VOLUME-7, ISSUE-7, JULY-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Biological Sciences

SEROPREVALENCE AND CLINICAL MANIFESTATION OF BANCROFTIAN FILARIASIS IN OTUKPO LOCAL GOVERNMENT AREA OF BENUE STATE, NIGERIA

Anyebe G. E.	Department of Zoology, Federal University, Lafia, Nasarawa State, Nigeria
Atu, B.O.*	Department of Biological Sciences, Benue State University, Makurdi, Nigeria *Corresponding Author
Rwang, P. G.	Department of Zoology, Federal University, Lafia, Nasarawa State, Nigeria
Ombugadu, A.	Department of Zoology, Federal University, Lafia, Nasarawa State, Nigeria

ABSTRACT This study assessed the prevalence of Bancroftian filariasis and the socio-economic factors responsible for the disease prevalence among the people of Otukpo Local Government Area (LGA) of Benue State, using immunochromatographic Card Test (ICT) procedure, questionnaire administration and physical examination of respondents for clinical manifestation of lymphatic filariasis morbidity. The overall prevalence of lymphoedema and hydrocoel in the area was 3.4% as was evenly observed in both male and female subjects. There was no statistical difference between communities ($X^2 = 8.45$, df = 5, $P > 0.05$) and as well as across age groups ($X^2 = 2.72$, df = 5, $P > 0.05$). Antigenaemia was found in 41 (11.3%) of 363 individuals examined using a randomized design. Majority of the infections (23.3%) were recorded at Aturukpo with the lowest prevalence (3.1%) recorded at Iwili. Statistically, prevalence rate differed significantly between communities ($X^2 = 5.4$, df = 1, $P < 0.05$). On the whole, the highest prevalence of 23 (11.1%) of the 41 antigenaemic individuals was recorded among farmers. A significant difference was observed in the distribution of positive cases of antigenaemia according to occupation ($X^2 = 87.4$, df = 7, $P < 0.05$) but showed no significant variation across age groups ($X^2 = 15.65$, df = 11, $P > 0.05$). The findings from this study showed that lymphatic filariasis is a problem in Otukpo Local Government Area (the headquarters of the Idoma's) hence, an urgent need to institute an elimination and morbidity management programme with a view to halting further transmission of the disease in the area.

KEYWORDS : *Wuchereria bancrofti,* Immunochromatographic Card Test (ICT), Antigenaemia, Lymphoedema, Lymphatic filariasis.

INTRODUCTION

Bancroftian filariasis is a disabling parasitic disease of the lymphatic system that is caused by infection with the filarial nematode roundworm called *Wuchereria bancrofti*. Infection occurs when the filarial parasite is transmitted to humans through mosquito bite. The female adult worm produces millions of microfilariae during its life-span of 4-6years (Nwoke *et al.,* 2010). The disease is characterised by Lymphangitis, Lymphangiovarix, Lymphorrhagia, Hydrocoele, Lymphoedema, Elephantiasis and filarial fever (Pani*et al*., 2005).

In Nigeria, the microfilaria of *Wuchereria bancrofti* exhibits nocturnal periodicity which synchronizes with the biting pattern of the local mosquito vector, appearing between 10p.m and 2a.m in the peripheral circulation before receding. The appearance of these microfilariae during this period is crucial to their development into the infective filariform (L3) larva in the gut of mosquito vector. When a mosquito with this infective L3 larva stage bites a person, the parasites are deposited on the person's skin from where they enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms in the human lymphatic system. This can result in an altered lymphatic system and the abnormal enlargement of body parts, causing pain and severe disability.

Bancroftianfilariasis is the only type of lymphatic filariasis that is endemic in Nigeria (Nwoke*et al.,* 2010). It has been listed among the Negleted Tropical Diseases (NTDs). This is so because it afflicts the poorest people that are often restricted to the tropical zones where the mosquito vectors thrive, imposing intolerable economic, health and social burden on the society. It does not cause death outrightly. The affected people live in rural areas and third world towns with ideal vector conditions of open sewage, poor drainage, inadequate housing and poor people (Brooker and utzinger, 2007; Dean, 2001, WHO, 2000)

Parasitological methods for diagnosis of *Wuchereriabancrofti* infection depend on microscopic detection of microfilariae (mf) in peripheral blood. However because of the difficulties often encountered when collecting night blood samples for microfilaria detection, an alternative method for the diagnosis of filariasis such as immunological detection of soluble parasite antigens in human blood has been developed (Targema*et al.,* 2005). Detection of circulating Filarial Antigen (CFA) being a useful non-microscopic method for diagnosis of bancroftianfilariasis is currently making the transition from the research laboratory to the field and can detect parasite antigen in both diurnal and nocturnal blood. To this end, the use of Immunochromatographic Card Test (ICT) for Circulating Filarial Antigen (CFA) detection of *Wuchereriabancrofti* was developed. It has been shown to provide more accurate information (99% sensitivity and specificity) on infection status than microscopy (Simonsen and Dunyo, 1998; Sunish*et al.,* 2002, WHO, 1999). This method has therefore been recommended as a rapid tool for defining the prevalence and distribution of filariasis as part of the global programme to Eliminate Lymphatic filariasis (PELF) (Schuetz*et al.,* 2000).

In Nigeria, Bancroftianfilariasis has been reported in rural communities in the lower Cross River Basin (Braide*et al.,* 2003; Eigege*et al.,* 2002). There are however other potentially endemic areas in the country that are yet to be studied. This study therefore investigated the status of the disease in some rural communities within Otukpo Local Government Area, Benue State Nigeria with a view to enriching the epidemiological baseline data in Nigeria hence morbidity management.

MATERIALS AND METHODS

Study Area

Benue State is administratively sub-divided into twenty-three (23) Local Government Areas (LGA including the study area; Otukpo LGA. The study area lies between latitude $7^{0}00^{'}$ and 7^{0} 40N and longitude $7^{0}30^{'}$ and 8^{0} 40E with a rapidly growing population of 261.666 (National Population Commission, 2006). It is bordered in the North by Apa, North East and East by Gwer and Obi LGAs, South and South-West by Ado and Ohimini LGAs while it shares a common boundary with Olamboro LGA to the west in Kogi State.

Study Sites: These included Ejoh, Adim, Aturukpo, Ojinebe, Iwili and Ojantele.These sites share common boundaries with other LGAs in

the state; some of which are endemic with Bancroftianfilariasis (Omudu and Okafor, 2010; Omudu and Ochoga, 2011). There are many seasonal streams in the study area which dries up during the dry season leaving stagnant ponds and ditches. Rice paddies and broken clay pots are common sites around residences and these could constitute ideal breeding sites for the mosquito vector during the wet season (Mid June to October).

IMMUNOCHROMATOGRAPHIC CARD TEST PROCEDURE

Study Subjects

Fifty-nine (59) study subjects aged >15 years were tested for daytime filarial antigenaemia using immunochromatographic card in each sampled community. A total of 363 persons (149 males and 214 females) constituted the sample size from the six communities. The participating subjects were assigned an identification number each. The number, sex and age of each subject were written on each immunochromatographic card in order to keep correct records of each subject and to avoid mixing up blood samples.

Data collection and physical examination for Bancroftianfilariasis morbidity

Basic demographic information (age, sex, educational status, occupation and ethnicity) on each study subject was obtained and recorded on the survey form. Confirmed oral consent of the subjects was sought before the clinical examinations were carried out. Subjects were asked to partially disrobe after which observation and palpation of the limbs and genitals of the subjects were carried out by trained health professionals of both sexes behind closed doors. Physical examinations of males included the genitals, the lymph glands in the groin and axila, the legs and arms. Female examination was restricted to the legs, arms and breasts because of cultural inhibitions of the people. Assessment of chronic lymphoedema and urogenital problems in filariasis was done according to guidelines provided by Dreyer *et al.,* (2002). The features that were used in staging lymphoedema were reversible swelling, shallow and deep folds, knobs, mossy and entry lesions, bad odour and cracks or peeling of the skin. For genital problems, assessment was done based on enlargement and deformity of the genital organs (the penis and the scrotum). The appearance of the genitals was also assessed and the findings for each study subjects were recorded accordingly.

Blood collection

Blood samples were collected from willing individuals only. The left thumb of each study subject was cleaned using a cotton swab (cotton wool dipped into methylated spirit). Pressure was exerted on the thumb to increase the pressure of the blood by simply pressing the thumb. Using a sterile lancet, the thumb was pricked, the first blood that came out was wiped off with cotton wool and subsequent blood was collected using a heparinised calibrated capillary tube. Blood sample collected was used immediately for the test.

Immunochromatographic card test procedure

- Cards were removed from pouch just prior to use
- The patient's left thumb was swabbed following confirmed oral consent and then punctured using a sterile lancet.
- The initial sample of blood was removed using a cotton swab, and sufficient fresh blood was obtained to fill a one hundred micro litres (100µl) heparinised calibrated capillary tube. Care was taken to avoid collection of air bubbles along with the blood. Where air bubbles were found, the capillary was filled past the 100µl line to compensate for the space occupied by the air bubbles. Blood was not applied directly from the finger either to the white or pink portion of the sample pad.
- Blood sample from the heparinised calibrated capillary tube was then added slowly to the white portion of the sample pad. It was left uncovered for approximately 30seconds to allow blood sample migrate to the pink portion of the card.
- The adhesive liner was later removed and the card closed. The starting time was noted on each card. The researcher ensured that the cards were read only after the plasma had flowed all the way down the strip.
- Test results were read 10minutes after closing card not at any other time to forestall false positive readings.
- A positive result showed two pink lines (Test and Control) on the card's window, and a negative result showed a single line (Control). Invalid tests were recorded when the control line did not appear.
- Test result with the individual's identification code was recorded on the patients' diagnostic data sheet.

Ethical Clearance

This was received from the Benue State Ministry of Health and the postgraduate Research committee of the Benue State University. The purpose of this study was explained to the local health authorities, village chiefs so as to obtain their permission and consent. Informed oral consent was sought from all the participants after the explanation of the procedures and the likely benefits because it is the traditional way of making agreements in the study areas as against written consent which could cause suspicion and refusal to participate. The study took care to respect confidentiality and all data were kept anonymously.

RESULTS

Antigenaemia in Relation to Different Parameters

Infection in Relation to Communities

The overall prevalence of infection in the study area was 41(11.3%) (Table 1). A total of 363 individuals (149 males and 214 females) constituted the sample size. The self-reported ages ranged between 15 to ≥ 89 years old with a median age of 53 years. Antigenaemia were found in 41 (11.3%) out of the 363 individuals. The overall prevalence of 11.3% antigenaemia was observed in Aturukpo, Ejoh, Iwili, Adim, Ojantele and Ojinebe. This record was highest in Aturukpo (23.3%) followed by Ejoh and Adim (13.3% & 13.3%) while the least were Iwili and Ojinebe (3.1% & 3.4%) (Table 1). Prevalence differed significantly between the six communities ($X^2 = 14.77$, df = 5, $P < 0.05$).

Table 1. Prevalence of *Wuchereria bancrofti* Antigenaemia in the Surveyed Communities in (%)

Communities	No. Examined	No. +ve (%)
Aturukpo	60	14 (23.3)
Ejoh	60	8 (13.3)
Iwili	64	2 (3.1)
Adim	60	8(13.3)
Ojantele	60	7 (11.7)
Ojinebe	59	2(3.4)
Total	**363**	**41 (11.3)**

Distribution of *Wuchereriabancrofti*Antigenaemia According to Gender and Age-group

Figure 1 showed the infection rate in males to be considerably higher than females. Statistical analysis did show a significant difference between the rates of infection in males and in females ($X^2 = 5.4$, df = 1, $P < 0.05$). Figure 2 showed antigenaemia prevalence by age in the entire study area. A gradual increase was observed in the youngest age groups, reaching a peak amongst the age group (75-89) and followed by a sharp decrease in age group > 89. The antigenaemia prevalences in the different age groups is summarized in figure 2. Statistical analysis did show a significant difference between the rates of infection in the age-groups ($X^2 = 15.65$, df = 1, $P < 0.05$).

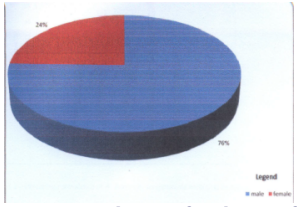

Figure 1- Percentage prevalence of W. bancrofti antigenaemia according to gender

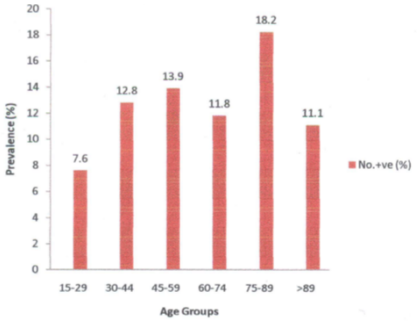

Figure 2: Percentage prevalence of *W. bancrofti* antigenaemia according to age-groups

Distribution of Chronic Morbidity

The overall prevalence of lymphoedema and hydrocele in the study area was 3.4 as was observed in both male and female (Table 2). Of the six communities visited, only Adim showed some clinical presentations of Bancroftianfilariasis among its people. Chronic manifestations such as lymphoedema of both the left and right legs and hydrocele were encountered during the study. Out of the three hundred and sixty-three individuals examined for clinical manifestations of the disease, (1.7%) had lymphoedema (Table 2) and the youngest person with lymphoedema was 15 years old. There was no statistical difference between people examined in the communities ($X^2 = 8.45$, df = 5, P > 0.05). One (1.7%) of the males examined aged 35 years had hydrocele. Figure 3 shows the distribution of the disease between males and females, however there was no statistical difference ($X^2 = 0.00$, df = 1, P > 0.05) between gender. Chronic clinical morbidity was not statistically associated with age ($X^2 = 2.72$, df = 5, P > 0.05). Plates 1 and 2 show the type and grades of lymphoedema observed during the study.

Table 2: Prevalence of Morbidity in the Surveyed Communities in (%)

Communities	No. Examined	Lymphoedema	Hydrocele
Aturukpo	60	0(0.0)	0(0.0)
Ejoh	60	0(0.0)	0(0.0)
Iwili	64	0(0.0)	0(0.0)
Adim	60	1(1.7)	1(1.7)
Ojantele	60	0(0.0)	0(0.0)
Ojinebe	59	0(0.0)	0(0.0)
Total	**363**	**1(1.7)**	**1(1.7)**

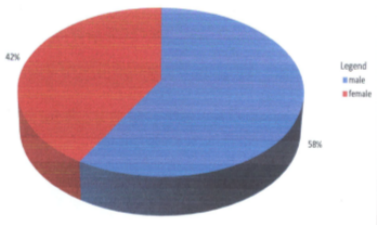

Figure 3: Percentage prevalence of morbidity by gender

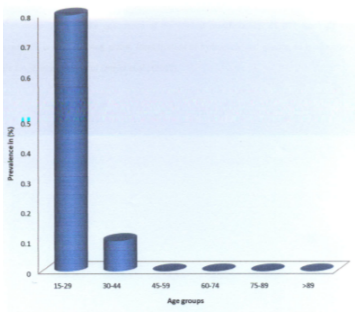

Figure 4: Percentage prevalence of morbidity according to age-group

Clinical Observations

Observations made on the occurrence of the clinical manifestations of *W. bancrofti* were documented in the following plates. Identification of hydrocoele and grading of lymphoedema were based on guidelines of Dreyer *et al.* (2002).

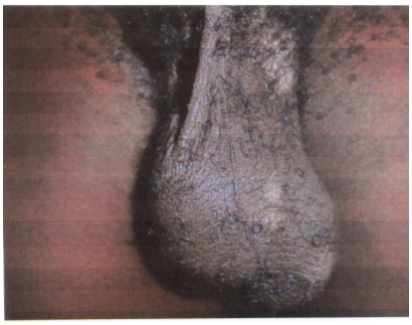

Plate 1: Hydrocele at its early stage

Plate 2: Lymphoedema of both legs showing both shallow and deep folds with lesions producing smelly secretions

DISCUSSION

This study presents a baseline data on the study area. Antigenaemia prevalence of 11.3% recorded in 6 communities showed that Otukpo LGA is endemic with the parasite. The observed variation in the prevalence of the infection in relation to the communities under study possibly suggests that the mosquito vectors thrive more some of areas due to favourable weather condition. These results make the LGA eligible for mass drug administration (MDA) for the elimination of lymphatic filariasis. According to WHO (1999), prevalence of antigenaemia or microfilaraemia of 1% or greater is enough to declare an area endemic and "countries should be prepared to implement mass treatment in all areas where the prevalence of infection is above 1% in order to achieve elimination of lymphatic filariasis". The higher prevalence among the males 28(18.8%) than females 13 (6.1%) may be explained by the exposure frequency. The males spend more hours outdoors at nights in social activities. Variations in clothing could be another reason for the higher prevalence in males. In most of the rural communities, after the activities of the day, the men in most cases are casual in their dressing, exposing a greater portion of their bodies whereas the women dress to cover their bodies. Antigenaemia variation between males and females may possibly be due to more exposure of the males to the vectors.

The high prevalence rate of antigenaemia as was observed among age groups 30-44 and 45-49 is not surprising because both men and women in that age bracket are heads of their families struggling to carter for responsibility which most times leave them exhausted and may want to stay out late for a longer time. The prevalence rate of 7.6% in age group 15-29 in this study showed that with the use of the antigen detection cards, it is easier to diagnose infection even in the younger children. According to WHO (2000b), infection can be acquired early in life but pathology develops slowly and overt disease becomes expressed in the teens or in early adult years. The results obtained from this study have indicated clearly that Otukpo

LGA in Benue State is endemic with *Wuchereriabancrofti*. The entire six communities in the LGA were found to be endemic although Iwili and Ojinebe had just two positive cases each. The current information showed high endemicity, the devastating impact of the disease and the need to begin intervention measures for the control of lymphatic filariasis (LF) in the area.

Environmental factors and human behaviour are thought to be the main reasons for the endemicity recorded in this study. Some of the communities have thick vegetation as well as seasonal streams. These streams dry up during the dry season leaving behind stagnant water bodies which are ideal breeding sites for the mosquito vectors. Cultivation of crops is usually in the rainy season when transmission is active due to an abundance of ideal breeding sites which when disturbed could force the mosquito out to bite especially during the early hours of the morning and thus transmit the parasite. Jaoko *et al.* (2001) had also observed that the highest transmission intensity occurred during and immediately after the rainy season (May-June) while the lowest intensity was during the dry season. More so, there is so much poverty in the land and most of the rural population cannot afford a well treated mosquito net let alone employ the use of insecticides or any other vector control measures. Most windows were without nets and their doors were left open while they stayed out late to relax themselves. Poverty and poor living conditions have been reported to be the leading causes of the persistent lymphatic filariasis in Haiti and other resource Poor countries (Coreil *et al*., 2003). In the philipines, lymphatic filariasis endemic areas are in the regions with the highest incidence of poverty (Galvez, 2003). Lymphatic filariasis as a disease of poverty has been reported elsewhere (Pani *et al.,* 2005).

The prevalence of chronic disease (Lymphoedema) in Adim could be attributed to environmental and health seeking behaviours that promoted rapid progression of the disease. The prevalence of chronic and acute manifestations of the disease was low, but consistent with previous studies from the other parts of sub Saharan Africa (Estambale *et al.,* 1994) and Ghana (Gyapong *et al*., 1998) where scrotal lymphoedema was the most common clinical manifestation in communities, lymphoedema and hydrocoele had equal prevalence in this study. In some of the communities visited, the subjects complained of having gone through hydrolectomy before our arrival. In their own words:

"So many men in this village used to have swollen scrotum, but they have all operated them so that there is hardly anyone left".

These percularities are responsible for the seemingly low record of hydrocoele in the study area. Lymphoedema of both the left and right legs was observed in a female at Adim whereas hydrocoelwas observed in a male. As reported in similar studies (Estambale *et al.,* 1994; Gyapong *et al.,* 1996; Meyrowitsch *et al.,* 1995; Weerasooriya *et al.,* 2001), the condition occurred later in life and was very rare among children below 15 years old.

The high prevalence of antigenaemia coupled with cases of morbidity observed is an indication that transmission has been on-going for a long time and it still continues.In most diseases the presence of disease often correlates well with infection status but for reasons unclear, there was no correlation between antigenaemia and lymphoedema of the legs. However antigenaemia was found in the male with scrotal lymphoedema but absent in the female with leg lymphoedema. Similar interactions were observed in Haiti (Addiss *et al.*, 1995), Tanzania (Simonsen *et al.,* 1995) and Ghana (Gyapong *et al.,* 1998).

CONCLUSION AND RECOMMENDATION

The WHO (1998a) recommended that ''countries should be prepared to implement mass treatment in all areas where microfilaraemia or antigenaemia is above 1%...'' The findings from this study showed that lymphatic filariasis is a problem in Otukpo local government area and Benue State at large. There is therefore an urgent need to institute an elimination and morbidity management programme with a view to halting transmission in the area. This should be further strengthened because, even if the LF elimination succeeds in halting transmission, many people will continue to suffer clinical manifestations.

REFERENCES

1. Addiss, D. G., Dimock, K. A., Eberhard, M. L. and Lammie, P. J. (1995).Clinical, Parasitological and Immunology observations of patients with hydrocoele and elephantiasis in an area with endemic lymphatic filariasis.Journal of Infectious Diseases 171: 755-758.
2. Braide, E. I., Ikpeme, B., Edet, E., Atting, I.And Kale, O. O. (2003). Preliminary observations on the occurrence of lymphatic filariasis in Cross River State, Nigeria. Nigerian Journal of Parasitology, 24:9-16.
3. Brooker, S. and Utzinger, J. (2007).Integrated Disease Mapping in a polyparasitic World. Geospatial Health, 2:141-146.
4. Coreil, J., Mayard, G. and Addis, D. (2003). Support groups for women with lymphatic filariasis in Haiti. Social, Economic and Behavioural Research Report Series, 2:1-48.
5. Dean, M. (2001).Lymphatic Filariasis. Hollis Publishing Company, U.S.A. 1-43.
6. Dreyer, D., Addiss, D., Dreyer, P. And Noroes, J. (2002). Basic Lymphoedema Management: Treatment and Prevention of Problems Associated with Lymphatic Filariasis. Hollis Publishing Company, Hollis, U.S.A., 7-66.
7. Eigege, A., Richards, F.O.,Jnr. Blakey, D.D., Milri, E. S., Gontor, I., Ogah, G., Umaru, J., Jinadu, M. Y., Mathai, W., Amadiegwu, S. and Hopkins, D. R. (2002). Rapid Assessment for Lymphatic Filariasis in Central Nigeria: A Comparison of the Immunochromatographic Card Test and Hydrocoel Rates in an Area of High Endemicity. American Journal of Tropical Medicine and Hygiene, 68(6):643-646.
8. Estambale B. B. A., Simonsen, P. E., Knight, R. and Bwayo, J. J. (1994). Bancroftianfilariasis in Kwale district of Kenya. I. Clinical and Parasitological survey in an endemic community. Annals of Tropical Medicine and Hygiene, 88(2), 145-151.
9. GalvezTan, J. Z. (2003). The elimination of lymphatic filariasis.A Strategy for Poverty Alleviation and Sustainable Development-Perspectives from the Philipines.Filarial Journal, 2(12).http://www.filarialjournal.com/2/1/12.
10. Gyapong, M., Gyapong, J.O., Adjei, S., Vlassoff, C., Weiss, M. (1996). Filariasis in northern Ghana: some cultural beliefs and practices and their implications for disease control. Social Science and Medicine, 43: 235-242.
11. Gyapong, J.O., Webber, R.H., Morris, J.O. and Bennett, S. (1998). Prevalence of hydrocoeleas a rapid diagnostic index for lymphatic filariasis.Transactions of the Royal Society of Tropical Medicine and Hygiene. 92:40-43
12. Jaoko, W.G., Simonsen, P.E., Meyrowitsch, D.W., Pederson, E. M., Rwegoshora, R. T. And Michael, E. (2001).Wuchereriabancrofti in a community with seasonal transmission: stability of microfilaraemia, antigenaemia and filarial specific antibody concentrations. Annals of Tropical Medicine and Parasitology 95(3):253-261.
13. Meyrowitsch, D. W., Simonsen, P. E. and Makunde, W. H. (1995). Bancroftianfilariasis: An analysis of infection and disease in five endemic communities of north-eastern Tanzania. Annals of Tropical Medicine and Parasitology, 89:653-663.
14. National Population Commission, (2006).National Policy on Population for Sustainable Development.Federal Government of Nigeria. Lagos 38.
15. Nwoke, B.E.B., Nwoke, E.A., Ukaga, C. N. and Nwachukwu, M. I. (2010).Epidemiological characteristics of Bancroftianfilariasis and the Nigerian Environment.Journal of Public Health and Epidemiology, 2(16):113-117.
16. Omudu, E. A. And Ochoga J. O. (2011).Clinical Epidemiology of Lymphatic filariasis and Community Practices and Perceptions among the Ado People of Benue State, Nigeria.African Journal of Infectious Disease, 5(2): 47-53.
17. Omudu, E. A. and Okafor, F. C. (2010). Gender Dimensions of Knowledge, Physical and Psycho-social Burden due to Lymphatic filariasis in Benue State, Nigeria. Journal of Parasitology and Vector Biology, 3(2):22-28.
18. Pani, S. P., Kumaraswami, V and Das L. K. (2005).Epidemiology of lymphatic filariasis with special reference to urogenital manifestations.Indian Journal of Urology, 21(1):44-49.
19. Schuetz, A., Addiss, D. G., Eberhard, M. L. and Lammie, P. J. (2000).Evaluation of the Whole Blood Filariasis ICT Test for Short-term Monitoring after anti-filarial Treatment.American Journal of Tropical Medicine and Hygiene, 62 (4): 502-503.
20. Simonsen, P. E. and Dunyo, S. K. (1998).Comparative Evaluation of three New Tools for Diagnosis of Bancroftianfilariasis on Detection of Specific Circulating Antigens. Transactionsof the Royal Society of Tropical Medicine and Hygiene, 93:278-282.
21. Simonsen, P.E., Meyrowitsch, T. W., Makunde, W. H. And Magnussen, P. (1995).BancroftianFilariasis: The Pattern of Microfilaraemia and Clinical Manifestations in three Endemic Communities in North-eastern Tanzania. ActaTropica, 60:179-180.
22. Sunish, I. P., Rajendran, r., Mani, T. R., Munirathinam, a., Tewari, S. C., Hiriyan, J., Gajanana, A. and Satyanarayana, K. (2002).Resurgence in filarial transmission after withdrawal of mass drug administration and the relationship between antigenaemia and microfilaraemia-a longitudinal study.Tropical Medicine and International Health 7(1):59-69.
23. Targema, N., Onwuliri, C.O.E., Mafuyai, H. B. And Mwansatl, G. S. (2005).Mapping of Lymphatic Filariasis in Benue State Nigeria.NigerianJournal of Parasitology,29(1):55-60.
24. Weerasooriya, M. V., Weerasooriya, T. R., Gunawardena, N. K. And Samarawickrema, W. A. (2001).Epidemiology of Bancroftianfilariasis in three Sub-urban Areas of Matara, Sri Lanka. Annals of Tropical Medicine and Parasitology, 95(3):263-273.
25. World Health Organization (1998a). Report of a WHO Informal Consultation Epidemiological Approaches to Lymphatic Elimination: Initial Assessment, Monitoring and Certification. WHO/FIL/99.195:1-32
26. World Health Organization (1999).Tropical Disease Research. Progress 1997-98. Fourteenth Programme of Bancroftianfilariasis.TDR/TDF/ComDT 98(2):1-16.
27. World Health Organization (2000). Preparing and implementing a national plan to eliminate lymphatic filariasis: In Countries where Onchocerciasis is co-endemic. WHO/CDS/CPE/CEE/2000.16