

A Survey of Bancroftian Filariasis by Detecting Microfilaria and Circulating Antigenaemia in Biase Cross River State, Nigeria

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Abstract

The estimation of filariasis prevalence in Biase Local Government has previously relied upon clinical evaluation and examination of night blood smears. However, night blood smears examination fail to detect the infection in individuals having low parasitaemia and cryptic filarial infection. The present study was undertaken to evaluate the prevalence of filariasis in nine wards of Biase local government by immunochromatographic test (ICT). Methods : Clinical examination was performed according to WHO criteria to classify filarial disease. Night blood smears collected between 21.00 to 00.00h were examined to detect microfilaria (MF). For estimation of circulating filarial antigen (CFA) by Binax Now filariasis, 2ml of blood was collected from each individual by venepuncture at any time of the day. Results : A total of 425 participants made up of 260 males and 165 females were examined randomly from the community with particular emphasis on those with suspected cases of infection such as elephantiasis of the leg.

Index terms— Circulating filarial antigen-filariasis-biasemicrofilariaemia.

1 I. Introduction

lymphatic filariasis (LF) caused by the filarial nematode *wuchereria bancrofti* affects more than 120million people worldwide (1).

In Africa, the Prevalence of lymphatic filariasis is especially striking, affecting over 40 million people in the sub-Saharan region alone (2). Overall, Africa is thought to account for 40 percent of all cases of lymphatic filariasis in the world (3).

The third most endemic country in the world for this disease (after India and Indonesia) is Nigeria, where it is caused by *W. bancrofti*, and 22.1 percent of the population is thought to be infected (4).

In 2003, a survey was carried out in Plateau and Nassarawa state in Nigeria where the prevalence of lymphatic filariasis determined by ICT test was 22.5 percent and 22.4 percent respectively (5). The diagnosis of filarial infection by clinical examination and parasitological methods was the mainstay in detecting filarial infection up to early nineties. These methods though correctly assess the clinical cases and microfilaraemic subjects with high microfilariae MF count, but fail to identify low MF count and cryptic filarial infection in asymptomatic amicrofilaraemic individuals (6). In recent years, with the introduction of new diagnostic methods such as rapid diagnostic tests (RDTs), the prevalence of filarial disease was redefined in many parts of the globe. The antigen and antibody assays have several advantages over microscopic identification of MF in blood, which is the traditional method of diagnosing Lf infection (14,15, 16). They are more sensitive (i.e., MF-negative persons with positive antigen or antibody test are frequently identified) (17) and both overcome the logistical constraint of obtaining blood at night, which is necessary in the many endemic areas where MF have nocturnal periodicity. The purpose of this study was to Study the infection status of the human population and finally to assess the impact of mectizan distribution for Onchocerciasis control on lymphatic filariasis in area where the two diseases are co-endemic. To date such study has not been done in Biase thus, the results of the present study may be

relevant to determine the geographic distribution of lymphatic filariasis and the location of communities that requires treatment beyond Biase Local Government area, Cross River State, Nigeria. Informed consent was obtained from study individuals (parents in case of minor). Children less than 16 years old and individuals who did not give their consent were not part of the study.

2 II. Materials and Methods

Sample collection, a door-to-door survey was carried out from October 2008 to November 2009 in the local government to include individuals (adults and children aged 16 years and above in the study. History suggestive of filariasis and diethyl-carbamazine citrate (DEC) or mectizan consumption was recorded.

Mf detection; Mf was detected by making two thick blood smears of 20 μ l each on a clean glass slide from 21.00 to 00.00h. The smears were air dried, dehaemoglobinised and stained with Giemsa stain to detect Mf.

Antigenaemia detection : About 2ml of blood was collected from all the individuals (n=425; 260 males and 165 females) enrolled in the study. Sera were separated in the field and brought to the laboratory and stored at -20 $^{\circ}$ C until tested. The binax now filariasis was used for detecting and quantifying w. bancrofti antigen. The test card was removed from the pouch just prior to use. The card was laid flat on the work surface. The capillary tube was filled to the 100 μ l mark using capillary action with venous blood. The 100 μ l of sample was added slowly from the capillary or pipette onto the top of the pink and white pad. The 100 μ l of sample was added slowly from the capillary or pipette onto the top of the pink and white pad. Important : Each drop was allowed to soak in before adding the next drop onto the pad. Incorrect addition of sample may result in device failure.

It was allowed until the sample has flown into the pink area and was completely wet (this should take about 30 seconds to 1 minute).

The adhesive liner was removed and discarded and the adhesive of the test card was exposed.

The card was closed. To ensure good test flow, the card was pressed very firmly along the entire area to the right of the window.

The timing started. The result was read through the viewing window after 10 minutes.

3 III. Result Interpretation a) Positive result

The test was positive if two lines (T and C) were seen in the viewing window. Any pink line in the T area indicated a positive result. The test was positive even when the T line appeared lighter or darker than the C line.

4 b) Negative result

The test was negative if only the C line was seen. To ensure that low positive samples had sufficient time to develop, a negative result was not to be recorded until 10 minutes have elapsed from when the card was closed.

Statistical analysis : The Pearson correlation coefficient, student T-test and chi-square test were used to analyse the data. X² for trend was used to find the relation of age with Mf and CFA prevalence while the Pearson Correlation was used to find out if there is any relationship between Knotts concentration method and the antigen detection.

5 IV. Results

A total of 425 individuals were examined from Biase local government. The prevalence of Mf and CFA was 13.2 percent and 48.7 percent respectively. There was a statistically significant difference in the prevalence of W. bancrofti microfilaria and circulating filarial antigenaemia by method of detection (X²=11.004, P<0.05). The correlation analysis showed that there is no relationship between the two methods of detection of filarial infection (r=0.967, P>0.05). The percentage of Mf and CFA positive individual increased steadily with age reaching a peak in the 16-26 year age group. The prevalence of Mf and CFA decrease steadily between 49-70 year age group. Beyond 70 year there was a fall in CFA prevalence while no individual was positive for Mf. There was a statistically significant difference in the distribution of circulating antigen of lymphatic filariasis in the blood of subjects by age (P<0.05) table 1 (1).

The Relationship between circulating filarial antigen (CFA) and microfilaria (MF) detection with clinical status is presented in table 2. It was observed that in asymptomatic individuals (n =399), ICT Now Filariasis Kits could detect infections in 203 (53.4%) individuals while night blood smear had 48 (12.6%) positive cases only. In symptomatic individuals (n=26), the prevalence was 61.5 and 46.1 percent by ICT and night blood smear respectively. Infection rate detected by CFA was significantly (P<0.05) higher compared to that by night blood smear examination.

Of the 425 individuals included in the study, 26 had clinical symptoms of filariasis (elephantiasis and hanging groin). Among the 24 individuals presenting with elephantiasis, Mf was present in 10 (38.5%) and CFA in 14 (53.9%) cases. All the 2 individuals presenting with hanging groin were microfilaraemic and were also found positive for CFA (Table 3). It was observed that all the microfilaraemic individuals were CFA positive but all the CFA positive individuals were not microfilaraemic. A total of 159 individuals were CFA positive but having no circulating Mf. From the 159 amicrofilaraemic antigen positive individuals, 151 were asymptomatic and amicrofilaraemic having cryptic infection detected by ICT now filariasis test kits.

Table ?? shows the prevalence of lymphatic filariasis according to the knotts concentration methods and ICT. Among participants who had meaningful results, 56 (13.2 per cent) were positive for the thick blood film technique and 207 (48.7 per cent) by ICT card test. Out of 56 mf positive persons by the Knotts concentration method, only 2(3.6 per cent) were negative by the card test, whereas 151(41.1 per cent) individuals were negative by the Knotts concentration method. 216(58.8 per cent) were negative according to both Knotts concentration and ICT card test whereas 218(51.3 per cent) were negative for ICT card test alone. The overall sensitivity of the whole blood ICT card test was 96.5 per cent (56/58) while the specificity of the test was 58.8 per cent (216/367). The two false negative were males in the 37-47 year of age group.

6 V. Discussion

Filariasis is a major public health problem in Nigeria. With the continuous change in environmental factors, urbanization and availability of newer diagnostic tools (8), the estimation of 22,1 percent of the population thought to be infected is bound to be increased (4). With the widespread availability of the CFA assay which reflects adult worm burden (9), it can now be demonstrated that a majority of the earlier studies underestimated the prevalence of filariasis in endemic communities (7).

The prevalence of CFA was considerably higher than Mf prevalence in all the age groups (10), (11), (12). In the present study, the prevalence of filarial infection in the population was approximately four times higher when determined by CFA positivity compared to Mf examination in all the age groups except 49-59 and 60-70 year age groups that the infection was one against sixteen and two against nineteen for Mf and CFA respectively (table 1). In the context of filariasis elimination programme, use of antigen detection in the diagnosis of filariasis, particularly in young children is important as treatment at an earlier age may prevent subsequent development of clinical disease. also most of them were engaged in farm work during the period of blood collection. Cynthia et al. (2003) had similar results where the prevalence of microfilaremia and antigenaemia was slightly higher in males than in females in Brazil. In this case however, Mf prevalence was estimated by a relatively less sensitive 20% blood smear and the present CFA+/Mf-might include low density Mf carriers. The prevalence of microfilaraemia and antigenaemia seemed to decrease with age (table 1). This is contrary to the work done in Cook Islands where the percentage of CFA positive subjects increased steadily with age reaching a peak in the 30-40 year age group (7).

A cost analysis of the ICT card test was carried out during the research. The Knotts concentration method was shown to have lower price (ICT cost per unit US\$8 vs. Knotts concentration cost per unit US \$0.3). However, certain features of the ICT card test proved to be extremely advantageous high sensitivity, the ability to offer prompt diagnosis, no need for complicated laboratory procedures, and no need for specialized technicians. These combined characteristics overcame the low price of the Knotts concentration making to be the overall more cost effective option, thereby justifying its use as a diagnostic tool in screening in endemic areas.

In conclusion, about 60 percent antigenaemia in the study population is a matter of concern and necessary control programme is needed to check the transmission of filariasis in the local government and neighbouring local government.

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The average CFA prevalence was about 4 times higher than the Mf prevalence indicating that majority of antigenaemia were slightly higher in males than in infection was antigen positive but Mf negative. In this study, the prevalence of lymphatic filariasis was 3.56 times higher when determined by ICT compared to microfilaria examination in all age groups. This also confirms the work done by Cynthia et al., (2003) ^{1 2}

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Figure 1: Table 2 :

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Figure 2: Table 3 :

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Age Group (Year)	No Exam- ined	No(%) positive for Mf	No(%) Positive for CFA
27 -37	105	18 (17.1)	44 (41.9)
16 -26	178	29 (16.3)	90 (50.6)
38-48	67	6(8.9)	33 (49.2)
49-59	25	1 (4)	16 (64)
60-70	37	2(5.4)	19 (54.3)
71-81	13	-(0)	5 (38.5)
Total	425	56 (13.2)	207 (48.7)
Mf-Microfilaria			
No-number.			
-denotes absence of positive cased use in results section.			

[Note: A]

Figure 3: Table 1 :

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