ORIGINAL ARTICLE

Sensitivity and Specificity of ELISA in Detection of Microfilariae

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Abstract

***Background**: Filariasis is a global problem and India has a major burden of disease in states like Andhra Pradesh, Odisa, Tamil Nadu, Kerala, Bihar and Uttar Pradesh. We tried to evaluate the Sensitivity and Specificity of ELISA for detection of microfilariae in Govt General Hospital, Guntur District of Andhra Pradesh. **Methods**: 100 samples were collected from those attending the filarial clinics under the control of district filarial officer were designated as Group A. 10 normal serum samples [Group B] and 10 serum samples (endemic normal) [Group C] from normal individuals in the endemic pockets identified by filarial control program officers which were detected smear negative were collected as controls. **Results**: Comparing the sensitivity and specificity of ELISA test between all groups Sensitivity of ELISA was calculated to 94% with Confidence Interval (CI 87.4 – 97.77%) and Specificity of 70% (CI 34.75 % - 93.33%). The positive likelihood value is 3.13 (CI 1.21 – 8.09) and negative likelihood value is 0.09 (CI 0.04 to 0.21) **Conclusion**: Antigen detection by ELISA has superior sensitivity and specificity in the diagnosis of filariasis both acute and chronic cases and occult filariasis which were failed to be diagnosed by smear examination alone.*

***Keywords**: Filariasis, microfilariae, ELISA*

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Introduction

Human lymphatic filariasis caused by Wuchereria bancrofti, Brugia malayi and B timori affects approximately 120 million people worldwide and nearly 1 billion people across the world are at risk. W *bancrofti* accounts for 90% of all filariasis cases in the world. [1] In India there are approximately 21 million people with symptomatic filariasis and nearly 27 million are (mf) carriers. [2] 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, hydrocele and elephantiasis. National Filaria Control Programme, launched in 1955 has operational, training and research components. [3] The strategies include (a) vector control (b) detection and treatment of filarial cases and (c) delimitation of endemic areas. 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 [4] 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.[5-8] 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 slide test between different groups.

Materials and Methods

This study was carried out in Govt. General Hospital, Guntur District of Andhra Pradesh, Ethical Permission for the study was obtained. A total of 120 samples were collected from 3 different groups. Cases were divided in Groups A, B and C. The group A which included the suspected filarial infection based on clinical presentation were taken it included 37% male and 63% females. Similarly Group B of normal controls were taken (n=10) 4 (40%) male and 6 (60%) female and the third group C (n=10) included Endemic normal 7 (70%) male and 3 (30%) females. All the 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 laxbrow 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, Sevagram.

For filarial antibody detection: 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.

ELISA: Coupling of pencillinase to produce conjugation of antihuman immunoglobulin and pencillinase was achieved by using glutraldehyde.

Procedure-Indirect ELISA for antibody detection

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 labeled 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 (e.g 10 ul conjugate to 10 ml PBS/T) Incubate each stick with 0.5 ml of diluted antihuman IgG penicillinase conjugate at 37ºC 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 37ºC 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 ELISE for antigen detection

Dilute test sera 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 duplicates) 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 (Brugia malayi) microfilaria ES pencillinase 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 37ºC 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 37ºC. 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

Table 1 shows the comparison between Group A and Group B for sensitivity and Specificity of ELISA. Out of 100 cases in group A when subjected to slide examination of microfilariae (mf) positive results were only shown in 6 cases. Whereas on ELISA test 94 cases appeared to be positive and 6 had negative results. In Group B

no positive cases were detected by (mf) slide test but ELISA showed (3) 30% positive and (7) 70% negative cases. Comparing the sensitivity and specificity of ELISA test in both groups Sensitivity of ELISA was calculated to 94% with Confidence Interval (CI 87.4 – 97.77%) and Specificity of 70% (CI 34.75 % - 93.33%). The positive likelihood value is 3.13 (CI 1.21 – 8.09) and negative likelihood value is 0.09 (CI 0.04 to 0.21).

The table 2 shows the values of smear examination and ELISA between Group A and Group C. whereas in group C no smear examination appeared positive 3 out of 10 cases tested positive for ELISA antibody and 7 cases were negative even in ELISA. Comparing the sensitivity and specificity of ELISA test in both groups sensitivity of ELISA was calculated to 94% with Confidence Interval (CI 87.4 – 97.77%) and specificity of 70% (CI 34.75 % - 93.33%). The positive likelihood value is 3.13 (CI 1.21 – 8.09) and negative likelihood value is 0.09 (CI 0.04 to 0.21).

The most commonest clinical presentation in the suspected cases Group A was Fever with lymphedema 54 cases and by slide test for microfilariae (mf) 3 were found to be positive and rest as negative where as when subjected to ELISA 51 were found to be positive and 3 were negative for antigen and antibody (Table-3). Similarly 41 cases with chronic filariasis with different grades were present. Out of this 3 were tested positive by slide test and rest were negative by ELISA 38 were found to be positive and 3 were found to be negative.

Table 1: Sensitivity and Specificity of ELISA between Groups A and B

Study group	Sex	No tested	Mf+	Antigen ELISA positive	Antibody ELISA positive	Both antigen – antibody positive	Negative
A	Males	37	2	3	15	18	3
	Females	63	4	8	17	33	3
B	Males	4	-ve	1	-ve	-ve	3
	Females	6	-ve	-ve	1	1	4

Table 2: Sensitivity and Specificity of ELISA between Groups A & C

Study group	Sex	No tested	Mf+	Antigen ELISA positive	Antibody ELISA positive	Both antigen – antibody positive	Negative
A	Males	37	2	3	15	18	3
	Females	63	4	8	17	33	3
C	Males	7	-ve	-ve	2	-ve	5
	Females	3	-ve	-ve	1	-ve	2

Table 3: Study group A – Results of study with clinical correlation

Symptoms	No tested	Mf+	Antigen +ve	Antibody +ve	Ag-Ab +ve	Negative
Fever with lymphedema	54	3	8	18	25	3
Fever with lymphadenitis	2	-	-	1	1	-
Fever with lymphangitis	2	-	-	-	2	-
Chronic filariasis Grade 1	1	-	-	1	-	-
Grade 2	12	2	-	3	8	1
Grade 3	18	1	2	6	10	-
Grade 4	10	-	1	3	4	2
Scrotal swellings	-	-	-	-	-	-
Tropical eosinophilia		-	-	-	-	-
Asymptomatic microfilaraemia	1	-	-	-	-	-
Total	100	6	11	32	51	6

Discussion

Recent advances in various areas of knowledge related Lymphatic Filariasis has led to the use of new approaches to lessen the severity and impact of the disease.[9] The introduction of new diagnostic tools for detection of infection by *W. bancrofti* in the human population have opened up new possibilities in terms of interrupting transmission and consequently the elimination of filariasis [9-13] The present study is aimed at and effective method for early diagnosis of filariasis. Night Blood Examination, Wet Film and peripheral Smear Examination after staining were time test procedures for the diagnosis of filariasis but sometimes are likely to miss the diagnosis. A clinically suspected case may have circulating filarial Antigen/Antibody and therefore Antigen/Antibody detection by ELISA utility as a diagnosis too was taken up in the present study. In this study we found that slide test for detection of microfilariae was less effective as it tends to miss most of the cases of microfilariae as shown by low number of positive results in the groups. Low sensitivity of this method has been demonstrated by other studies [14] it was also found that this method does not allow accurate determination of true prevalence of infection.[15] In the present study it was found that ELISA was 94% sensitive and 70% specific for detection of microfilariae when compared with the study Group A and Group B and C. In a similar study by wattal S et al; found that in non endemic normals and microfilariae carriers the ELISA test was found to have 100% sensitivity and 94.12% specificity. [16] In another study by Hoti SL et al; comparing the ELISA test between mf chronic carriers and acute filariasis the specificity of assay on sera samples collected during night was found to be 100% and the sensitivity was 96.8%. [17] Circulating Filarial Antigens CFA is now considered to be a promising tool for diagnosis of filariasis at both individual and community levels. According to AW Wong and Guest 1969 noted that microfilarial antibodies were present in all patients with elephantiasis but were found in none in patients with circulating mf. [18] 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 94% sensitivity of and 70% specificity in the groups. It clearly means that there is a significant association between density of microfilariae and CFA. Similar findings were reported by Rocha et al; therefore it can be said that ELISA was sensitive and specific for detection of microfilariae.

Conclusion

Antigen/Antibody detection by ELISA has superior sensitivity and specificity in the diagnosis of filariasis both acute and chronic cases and occult filariasis which were failed to be diagnosed by smear examination alone. Age and sex prevalence for the disease were affected by factors like local population susceptibility and geographical variations. Therefore ELISA test should be done in all cases of suspected of filariasis.

Conflict of Interest: *None declared*
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