.***

Keywords: Filariasis, Filarial skin test, Brugia malayi

1. Introduction

Filariasis is one of the oldest diseases known to mankind. The term, synonymous with Elephantiasis, find description in chapter XII of Sushrata Samhita, the epic treatise in surgery written around 6th century BC. The disease finds elaborate description in later treatises, particularly in Medhava Nidana (Chapter XXXIX).

The word filarial is derived from filar, that is thread like. In 1709 Clark in Cochin described Elephantiasis of legs and called it Malabar Legs. In 1866, Wucherer in Brazil found microfilariae in chylous urine. In 1872, Lewis working in Calcutta, found microfilaria in peripheral blood. In 1870, Manson, working in China discovered the development of W. Bancrofti in mosquitoes. In 1927, Brug discovered microfilaria of Brugia malayi. In 1940, existence of Brugia malayi was reported by Rao and Maplestone. Diethylcarbamazine was discovered in 1946 and has been the mainstay of treatment of filariasis since then.

Filariasis is a disease caused by certain nematodes (round worms) placed under the superfamily filarioidea (Filarial worms). There are nine recognized species of filariid for which man is the normal definitive host. Of these, five are responsible for different disease states, viz:-

1) Wuchereria bancrofti
2) Brugia malayi
3) Brugia timori
4) Onchocerca volvulus
5) Loa Loa

Vectors: The principal (and practically only) vector of W. bacrofti in Culex quinquefasciatus. Its breeding places tend to increase with urbanization and its relative resistance to insecticides makes it difficult to control. The vectors of B. malayi are various species of Mansonia especially M. uniformis.

Parasitology

The immature first stage larva of filarial worm is called microfilaria (MF). The pathogenic microfilaria live in the lung blood and are released periodically in to peripheral circulation. This interesting phenomenon is termed as "Periodicity" and is thought to be an adaptation by the microfilaria to the sitting habits of their insect vectors. Mechanism of the phenomenon is not fully understood and is influenced by sleeping working body activities and may depend on changes in body temperature, chemical composition and differences in oxygen tension between arterial and venous blood. Periodicity may be nocturnal e.g. in periodic W. bancrofti, B. malayi and B. timori or diurnal i.e. greatest number of microfilariaere present in day hours e.g. Loa Loa. Microfilaria can be found is the peripheral blood throughout the 24 hours with only slight increase in numbers during day or night. The strain is then, said to be sub periodic.

Pathogenesis

Invasion of man by the infective stage (L3) of microfilaria occurs without causing any symptoms. After molting to fourth stage larvae and young adults which usually takes 1-3 months these lymph dwelling filariae begin to induce local in inflammatory reactions. Most of the pathology associated with the infection occurs around adult worms in lymph nodes and afferent lymphatics. Sometimes pathological changes may also be observed when the microfilaria are cleared from blood or when the adult worms are located in ectopic site. These pathological changes have cell-mediated, humoral and foreign body components but little is known about the factors influencing sequence and intensity of the above mentioned pathological changes. Pathology for all three species in man is similar except that genital and renal lymphatic involvement is limited almost exclusively to W. bancrofti infections.

Common diseases manifestation of filariasis have been reported from various parts of the country as under:

(In a typical large series)	
1. Hydrocoele	60%
2. Elephantiasis of lower limb	25%
3. Elephantiasis in upper limb	13%
4. Chyluria	1.3%

The characteristics pathological lesions are in the form of lymphadenopathy, lymphangitis, genital lesions (funiculities epididymo-orchitis hydrocoele) lymphadema (Elephantiasis and tropical) pulmonary Eosinophilia. Other forms of filarial involvement includes chyluria, filarial granulomas, nephritis, ocular manifestations and amyloidosis. The involved lymph nodes become enlarged tender discrete or matted but not attached to the skin.

Filarial funiculitis is lymphangtitis of spermatic cord and inflammation of surrounding connective tissue. Lesions are similar to that of lymphangitis elsewhere. Epididymis become large smooth soft and tender. Orchitis is characterized by a boggy oedamatous testis due to inflammation of tunica and adventitia rather than the inflammation of tests itself.

Hydrocoele is the most common genital manifestation of chronic bancroftian filariasis characterized by a distended, thickened tunica vaginalis. Lymphadema and elephantiasis develop as a result of lymphagitis and associated lymphatic obstruction.

Tropical pulmonary eosinophilia is a relatively uncommon presentation of Filariasis and is characterized by clinical and immunological hyper- responsiveness, which is effected by both suppression of cell mediated immunity (CMI) response and humoral response.

2. Material and Methods

Study population: The present study was carried out in patients and normal healthy individuals. The name, age, sex, clinical history, symptoms associated with filariasis as well evidence for signs of clinical and occult filariasis were recorded of all patients.

The subject material comprised of:

1) Patients admitted or attending the medical outdoors of the respective hospitals.

A. Filaria related subjects: 108
B. Non Filaria related subjects 233

Exclusion criteria: In hospitalized group of patients, 31 were common for both filarial related and non-filaria related subjects.

1) Healthy asymptomatic subject: 210

This group included:

A. Volunteers from university and medical students.
B. Healthy willing attendants of the patients.

Microfilaria demonstration;

Conventional Thick Night Blood Smear

Peripheral capillary night blood for demonstration of microfilarial was taken by finger puncture method. After clearing the tip of a finger (preferably ring finger) with alcohol, it was puncture and 20U mm blood was sucked in a pipette and a thick blood smear was prepared on a clean glass slide covering an area of about half a square inch and was allowed to dry. The blood film was dehaemoglobinized and fixed with methanol. The fixed slide was them stained with Giemsa stain. Care was taken to stain and examine the smears the next morning after the sample collection so as to prevent shrinkage and distortion of the microfilariae. The microfilarial count was made under the low power of the binocular microscope and expressed as per 20 cubic mm of blood.

Venous blood concentration technique

The venous blood from a superficial vein of the upper extremity by applying tourniquet pressure (to make the vein prominent) was collected in a 10ml disposable syringe after cleaning the area with absolute alcohol. The sample collection was done between 10:00 p.m. to 1:00 a.m. The blood was transferred to a heparinised vial and shaken gently to mix heparin and the blood. The remaining blood was kept in a non-oxlatated vial for separation of serum for the study of filarial antibodies.

The heparinized blood was filtered next morning using membrane filters of 5micron porocity (Millipore filters) by the technique [1]. Five ml of the blood was haemolyzed by the addition of distilled water for one minute and the tonicity of the medium was maintained by adding concentrated sodium chloride solution. It was filtered thorough Millipore filters using a suction apparatus to facilitate filtration under positive pressure

Filters were taken out from the filters holder and immersed in normal saline for 10-15 minutes at 37^0 C to loosen the attached microfilariae from the filter. The suspension was then centrifuged at 3000 rpm for 10 minutes. The separated pellet containing microfilaria was re-suspended in saline and entire content was examined under iris setting sufficient to give good contrast. The microfilare (mf) by this technique are seen live and are motile. The microfilaria count was expressed as microfilarae per 5ml of blood (mf/5ml).

For demonstration of microfilaria in blood certain factors must be kept in mind. First and foremost is the phenomenon of periodicity. The ideal collection time for different species has been suggested as follows:

Species	Collection time
W. bacrofti	
Periodic (Nocturnal)	22.00-04.00hrs
Sub-periodic (Nocturnal)	20.00-22.00hrs
Brugia Malayi	
Periodic (Nocturnal)	22.00-04.00hrs
Sub-periodic (Nocturnal)	20.00-22.00hrs
Brugia timori	
Nocturnal	22.00-04.00hrs
Loa loa	
Diurnal	10.00-15.00hrs

Even collection at appropriate time may not yield positive result because numbers of microfilariae in blood is often few and therefore larger the volume of blood greater the possibility of demonstration of microfilaria. In chronic infection microfilarial yield is very low. Microfilaria are higher in capillary than in venous blood. Similar number of W. bacrofti microfilare can be recovered from 0.1ml of ear lobe capillary blood as from one ml of venous blood [2] . Also, more microfilarial can often be found in capillary blood collected from ear lobe than from the finger.

Filarial skin Test

Recently, Central Drug Research at Lucknow has developed a skin test using B. malayi larval antigen for the diagnosis of human filariasis. It has been claimed to have a very high specificity and sensitivity in earlier reports [3].

Antigen Preparation:

Collection of Brugia malayi infective larva:

Laboratory bred susceptible strains of Aedes aegypti female mosquitoes were feed on microfilaraemic Mastomys natalensis (rodent) between 7.30 to 8.30 pm when circulating microfilariae of subperiodic strain of Brugia Malayi (L3 stage) are in abundance in systemic circulation. The fed mosquitoes were kept for 10 to 11 days in a temperature (2^0 C) and humidity (5%) controlled in setarium for collection of infective larvae (L3 stage) the mosquitoes were dissected on 10^{th} or 11^{th} day in 0.85% sterile saline under a dissecting microscope. The 3^{rd} stage (L3) infective larvae were extracted and stored at -4 centigrade until required number of larvae were obtained. Larvae thus obtained were finally homogenized, sonicated and centrifuged under chilled conditions. The soluble fraction was finally sterilized by filteration (0.22 U filter), using the technique described by [4]. The protein content of this fraction was determined and used as antigen as described by [5] . Merthiolate was added in the concentration of 1;10,000 (Indian Pharmacopia, 19850) as preservative.

Lyophilization

The antigen solution was distributed in several sterilized ampoules and lyophoilized by the technique described in Recent advances in Researches on Filariasis and Schistosomiasis [6]. The ampoules were sealed under vaccum at 102 Torr (Lyophilab 80C, The Scientific Instrument Company Ltd).

On the day of testing the stored lyophoilized antigen was reconstituted with the distilled water to its original volume (0.2ml) and then diluted with normal saline (0.85%) of get the required antigen protein concentration (40µg/l).

Storage:

The lyophoilized antigen in vaccum sealed vials was kept at ordinary room temperature. The protein estimation of the stored antigen was made immediately before use. The skin reactivity (based on protein concentration at the time of storage) was evaluated and compared with freshly prepared antigen. The stability of the antigen have been evaluated and found to retain is biological activity as evidenced by skin reactions. There was also no alteration in the protein contents[3] .

Procedure of the test:

This reactions is based on the interaction between the sensitized mast cells fixed in the dermis and the homologous antigen inoculated. Antigen (0.05ml containing 2µg protein) were injected intraderamly on the volar surface of the forearm. Care was exercised not to deposit antigen in the deeper tissues and spilling of antigen on skin surface. The original wheal and that formed 15 minutes after injection of antigen were marked with a ball point pen and impression taken on butter papers moistened with Alcohol . The reaction ratio on which the present work is based on was to determined as follows as described by [4].

Reaction Ratio (RR)

Wheal area immediately after inoculation of antigen was recorded. The reaction being immediate hypersensitive type, full wheal of the antigen develops about 15 minutes after the antigen injection. The impressions recorded on butter paper as described above were transferred on a tracing paper and later translated on to mm2 graph paper. The area covered by the original wheal and that by the final wheal were determined. The reaction ratio was obtained by dividing the final wheal area with the initial wheal area. The reaction ratio of 2 and above was taken as positive reaction as this was found to be most specific and yielded least false positive, the Youden's Index being 0.9864 [7]**.**

Demonstration of microfilaria after provocation with diethylcarbamazine (DEC)

In certain patients in whom FST was positive but blood negative, a dose of 100 mg of diethycarbamazine citrate was given and blood examined after 30 minutes for microfilaria. It has been demonstrated that microfilaria are provoked to come out from lung capillaries to systemic circulation under effect of DEC. This test can be performed both during the day time as well as during the night time. Microfilaria positivity as well as microfilaria count significantly improved after DEC provocation.

In this group two sets of subjects were included:

i. Night survey
ii. Day survey after provocation with DEC.

The night survey was done on patients admitted to medical wards of Safdarjung Hospital, New Delhi. The residences of the patients included from Delhi. It also included migrant population from other states. This part of the study was carried out with the active support of Dr. CP Singh, Head of Medicine,Safdarjung Hospital, Lucknow.

The day survey after provocation with diethylcarbamazine was carried out on attendants of Shahdara Mental Hospital. The population in this group belonged to Trans-Jamuna area Delhi and adjoining districts of Uttar Pradesh. This survey was carried out with the active support and help of Dr. RC Agrawal, Psychiatrist, mental Hospital, Shahdara, Delhi.

Statistical analysis

Data collected were cleaned, filled in the excel sheet and analyzed. Percentages and proportions were used to express data.

3. Observation and Results

The present study was undertaken with the aim to determine reliability and validity of filarial skin test (FST) using Brugia malayi infective larval (L3) antigen.

Of the various method available for the detection of filarial infection, most conclusive evidence is demonstration of microfilaria in peripheral blood. However, traditional methods of demonstration of microfilaria give positive result when the density of the same in blood is very high. Chances of detecting microfilaria increase when large amount of blood is examined. As in thick blood smear, detection rate is very low and in centrifugation after saponification, microfilaria are desheathed and broken along with large number of blood cell debris resulting in unreliable counts.

The present study therefore utilizes concentration techniques using Millipore filters. This techniques is expensive and costs about Rs. 150 per sample. Yet, this is at present best available techniques to demonstrate microfilaria with accuracy and with minimum change in its morphology. Effort is being made to determine the reliability and validity of filarial skin test (FST). The need for such a test has felt since long for the diagnosis of filariasis as the above mentioned techniques are inadequate, expensive requires night blood sample and pathological support services

The study was carryout out in a total of 520 cases. The selection of cases was a random covering filarial related as well as other patients and healthy subjects. The subjects mainly comprised of adult age group ranging form 14-80 years. Patients of both sexes were included.

A total of 241 cases were examined for evidence of microfilaria in their blood by traditional night blood smear and Millipore filtration technique using 5 ml blood.
Efficacy of the traditional thick night blood smear (20 cumm blood) in detecting filarial positivity was compared with the concentration technique using 5ml of night blood by filtering the blood through the Millipore filter. The results obtained are tabulated below.

Table 1: Comparison of the MF demonstration by conventional night blood smear and concentration technique

No. of cases	Thick smear (20 cumm)	Concentration Technique (5ml blood)
241	17 (7.05%)	90 (37.34%)

The results of these two methods (table no. 1), clearly indicate the superiority of concentration technique using 5ml blood (and filtering the same through Millipore filters), in the detection of filariasis as compared to the traditional thick night blood smear as being practiced at present.

Out of a total endemic area study population of 520 cases, 108 (20.76%) cases revealed signs and symptom related to filaraisis, including 40 (7.69%) cases of prolonged pyrexia of more than 14 days duration and where routine investigations could not be reveal the cause of fever. The mf demonstration was positive in 147 (28.26%) out of a population of 520.

Table 2: Prevalence of Filariasis as assessed by clinical examination, mf demonstration (5mf blood) and FST

Region	N	Clinical	Mf positive	FST
Total	520	108 (20.7%)	147(28.2%)	317 (60.9%)

Table 3: Microfilaria positivity and FST in patients presenting with filarial related symptoms

Clinical diagnosis	N	Mf positive	FST positive
Pyrexia	38	8	29
Eosinophilia (AEC 700-1500)	14	8	12
Localized swelling of arm and leg	13	6	12
Effusion and Ascitis	11	5	8
Hydrocoele	10	7	9
Lymphadenitis and Lymphangitis	9	4	6
TPE (AEC >3000)	7	0	7
Epididymo-orchitis	4	2	4
Chyluria	2	0	1
Total	108	40 (37.03%)	88 (81.48%)

The study was carried out selecting the patients at random including patients with symptoms of filariaiasis numbering 108 cases, and patients with non-filaria related diseases numbering 202 (after exclusion of 31 common cases, i.e., patients having filariasis as well as other system involvement). Of the 520 cases, 210 cases were of asymptomatic category. The disease distribution of cases under the filarial relation symptoms category and pattern of mf recovery and skin reactivity with FST is given in the table no. 3.

Out of the 108 cases presenting wit filaria relates symptoms 40 (37.03%) showed microfilaraemia. Skin sensitivity with FST was present in 88 (81.48%) cases. Positivity of FST in hydrocoele is 90% and in TPE is 100%. From the table it is also apparent that none of the TPE case showed mf recovery in peripheral blood.

Table 4: Microfilaria positivity and FST in non-filaria related patients

System involved	N	Mf recovery (5mf blood)	FST positive
CNS	54	12	30
CVS	29	6	16
Respiratory	49	12	25
GI tract	39	12	21
Diabetes	9	3	4
Hepatobility	15	6	8
Renal	11	0	7
Hematological	11	3	8
Others	16	4	6
Total	233	58 (24.89%)	125 (53.64%)

The data in table No.4 shows diseases systems and patterns of mf recovery and FST positivity in the patients primarily presenting with a systemic illness, not related to filariasis. The results show mf recovery in 24.89% and FST positivity in 53.64% randomly selected cases belonging to various system involvement, not related to filariaisis directly. One of the notable finding is absence of mf recovery in patients presenting with renal diseases.

The table no. 5 shows patterns of MF recovery and FST reaction in normal healthy population. These subjects were

healthy relatives of patients, university students and healthy medical student of KGMU, Lucknow. Out of a total of 210 normal subjects mf recovery was possible in 69 (32.85%) and the FST was positivity in 61.90%. it is interesting to note that mf recovery rate in normal population is comparable with patients having filarial related symptoms (32.85%) as against 37.03%).

Table 5: Microfilaria positivity and FST in healthy subjects

No of cases	Mf recovery	FST positive
210	69 (32.85%)	130 (61.90%)

Filarial skin test (FST) was performed in 7 cases of tropical pulmonary eosinophilia (a representative group of occult filariasis) and 10 cases of non traumatic hydrocoele (a known manifestation of filarial infection). The finding are present in Table no.6 below.

Table 6: FST reactivity in TPE and hydrocoele

	N	FST positive	FST negative
TPE	7	7 (100%)	0
Hydrocoele	10	9 (90%)	1 (10%)

The table (6), indicates a positive reaction with FST in all cases of TPE and 9 out of 10 cases of hydrocoele. The reaction ratios were divided in four group (0-2, 2-4, 4-6, and >6). The blood counts of the study population in relation of these groups is presented in table 7 below.

Table 7: Blood counts in relation to reaction ratio of FST

Reaction ratio	TLC	AEC
0-2	6825.71	256.82
2-4	6916.92	360.82
4-6	7206.58	625.82
>6	7328.23	670.34

Total leucocytes counts shows no statistically significant difference in different reactions ratio groups. Similarly, there is no statically significant difference in lymphocytes count. However, absolute eosinophil count (AEC) was significantly higher in group having reaction ratio of 4-6 or >6 (AEC 625.82 and 670.34) as compared to FST negative cases RR <2 , (AEC 256.82).

Absolute eosinophil count (AEC) levels were further analyzed in reaction to filarial realted cases, normal healthy cases and patients presenting with other diseases in the table no. 8 given below.

Table 8: AEC levels in Filaria related, normal and other disease individuals

Group	N	AEC
Filarial related cases	108	598.34
Normal cases	210	315.46
Other disease cases	202	310.30

The filarial related cases show a significantly high level of eosinophil counts ($p<0.001$) as compared to normal endemic and other diseases groups. There is no significantly different is AEC levels between normal endemic and other diseases cases.

During microfilaria demonstration a group of 24 cases who were negative for mf count were given 100mf of diethyl carbamazine and mf count was done after 30 minutes. The result are given in Table no. 9. The result shows a rise in mf recovery in 58.33% of cases. In a further subset of patients the pattern in rise of mf count after DEC provocation is given below (Table No. 10).

Table 9: Table depicting rise in mf positivity after provocation with diethylcarbamazine (DEC).

No. of cases	Mf count before DEC	Mf recovery after DEC	
		Present	Absent
24	0	14 (58.33%)	10 (41.66%)

Table 10: Rise in average mf count after DEC provocation

No. of cases	Before DEC mf count	After DEC mf count	% rise in count
5	3.4	13.2	388.2%

The above table indicates the efficacy of DEC provocation test on mf recovery done at night.

4. Discussion

The results conclusively prove the thick night blood smear technique failed to demonstrate mf in 81.12% of proven microfilaremics (as in the present study). Comparative figures in detecting microfilaraemia by these techniques is as under:

No. of cases examined	241
MF recovery rate	
By traditional night blood smear	7.05%
By concentration technique	37.34%

Similar observations has been made by [10].

In this study, selection of population was done on random basis including normal healthy, filarial related patients and patients with other diseases. Out of total population of 520,210 belonged to normal healthy individual, 108 patients had filarial related diseases and the rest of the individuals were having different other diseases. The patterns of MF recovery and skin reactivity with FST in the mentioned categories was found as follows.

Table 11: FST and MF recovery in different groups

Groups	MF rate (5ml blood)	FST positivity
Normal healthy	32.85%	61.9%
Filaria related disease	37.03%	81.48%
Other diseases	24.89%	53.64%

From the table, it is apparent that patients having filarial related symptoms had only slightly higher percentage of MF recovery and sensitivity of skin test. There are no significance difference in MF recovery and FST positivity amongst normal healthy and patients suffering from non-filaria related diseases.

A marginally low MF recovery rate and FST positivity in patients suffering from non-filaria related diseases may be attributed to the fact that the patients in later category were taking differences types of medicines which might have interfered with MF recovery and skin sensitivity. Higher figures for MF recovery (66.67%) in night blood smear in patients having acute filarial manifestation [8] . This might

have been due to selection of study population and smaller number of the patients examined (total 30 cases).

However, there is no significant difference in MF recovery rate (21.05%) and filarial skin test (76.31%) in patients clinically suspected of filarial fever. The MF recovery rate and FST positivity rate is 46.15% and 12.30% respectively in patients with localized swelling of arm and leg. These figures correspond to that obtained by [8]. In patients with non pyogenic exudative effusion and ascitis, the MF recovery rate and FST positivity rate were 45.45% and 72.72% respectively. In patients with acute epididymo-orchitis, MF recovery and skin sensitivity were found to be 50% and 100% respectively. In patients presenting with nontraumatic hydrocoele, MF recovery and FST positivity were found to be 70% and 90% respectively.

The patients with tropical pulmonary eosinophilia (AEC >3000/cumm) showed positive filarial skin test in 100% of the cases, but none showed presence of microfilaria in the peripheral blood. This findings is in tune with the observation of earlier workers. The rapid removal of microfilaria from blood is attributed to the raised levels of IgG antibodies, present in the serum of these patients [9].

This particular phenomenon may hold clues for development of effective vaccines against filariasis. At the same time studies in this area may also shoed light on different clinical manifestation of filariasis. For example, why some patients develop marked lymphadenopathy while others develop elephantiasis and why the majority of the infected persons escape development of filarial symptoms.

In the patients having other diseases than that related to filariasis, the patterns of MF recovery and FST positivity show no remarkable patterns in different disease states. One noteworthy exception was found in the patients suffering from chronic renal diseases. In this group, out of a 11 patients, 63.63% showed FST positivity. However, no microfilaria could be demonstrated in any of these patients. The reasons for this are not apparent and this needs further evaluation and confirmation.

The normal healthy group showed most interesting finding. In this group, out of a 210 cases, 32.85% showed MF recovery in peripheral blood. FST positivity was found to be 61.90%. Earlier estimate of asymptomatic microfilaremics have been placed in the range of 10-20% (National Filaria survey), which is grossly inadequate .These asymptomatic microfilaremics pose a serious threat for further transmission and expansion of filaraisis. This group being asymptomatic, never seeks medical attention and serves as a reservoir of infection for transmission to other individuals.

By comparing the findings of present study with those which were done for the evaluation of sensitivity of the filarial skin test (FST) using B. malayi larval antigen, It is important to emphasize at the outset that the test is still in evaluation stage and only a few studies have been done and the study population in those studies was not very large, i.e. [4] studied 72 cases, [8] studied 47 cases and [10] included 199 cases.

1) The sensitivity of FST was matched with diseases known to be caused by filarial worms (Hydrocoele and TPE). The reaction with FST was positive in 100% of the TPE group and 90% in hydrocoele group again placing the sensitivity of the test in the range of 90-100%.
2) The findings of previous studies and the present study in regard to validity are summarized in table no 12. Our findings are in agreement with earlier workers.

Table 12: The validity of FST in different studies

Chandra et al 1978	98.6%
Kumar et al, 1985	92.5%
Sircar et al, 1989	98.07%
Present study	93.57%

The table showing sensitivity of FST using B. malayi antigen gives fairly constant results as reported by previous workers and places the sensitivity at the 93.57%. As is clear from the table, the FST has a fairly constant reproducibility, that is validity of the test is in the order of 90-95%.On comparison and analysis of all the parameters, the specificity and sensitivity of the filarial skin test using B. malayi larval antigen (L3) is fairly well established by the results of the present study.

DEC provocative test

The phenomenon of provocation of microfilarae after diethylcarbamazine is well known for day period, but has not been used as an provocative test at night. Hence, night provocation after 100mg DEC was evaluated in the present study. the population was divided into two groups:

The first group were FST positive but negative for MF in night blood. In this group out of total 24 cases 14 (58.33%) patients showed MF positivity in peripheral blood after DEC provocation at night.

The second set were both MF positive and FST positive but with low MF count (average count 3.4). After provocation with Diethylcarbamazine, the MF count rose from 3.4 to 13.2 showing a 388.2% rise in MF count.

Therefore, the present study clearly demonstrates the usefulness of DEC provocation test in clinical practice, both for obtaining higher MF positivity at night as well as it's utility where the collection of the night blood samples is difficult for cultural or personal reasons. Also the test can be utilized where the MF count is very low i.e. in provocation symptoms.

Reactivity in different individuals to FST was studied in relation to hematological parameters. The individuals with reaction ratio of 4-6 and more than 6 showed a significant elevations in their AEC levels. 625 and 670.34 respectively. At the same time total leukocyte count and lymphocyte count showed no statistically significant differences in different reaction ratio groups. Further, AEC levels in filarial related groups were higher AEC 598.34 than in normal endemic and other diseases category. These findings are in true with earlier workers.

The elevation of eosinophils in filarial related group points towards link between eosinophilic response and

development of filarial symptoms and may have a common mode in triggering these reactions. Further work on these lines will be useful in understanding why some patients with filarial infection develop symptoms while majority of them remain asymptomatic. [9] have suggested that in asymptomatic microfilaremics there is hyposensitization to microfilarial antigens. When this hyposensitization, either due to drugs or other factors, is uncovered patients develop filarial symptoms. Further work will help elucidate the exact mechanism, factors affecting the immune response and may lead to development of modalities for prevention and treatment of filariasis.

5. Conclusion

The study revealed following significant conclusions:

1) Microfilaria recovery rate using concentration techniques resulted in 37.34% positivity in Lucknow as contrast to 7.05% only by conventional night blood technique.
2) The clinical disease in the study represents only a fraction of filarial infection. Out of 317 subjects with FST positive and MF positive cases, clinical diseases was apparent in only 108 (20.76%) cases.
3) Surprisingly, the FST positivity and MF recovery in normal healthy population was nearly as high as observed in those with filarial related disease. Out of a population of 520, normal healthy population were 210 in numbers, in whom MF recovery and FST positivity was found in 69 (32.85%) and 130 (61.90%) respectively.
4) No microfilaria could be demonstrated in patients with chronic renal diseases (11).
5) Microfilaria recovery and FST positivity in patients with hydrocoele was found to be 70% and 90% respectively.
6) The patients with TPE did not have microfilaraemia. FST positivity in these patients was found to be 100%.
7) In study population AEC levels amongst subjects with filarial related symptoms was found to be significantly higher 598 as compared to 315 (in normal endemic cases.
8) Evaluation of the DEC provocation test revealed significant clinical application of this test in demonstrating microfilaria, increasing MF positivity, by 58.33%, increasing MF count by 388.2% and by demonstrating microfilaraemia during day time in 20% of cases.
9) FST positivity in MF positivity was present in 93.5% cases.
10) Reaction ratio of more than 4 was associated with higher levels of AEC: 625 in cases reacting negatively to FST.

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