

Original Research Article

Comparison of ELISA versus conventional methods in diagnosis of microfilariae

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Abstract: Filariasis is a major burden in India in states like Andhra Pradesh. With launching of global filariasis elimination program it has become necessary to use sensitive and accurate methods for diagnosis of filariae as compared to conventional methods. We in this study tried to compare the results of detection of microfilariae with conventional slide methods compared with the ELISA method. 90 samples were collected from those attending the Government General Hospital with signs and symptoms of filariasis they were designated as Group I [Test]. 10 normal serum samples Group II [Normal Controls] and 10 serum samples Group III [Endemic Normal] from normal individuals in the endemic pockets identified by filarial control program officers which were detected smear negative were collected as controls. out of the 90 cases in Group I by the ELISA method it was found that the total numbers of positive cases were 53 and total numbers of negative cases were 37, only 4 cases were found to be positive by the conventional slide method. In Group II normal control samples revealed that all the cases were reported as negative by slide method however by ELISA 7 out of total 10 cases were detected with the antigens and antibodies to microfilariae. The group III had no case detected by conventional slide method as positive but ELISA showed the presence of antigens, antibodies in 7 out of the total 10 cases included in the study. The sensitivity and specificity of ELISA with conventional slide method in all the samples was calculated it was found that Elisa has specificity of 90.24% [CI 76.8 to 97.28%] and specificity of 100 % [CI 93.28 to 100%] the positive predictive value PPV was 92.98%. ELISA is superior in detection of all cases of microfilariae. The conventional methods may not accurately identify microfilariae in all samples therefore it is recommended that ELISA forms an integral part for detecting the presence of microfilariae especially in Endemic areas.

Keywords: Filariasis, Diagnosis, ELISA

INTRODUCTION

The human filarial infections comprise *Wuchereria bancrofti* and the similar *Brugia malayi*, *Loa loa* in West Africa, and *Onchocerca volvulus* in West Africa and Central America. Also mentioned are the non-pathogenic infections caused by *Dipetalonema perstans*, *D. streptocerca* and *Mansonella ozzardi*. Lymphatic filariasis is a devastating disease and it is found in more than 80 countries across the world [1, 2]. Approximately 1 billion people across the world are at risk. It affects countries like south and Central America, West and East Africa, East Mediterranean, South East Asia and Western Pacific. *W. bancrofti* accounts for 90% of all filariasis cases in the world [3]. The infection damages the human lymphatic system and its acute and chronic complications cause considerable disability in affected individuals. People with the disease are incapacitated or disfigured with swollen genitalia

(hydrocoele) or dramatically thickened limbs, with hard, rough and fissured skin (lymphoedema/elephantiasis). Affected individuals suffer social and economic consequences due to stigma and reduced productive capacity resulting from these complications [4].

In India there are approximately 21 million people with symptomatic filariasis and nearly 27 million are (mf) carriers [5]. Traditionally it has been accepted that there are three different groups of people found in a filarial endemic area. Those who are exposed but no evidence of disease called Endemic Normals, those with asymptomatic microfilariaemia and lastly those with chronic lymphoedema, hydrocoele and elephantiasis. National Filariasis Control Programme, launched in 1955 has operational, training and research components [6]. The main strategies of which were the

vector control, detection and treatment of filarial cases and delimitation of endemic areas. This is being carried out through control units, night clinics and survey teams. Formation of specialized centers for diagnosis and monitoring were also the part of the program. This program continues to be in place, in addition to the program to eliminate LF in India. Measurement of microfilaremia is a well-recognized gold standard for demonstrating the impact of interventions but is not an optimal method of monitoring or surveillance because of requirement of nocturnal blood collection and relatively less insensitive test for infection [7]. W bancrofti assessment of antigenemia offers the convenience of daytime testing for microfilaremia however both microfilaremia and antigenemia develop from months to years after exposure, reducing their utility for detection of lower levels of infections and greater sensitivity for testing microfilaremia. Antibody detection for diagnostic assays for filariasis has been the basis of diagnostic assay for filariasis [8-11]. The best of assay are sensitive for infection but are not specific because they have some degree of cross reactivity with other helminth infections. With this background we tried to evaluate the effectiveness of ELISA in detection of microfilariae when compared with conventional slide test between different groups.

MATERIALS AND METHODS

This study was carried out in Govt General Hospital, Guntur District of Andhra Pradesh, and Ethical Permission for the study was obtained. 90 samples were collected from those attending the Government General Hospital with signs and symptoms of filariae they were designated as Group I [Test]. 10 normal serum samples Group II [Normal Controls] and 10 serum samples Group III [Endemic Normal] from normal individuals in the endemic pockets identified by filarial control program officers which were detected smear negative were collected as controls. All patients confirmed by clinical picture, suspected cases and the normal and endemic normal samples were screened for the presence of microfilaria by collecting blood by finger prick before and after DEC provocation test by wet mounts, thin and thick smears. Smears were stained with Leishman stain. 5ml of blood was collected by venepuncture under aseptic precautions by sterile syringe. Clot formation was allowed to occur for 2 hours at room temperature and then centrifuged and preserved in sterile lax brow vials and refrigerated at 20°C. Stained smears were observed for microfilaria. Serum samples were tested for both antigen and antibody at JB Tropical Disease Research Centre, MGIMS, and Sevagram. Preparation of Excretory Secretary antigen (ES antigens) The ES antigens are

exoantigens or in vitro released antigen by the filarial parasites that have been used in different immune assays for the detection of antibody. It is generally believed that nematode secretions are strong immunogens and are expected to have adjuvant effect to evoke good humoral immune response in the host.

ELISA Enzyme-Linked Immunosorbent Assay:

Coupling of penicillinase to produce conjugation of antihuman immunoglobulin and penicillinase was achieved by using glutaraldehyde. Dilute test sera and positive and negative control sera 1:300 in PBS/T (10 ul serum is diluted to 1.5ml). Transfer 0.5 ml of diluted sera (in duplicates) to lab led plastic vials. Place on antigen coated stick in each vial and incubate at 37°C for 1 hr 30 minutes. Wash the sticks 5 times with PBS/T 9 each time add about 1.5 ml PBS/T, shake gently and discard after 4 minutes) While washing is going on dilute the enzyme conjugate 1:1000 times in PBS/T (10ul conjugate to 10 ml PBS/T) Incubate each stick with 0.5 ml of diluted antihuman IgG penicillinase conjugate at 37°C for 30 minutes. Wash stick 9 times with PBS/T. Change stick to labeled glass tubes. Add 0.5 ml of starch-iodine-penicillin substrate to each tube and incubate at 37°C Note decolorisation time for each tube. Serum sample complete decolorisation at least 4 minutes earlier than the negative control serum is considered positive for filarial antibody. Serum sample showing complete decolorisation at least 2-3 minutes earlier than the negative control serum is considered Borderline Positive for filarial antibody and the test need to be repeated.

Procedure for inhibition ELISA for antigen detection:

Dilute test sera and positive and positive control sera 1:300 in PBS/T (10 µl serum in diluted to 1.5ml). Transfer 0.5 ml of diluted sera in to labeled plastic vials. Place one antibody (FSIgG) coated stick in each vial and incubate at 37°C for 1 hr 30 minutes. Wash the sticks 5 times with PBS/T (each time add about 1.5 ml of PBS/T, shake gently and discard after 4 minutes). While washing is going on dilute the Bm microfilaria ES penicillinase conjugate 1:500 times in PBS/T (e.g 20 ul conjugate to 10 ml PBS/T) Incubate each stick with 0.5 ml of diluted conjugate at 37°C for 1 hour 30 minutes. Wash stick 5 times with PBS/T Change stick to labeled glass tubes. Add 0.5 ml of starch-iodine-penicillin substrate to each tube and incubate at 37°C. Note decolorisation time for each tube. Serum sample showing complete decolorisation at least 4 minutes later than the negative control serum is considered positive for filarial antigen. Serum showing

decolorisation at least 2-3 minutes later than the negative control serum is considered Borderline Positive for filarial antigen and the test is repeated.

RESULTS

The results of ELISA were compared with the microfilariae detection by slide method, it was found that the only 2 cases each in the male and female, a total of 4 cases were detected by the slide methods rest all

the cases were detected as negative. However by the ELISA method it was found that the total numbers of positive cases were 53 and total numbers of negative cases were 37 in the test group I. The comparison of the same results with the normal control samples revealed that all the cases were reported as negative by slide method however by ELISA 7 out of total 10 cases were detected with the antigens and antibodies to microfilariae. See table 1.

Table 1: Comparison of ELISA with conventional smear examination between Group I and Group II

Study group	Sex	No tested	Mf+	Antigen ELISA positive	Antibody ELISA positive	Both antigen – antibody positive	Negative
Group I [Test]	Males	35	2	7	11	7	12
	Females	55	2	10	16	2	25
Group II [Normal controls]	Males	4	-ve	1	-ve	-ve	3
	Females	6	-ve	-ve	1	1	4

The table 2 shows the comparison of the results of detection of microfilariae by ELISA in Group I [Test Group] versus the endemic control samples obtained from endemic area identified by their residence by District filariae control officer showing the endemic zones. Whereas total of 4 cases were detected

by the slide methods rest all the cases were detected as negative in the test group I. The group III had no case detected by conventional slide method as positive but ELISA showed the presence of antigens, antibodies in 7 out of the total 10 cases included in the study.

Table 2: Comparison of ELISA with conventional smear examination between Group I and Group III

Study group	Sex	No tested	Mf+	Antigen ELISA positive	Antibody ELISA positive	Both antigen – antibody positive	Negative
Group I [Test]	Males	35	2	7	11	7	12
	Females	55	2	10	16	2	25
Group III [Endemic Normal]	Males	7	-ve	-ve	2	-ve	5
	Females	3	-ve	-ve	1	-ve	2

The most common clinical presentation was fever with lymphedema detected in 51 (56.66%) of cases followed by lymphangitis in 24 (26.66%) of cases. The other clinical presentation includes fever with lymphadenitis in 7 (7.78%). Chronic filariasis of different grades were evaluated includes total of 6 patients none of them reported positive by slide method and 4 out of 6 were found to be positive with ELISA

test. 2 out of 90 patients were of asymptomatic microfilaremia one of which was detected as positive by ELISA. See table 3. The sensitivity and specificity of ELISA with conventional slide method in all the samples was calculated it was found that ELISA has specificity of 90.24% [CI 76.8 to 97.28%] and sensitivity of 100 % [CI 93.28 to 100%] the positive predictive value PPV was 92.98%.

Table 3: Study Group I [Test Group] - Results of study with clinical correlation

Symptoms	No Tested	Mf+	Antigen +ve	Antibody +ve	Ag-Ab +ve	Negative
Fever with lymphedema	51	4	11	20	5	15
Fever with lymphadenitis	7	-	2	1	1	3
Fever with lymphangitis	24	-	3	6	1	14
Chronic filariasis	-	-	-	-	-	-
Grade 1	-	-	-	-	-	-
Grade 2	2	-	-	-	1	1
Grade 3	1	-	-	-	-	1
Grade 4	3	-	1	-	-	2
Scrotal swellings	-	-	-	-	-	-
Tropical Eosinophilia	-	-	-	-	-	-
Asymptomatic microfilariaemia	2	-	-	-	1	1
Total	90	4	17	27	9	37

DISCUSSION

We in the present study tried to compare the results obtained by conventional slide method of detection of filarial versus the ELISA method; we found that ELISA was far superior in detecting the filariae antigen, antibodies where the conventional slide methods failed to identify the microfilariae. One study by Wattal S *et al.*; for the diagnosis of bancroftian filariasis under filariasis elimination program using Og4C3 antigen ELISA as the tool in comparison with the Thick Blood Films found that Og4C3 ELISA and TBF results were comparable and they concluded that the finger prick DBS Og4C3 can be used as an alternative to TBF microscopy for detection of bancroftian filariasis [12]. They also found that ELISA test was found to have 100% sensitivity and 94.12% specificity for detection of Mf carriers in sera samples [12] this is agreement with our observation where we found that ELISA has specificity of 90.24% and sensitivity of 100 % the positive predictive value PPV was 92.98%. In another study by Hoti s *et al*; for the detection of day blood filarial antigens by Og4C3 ELSIA test using filter paper samples found that samples collected on filter paper during the day can be used as an alternative to sera samples for detection of filarial antigens employing Og4C3 ELISA. Also, samples collected during morning hours yield a higher positivity. The assay when applied to serum samples will be useful especially when quantitative results are required [13]. According to AW Wong and Guest 1969 noted that microfilaria antibodies were present in all patients with elephantiasis but were found in none in patients with circulating mf [14]. Smithers *et al.*; has suggested that living mf are relatively inert antigenically and do no stimulate production of antibodies. Our results contradict such findings because we found the 100% sensitivity of and 90.24% specificity in the groups [15]. It clearly means that there

is a significant association between density of microfilariae and CFA. Similar findings were reported by Rocha *et al.*; [16]. In one study by Pani SP *et al* for comparing Immuno chematographic card test with night blood smear examination for detection of W bancrofti microfilaria carriers found that ICT was 100 % sensitive compared to blood smear examination alone [17]. We have now seen in several studies that Night blood examination; wet film and peripheral smear examination alone are not satisfactory methods for diagnosis of filariasis because they are many time likely to miss the diagnosis. With increasing understanding of the Lymphatic filariasis and development of newer methods it is apt to adopt the new suitable methods for diagnosis of filariasis.

CONCLUSION

ELISA is superior in detection of all cases of microfilariae. The conventional methods may not accurately identify microfilariae in all samples therefore it is recommended that ELISA forms an integral part for detecting the presence of microfilariae especially in Endemic areas.

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