Epidemiological And Biochemical Studies Of Human Lymphatic Filariasis And Associated Parasitoses In Oguta, Southeastern Nigeria

O Ojiako, G Onyeze

Citation

O Ojiako, G Onyeze. *Epidemiological And Biochemical Studies Of Human Lymphatic Filariasis And Associated Parasitoses In Oguta, Southeastern Nigeria*. The Internet Journal of Parasitic Diseases. 2008 Volume 4 Number 1.

Abstract

Possible organ infections associated with human filariasis, helminthiasis and malaria in Oguta Local Government Area of Imo State, Southeastern Nigeria were investigated. Blood, urine and stool samples were collected in appropriate containers from 200 male and female respondents aged 31 – 85 years. Parasitological studies were carried out on blood samples for malaria and/or microfilariae parasites while stool samples were tested for the presence of some intestinal parasites. The study showed a prevalence of intestinal protozoa (Entamoeba histolytica), Wuchereria bancrofti and the intestinal helminthes Ascaris lumbricoides and Hookworms. Biochemical parameters of liver integrity were also studied across the various infection cohorts among the respondents. Results obtained show that these parasitic infections depressed the haematological parameters relative to 'normal' respondents. Comparative biochemical analyses showed significant ($p<.05$) differences in some liver function parameters obtained for infected respondents relative to those not infected. There was also a positive correlation between age brackets with highest filarial infection (with no malarial coinfection) and age groups with elevated markers of liver dysfunction. This study can be of immense diagnostic value in the clinical management of the filariases especially in malaria-endemic and resource-poor areas.

INTRODUCTION

Till date the dream of eliminating some parasitic diseases like malaria and filariasis has not been fully realised. Instead, there appears to be a recrudescence of these old endemic debilitating parasitic diseases in some parts of developing countries. Malaria is still known to be the major cause of mortality and morbidity in the tropical and subtropical regions of the world (WHO, 2004) and is caused by Plasmodium species that have mosquitoes as their intermediate hosts and also serve as vectors of infective parasite stages to man. An added danger to malarial infections is that its effects and even recrudescence after intervention are usually worse with children (Borrmann et al, 2008).

Filariasis on its own is a major public health problem in many parts of Asia, Africa, the Western Pacific and the Americas (Anosike et al, 2005). It is actually a group of diseases whose causative parasites have different vectors. Filariases are a group of vector – borne parasitic diseases of humans and other animals, caused by long, threadlike worms (hence the name "filaria" from Latin) that in their mature adult stages reside in the lymphatics or in connective tissue. Of the eight filarial parasites that commonly infect man three species account for most of the pathology associated with these infections: the lymphatic dwelling filariae Wuchereria bancrofti and Brugia malayi and the skin dwelling Onchocerca volvolus (Ottesen, 1984). Infection by Wuchereria bancrofti is the most common (Anosike et al, 2001) and accounts globally for approximately 90% of all infections. Worldwide, over 120 million people are infected with lymphatic filariasis and in Africa, the prevalence is especially striking, affecting over 40 million people in the sub-Saharan region alone. Overall, Africa is thought to account for 40% of all global cases (Lenhart et al 2007).

The health, social and economic burdens of endemic tropical parasitic diseases have been assessed to include direct disease-related costs to individuals and households, costs to government-funded healthcare systems, lost productivity of infected individuals, and reduced productivity from structural changes in the economies of endemic villages (Evans et al, 1993; Gyapong et al, 1996; Haddix and Kestler, 2000). In Nigeria and elsewhere where diseases like helminthic infections, malaria and filariasis are endemic, the

conventional diagnostic techniques are invasive and often times repetitive as different blood samples will be required for parasitological examination of filarial and malaria patients and confirmation is based on positive parasitaemia. They are also highly technical, time consuming, expensive and in most cases fraught with poor cooperation from patients who are already anaemic. Yet due to several factors including poverty, development projects without environmental impact assessment and other activities that favour the breeding of the mosquito vectors (Haddix and Kestler, 2000) the burden of these parasitic diseases has virtually bent the back of the inhabitants of the tropics and does not seem to be abating as evidenced by recent studies (Anosike et al, 2005; Okoye and Onwuliri, 2007). Incidentally, both malaria and filariasis have feverish symptoms and in rural areas it is often difficult to distinguish drug-resistant falciparum malaria and periodic fever due to filaria infection.

These facts justify the need for more and possibly faster tools of diagnosis, management and control of these parasitic diseases. This work is therefore aimed at studying the patterns of some of these parasitic infections especially lymphatic filariasis as well as assessing the possibility of using biochemical parameters in its diagnosis in the presence or absence of malaria and intestinal helminthes whose febrile and nauseous symptoms oftentimes confuse with those of filariasis.

MATERIALS AND METHODS

Study Area: The study was carried out in Oguta Local Government Area, one of the 27 Local Government Areas of Imo State in the Southeastern part of Nigeria. The administrative headquarters, Oguta, is a sub-urban town. Most of the residents of the local government area (LGA) are predominantly farmers, fishermen and civil servants. Oguta town has some recreational facilities like an international golf course, the Oguta Lake and the Oguta Lake Motel among others in addition to a natural scenic beauty accentuated by the presence of rivers which nearly encircle the town and the adjoining towns in the LGA. The presence of these rivers which serve as mosquito breeding sites coupled with the continual interaction with the water bodies in normal daily activities and chores makes the area potentially endemic for mosquito- and water-associated parasitic infections.

Study Group: A total of 200 potential patients were recruited over 1000 volunteers. Informed consent of the volunteers was obtained as demanded by WHO (TDR, 2001). With the assistance of individuals recognised by WHO (2004) as key informants like school teachers, private medical practitioners, patent medicine dealers and traditional healers, certain villages were mapped out for study after which possible respondents to a preliminary questionnaire were identified. Male and female respondents aged between 30-85 years with clinical symptoms like presence of eye worm, skin depigmentation, itching, diarrhoea, fever, hydrocoele, lymphoedema and elephantiasis (TDR, 2002) etc were selected for the study. There were no language and cultural limitations. Blood, urine and faecal samples were collected from volunteers among the selected respondents who were fully aware of the objectives of the study and the possible benefit of free drugs should any eventually be confirmed positive.

Sample Collection: Urine, faecal and blood samples from each respondent were collected by qualified health personnel using appropriate containers which were then covered in black polythene bags and stored in small plastic food coolers loaded with ice before analyses.

Mid-stream urine samples were collected and examined for the presence/absence of cysts, ova etc as recommended by Zeibig (1997). A 5ml portion of blood was collected from each respondent using a disposable syringe and a needle. A 2ml portion of the blood sample was put into an E.D.T.A. bottle to prevent clotting while the remaining 3ml was allowed to clot in the syringe. Sera from the clotted samples were used for liver function tests after centrifuging for 10 minutes at 3000rpm with a Wisperfuge Centrifuge (Ojiako and Nwanjo, 2006) while the unclotted samples were used for parasitological examination for malaria and filaria parasites as described by Zeibig (1997).

Urinalyses using MediTest Combi 9 Test strips designed to detect and roughly estimate the following parameters: pH, glucose, ascorbic acid, protein, ketone, nitrite, bilirubin, urobilinogen and blood were carried out in all urine samples prior to other analysis. Urine samples were further analysed microscopically for the presence of epithelial cells, white blood cells (WBC), red blood cells (RBC), casts, crystals and organisms such as yeast cells, Trichomonas vaginalis, ova of Schistosoma haematobium, according to the methods of Baker et al, (2000) and Zeibig (1997). All samples that tested positive or slightly positive for infection or another disease condition different from those under study were discarded to avoid confusion with the possible role of

filariasis and the associated diseases under study.

Parasitological Tests: The direct wet smear technique of stool analysis was used to determine the presence of ova or larvae of helminthes. Thin and thick blood films stained by Giemsa staining technique were used to detect and confirm malaria and filaria parasites (Zeibig, 1997; Udonsi, 1999; Wanji, 2001).

Biochemical and Haematological Tests: Total and conjugated bilirubin levels in serum samples were measured according to the modified (Henry, 1974) method of Malloy and Evelyn (1937). The activity of alkaline phosphatase E.C. 3.1.3.1 (orthophosphoric monoester phosphohydrolase) was measured according to the method of King and Armstrong (1934) as modified in Tietz (1983) while aspartate aminotransferase (ASAT) E.C.2.6.1.1 and alanine aminotransferase (ALAT) E.C.2.6.1.2 activities were measured spectrophotometrically (Camspec M210 spectrophotometer) using RandoxTM kits based on the method of Reitman and Frankel (1957). Haemoglobin levels were determined spectrophotometrically using Drabkins's solution (potassium ferricyanide, BDH) while packed cells volume (PCV) was determined using the microhaematocrit method (Tietz, 1976; Baker et al, 2000).

Statistical Analysis: Normal values for the different parameters were obtained by measuring the same parameters (measured in parasite-positive cases) in a set of persons (matched for age and gender) in the same area that tested negative to all the parasitic infections investigated. Chi-square test of independence was used to test dependence/independence of measured parameters with respect to patterns of parasitic infection or age group of respondents. Chi-square test of homogeneity was used to ascertain any difference within cohorts. Student's t-test was used to measure differences between measured parameters and normal values. Differences at $P < 0.05$ were considered significant (Bailey, 1994).

RESULTS

Table 1 shows the patterns of infection of the studied parasitoses according to age while Table 2 shows the relative prevalence of the parasitic infections in the studied population. Table 3 shows the patterns of infections and coinfections of the diseases while Tables 4 and 5 show the results of the liver diagnostic and haematological parameters respectively. Results of the parasitological examination of blood and stool samples show that of the 200 chosen respondents, 40 (20%) had filariasis while 15% had malaria. Of the filaria- and malaria-positive cases there were mutual coinfections as well as coinfections by helminthiasis (Ascariasis lumbricoides and hookworms) and protozoasis (Entamoeba histolytica) as shown in Tables 1 and 3.

The respondents fall within the age bracket of 30 and 85 even though some of the respondents did not know their exact age and we had to use some well established historical events like the second World War, Nigerian Independence in 1960 and the Nigerian Civil
War (1967-1970) among others to place their age. Infection of lymphatic filariasis was highest among the age brackets 41-45 and 51-55 and no infection was observed among the age brackets 31-35, 36-40, 61-65, 71-75 and 76-80. There were however positive parasitoses in all the age groups even in the absence of filariasis except for age group 76-80 which had no sample representative in the study.

Analyses of gender-related prevalence (Table 2) show that though there were more males (112) than females (88) among the respondents, there were more positive cases of plasmodiasis, filariasis and helminthiasis among the female respondents in the study area. Of the 20% overall prevalence of filariasis there was a gender-specific significant difference ($p < 0.05$) between the female (26.10% of total sample) and the male respondents (15.18% of total sample).

Results of the liver function tests (Table 4) show that of the 40 filaria-positive cases 23 (57.50%) had elevated (above adult normal levels) levels of bilirubin and also elevated levels of alanine and aspartate aminotransferase. Statistical analyses of these results using sign test show that there were significant differences ($p< 0.05$) in the values of liver function parameters of respondents infected with filariasis and malaria and the corresponding values for those not infected. Bilirubin levels, for instance, were highest in the 41-45, the age range with the highest prevalence of filariasis, followed by the age range 61-66, the age range with the highest prevalence of malaria. The values for the haematological parameters, packed cells volume (PCV) and haemoglobin level however showed no significant differences among the various groups.

Figure 1

Table 1: Age Profile Of Respondents And Patterns Of Infection Of Studied Parasitic Diseases

Age (Years)	Sex	Number Examined	Filariasis	Plasmodiasis	Intestinal Helminthes	Intestinal Protozoa
31-35	M	6	-	2	-	2
	F	7	-	-	1	-
36-40	M	-	-	-	-	-
	F	12	-	2	-	2
41-45	M	17	7	-	1	-
	F	13	9	1	-	-
46-50	M	7	-	-	1	3
	F	11	3	-	1	1
51-55	M	19	5	2	-	-
	F	13	6	-	-	1
56-60	M	23	3	-	-	2
	F	11	-	-	-	1
61-65	M	22	-	2	-	-
	F	7	-	2	-	-
66-70	M	18	2	1	-	-
	F	5	4	1	-	-
71-75	M	-	-	-	-	-
	F	6	-	2	-	-
76-80	M	-	-	-	-	-
	F	-	-	-	-	-
81-85	M	-	-	-	-	-
	F	3	1	-	-	-
Total		200	40	15	4	13

Figure 2

Table 2: Prevalence of Studied Parasitic Diseases

Disease	**Sex**	**Number Examined**	**Number Infected**	**Prevalence (%)**
Filariasis	Male	112	17	15.18
	Female	88	23	26.14
	Total	200	40	20.00
Malaria	Male	112	7	6.25
	Female	88	8	9.10
	Total	200	15	7.5
Intestinal helminthiasis	Male	112	1	0.89
	Female	88	3	3.41
	Total	200	4	2
Intestinal protozoa	Male	112	9	8.04
		88	4	4.55
	Total	200	13	6.5

Figure 3

Table 3: Patterns Of Infection And Co-Infection Of The Studied Parasitic Diseases

Parasitic Infections	**Number Affected**	**Percentage of Sampled population**
Filariasis	40	20
Malaria	8	4
Filariasis + Malaria	7	3.5
Filariasis + Intestinal helminthiasis	4	2
Filariasis + Intestinal protozoasis	13	6.5

Figure 4

Table 4: Results Of Liver Diagnostic Parameters

Age (Years)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)
31-35	65.2 ± 18.38	8.00± 0.01	15.01 1.44	12.83 ± 3.59	5.99 ± 1.20
36-40	66.01 5.65	8.00 0.02	15.5 0.71	13.68±2.39	7.70 ±1.20
41-45	89.3 32.33	14.01 6.92	26.0 12.48	22.23± 7.70	7.18± 0.68
46-50	78.4 12.75	6.60 0.89	16.6 2.40	14.36± 0.86	11.29± 5.47
51-55	78.6 15.51	10.40 4.39	18.0 6.04	15.05± 4.45	8.55± 2.91
56-60	69.3 25.78	9.30 4.43	17.3 5.46	14.54 ±8.04	7.35± 3.93
61-65	85.0 22	12.2 4.26	22.4 7.16	18.13± 6.16	9.92± 6.33
66-70	68.5 10.24	8.80 1.25	16.3 1.71	11.12± 2.22	6.50± 0.86
71-75	72.01 0.01	8.01 0.02	15.0 0.06	13.68± 0.17	6.84 ±0.17
76-80	-	-	-	-	-
81-85	94.01 0.01	14.01 0.02	20.0 0.08	15.39± 0.17	8.55± 0.17
Control	**65.60± 12.83**	**8.40± 1.74**	**15.26 ±1.08**	**13.25±3.45**	**6.86 ± 1.21**

ALP = Alkaline phosphatase, ALT= Alanine aminotransferase,
AST = Aspartate aminotransferase.

Figure 5

Table 5: Results of Measured Haematological Parameters

AGE (Years)	PCV (%)	HAEMOGLOBIN (g/dL)
31-35	35.01 ± 7.07	10.30 ± 2.12
36-40	33.52 ± 6.36	10.30 ± 2.40
41-45	32.31± 5.50	9.80 ± 1.44
46-50	33.20 ± 3.11	10.06 ± 1.23
51-55	32.40 ± 2.88	9.80 ± 1.04
56-60	34.72 ± 5.13	9.60 ± 1.93
61-65	34.62 ± 2.79	10.20 ± 1.33
66-70	34.84 ± 4.57	10.40 ± 1.08
71-75	30.01 ± 0.01	8.80 ± 1.01
76-80	-	-
81-85	27.00 ± 0.02	8.40 ± 0.07
Control		
Male	40.54 ± 7.81	13.61 ± 1.95
Female	36.44 ± 9.05	11.54 ± 5.32

PCV = Packed cell volume

DISCUSSION

The findings of this study showed that 20% of all the respondents examined were infected with filariasis. This is ordinarily a confirmation of endemicity. The sampling procedure however was not a blind and random one and the aim of the study was not to determine endemicity. There was already a statewide distribution of ivermectin (MectizanR) and prevalence of disease has already been confirmed in different parts of the State (Nwoke, et al, 1994; Dozie, 2003). The respondents were recruited from a group of volunteers with clinical symptoms of filariasis who were already feeling sick and responded to invitation of health personnel with the hope of receiving free medication.

Also, disease prevalence was confirmed using presence of microfilaria in blood. It is widely known (Nutman, 2000, , et al., 2004) that zero microfilaria prevalence does not necessarily mean absence of infection since microfilaria tests may give false-negative results especially if individuals carry single-worm or single-sex infections or when there is some residual transmission due to low-density infection when the parasite presence may not be detected in a blood sample (Plaisier et al., 2004). In spite of the foregoing the demonstration of microfilaria in blood samples is still the most reliable means of confirming patent infection as well as determining the severity of infection (Nwoke et al, 1994; 1998; Nutman 2000). More over, the specificity and sensitivity of the study tool was further enhanced by the use of questionnaire prior to microscopy. The use of questionnaires is an acceptable epidemiological tool in filariasis survey (Mathieu et al, 2008). Since the objective of the work included comparing proven patients with 'normal' persons we had to use the most overt means of confirming infection. The results therefore at best, confirm the presence of filariasis in Oguta Local Government Area and not necessarily endemicity.

The highest prevalence of filariasis was observed amongst the age groups 41 – 45 and 51 – 55 years. Infection was not observed amongst respondents aged 31 – 35, 36 – 40, 61 – 65, 71 – 75, 76 – 80 and 80 – 85 years. This finding is interesting and does not agree with the findings in several other areas of the world and even in Nigeria. Filarial infection usually peaks around 30-35 years. The absence of infection in this age bracket in the study area may be due to several factors including the fact that people within this age range are young adults and may not be willing to present themselves for examination. Social stigma associated with filariasis has been severally reported as contributing to the reluctance of young adults and mature persons, to openly declare their filariasis status (Okoye and Onwuliri, 2007) and even in Imo State of Nigeria, respondents to a research questionnaire admitted that they will be unwilling to marry filarial patients (Dozie, 2003). Another possible reason for the absence of infection among young adults in the area could be the common and indiscriminate use of antifilarials. This practice among persons is not recent in Nigeria and has been reported even among rural dwellers in the northern part of the country (Anosike and Onwuliri, 1994). These drugs are freely available and are therefore open to abuse.

The age-related prevalence observed in the study area has some support from some other areas especially when considered in relation to male respondents. In males, two patterns of prevalence have been reported (Ottesen, 1998). In one, prevalence rises until early adulthood, then plateaus or falls slightly (Pani et al., 1991). In the other, prevalence rises continually with age or plateaus after the age of 50 years (Pani et al., 1991). Our findings of highest prevalence from 46-50 and 51-55 years of age therefore agree with the latter case.

The results of gender – related prevalence of filariasis showed that there was a significant difference ($p < 0.05$) in the prevalence of infection between male and female respondents. There have been reports that women generally have a lower level of prevalence of microfilaraemia and lower mean microfilarial densities than men (Penaia and Spears, 1985; Evans et al, 1993). Even in Nigeria Anosike and Onwuliri (1994) reported that prevalence of filarial

(onchocercal) infection was more in male than female respondents. The findings of the present study showed a higher prevalence of filarial and malaria infection amongst female respondents. This may be because more females than males in Oguta work around the riverine areas as traders and farmers and thus get exposed more to the sources of infection. According to Nwobi (2004), the two main occupations of Oguta dwellers, farming and fishing, have gender-related patterns of practice. Main farming activities like bush clearing and mound making are the areas men participate in more frequently. Women also participate in these activities but not as much as the men. The women however are more regular in the farms because the year-round jobs in the farm like weeding and harvesting of maize, melon etc are done by the women. As for fishing, the men go offshore mostly, while women fish around the riverbanks using nets and baskets set in the stagnant marshy fringes of the water bodies. The women therefore are more exposed to mosquito bites in the farms and at the riverbanks. Exposure to vectors of disease is a major factor in the endemicity of the disease. This may then explain the greater prevalence of parasitoses among women than men in the studied population.

Also in the reported case of greater filariasis prevalence among men in the northern part of Nigeria (Anosike and Onwuliri, 1994), the study was conducted in a predominantly muslim environment where the dress code of women ensured a covering of virtually the whole body thus reducing the biting rate of the vector insect. Our study area is peopled mainly by christians and animists who do not have such gender-related and mosquito bite-protective dress codes.

Results of the liver function tests show that most (57.14%) of the individuals infected with filariasis had elevated levels (above adult normal ranges) of liver diagnostic parameters. Infection prevalence pattern also correlated with the levels of liver parameters of respondents aged 41 to 60 years. Highest prevalence coincided with the age group having the highest levels of bilirubin and highest activities of alanine and aspartate aminotrantransferases as well as serum alkaline phosphatase which are all markers of liver integrity. Elevated levels of these parameters are indicative of liver disease (Peters, 1989). This would indicate therefore that filariasis has a direct influence on the functions of the liver. The association of malaria with liver damage is not in doubt and our results corroborate that. The association of filariasis with renal damage is also known. Ottesen (1984) had earlier associated filariasis with impairment of kidney function. The possible association of filariasis with liver function is novel and deserves further investigation in different locations to eliminate other confusing factors like nutrition, environmental factors and drug abuse among others.

This study has shown that filariasis has influence on liver and kidney functions at least in the studied area. Therefore the estimation of liver function parameters and the subsequent interpretation will be of diagnostic value if it also accommodates the possibility of filarial infection even in proven cases of malaria parasites from microscopy. This will improve the clinical diagnosis and management of filariasis especially as most fever patients that report for treatment assume that they had malaria (as we found out through our questionnaire) and it is a common medical practice to treat malaria in almost all patients that present symptoms of fever.

References

r-0. Anosike, J. C. (1994). Studies on filariasis in Bauchi State, Nigeria V: The distribution and prevalence of Mansonellosis with special reference to clinical signs. Applied Parasitology 35: 189 – 192
r-1. Anosike, J. C. and Onwuliri, C.O.E. (1994). Studies on filariasis in Bauchi State, Nigeria 1: Endemicity of human onchocerciasis in Ningi local Government Area. Annals of Tropical Medicine and Parasitology 81: 31-38.
r-2. Anosike, J.C.; Nwoke, B.E.; Ajayi, E.G.; Onwuliri, C.O.; Okoro, O.U.; Oku, E.E.; Asor, J.E.; Amajuoyi, O.U.; Ikpeama, C.A.; Ogbusu, F.I. and Meribe, C.O. (2005). Lymphatic filariasis among the Ezza People of Ebonyi State, Eastern Nigeria, Annals of Agric and Environ Medicine, 12:181-186.
r-3. Bailey, N.T.J. (1994). Statistical methods in biology, 3rd edn., University Press, Cambridge, 38-87.
r-4. Baker, F.J. Silverton, R.E. Pallister, C.J. (2000). Introduction to Medical Laboratory Technology. Bounty Press Limited Nigeria. pp 353-362.
r-5. Borrmann S, Matsiegui PB, Missinou MA, Kremsner PG 2008 The effects of Plasmodium falciparum parasite population size and patient age on early and late parasitological outcomes of antimalarial treatment in children. Antimicrob Agents Chemother.
r-6. Dozie, I.N.S. (2003). Onchocerciasis and its socioeconomic effects in some parts of Imo State, Nigeria. Unpublished PhD Thesis, University of Jos, Nigeria, 240-243.
r-7. Evans, D.B.; Gelband, H. and Vlassoff, C. (1993). Social and economic factors and ther control of lymphatic filariasis. Acta Tropica 53:1-2.
r-8. Gyapong, M.; Gyapong, J.O.; Adjei, S.; Vlassoff, C. and Weiss, M. (1996). Filariasis in northen Ghana: some cultural beliefs and practices and their implications for disease control. Social Science in Medicine 43:235-242.
r-9. Haddix, A.C. and kestler, A. (2000). Elimination of lymphatic filariasis: economic aspects of the disease and programmes for its elimination. Transactions of the Royal Society of Tropical medicne and Hygiene 94:592-593.
r-10. Henry, J.B. (1974). Clinical diagnosis and management

by labvoratory method, W.B. Saunders Co., Philadelphia, 332-335.
r-11. Lenhart, A, Eigege, A.; Kal, A.; Pam, D.; Miri, E.S. Gerlong, G.; Oneyka,J.; Sambo, Y. ; Danboyi, J.; Ibrahim, B.; Dahl, E.; Kumbak, D.; Dakul, A.; Jinadu, M.Y.; Umaru, J.; Richards,F.O. and Lehmann, T. (2007). Contributions of different mosquito species to the transmission of lymphatic filariasis in central Nigeria: Implications for monitoring infection by PCR in mosquito pools.Filaria Journal, 6:14 doi:10.1186/1475-2883-6-14.
r-12. King, E.J. and Armstrong, A.R. (1934). A convenient method for determining serum and bile phosphatase activity. Canadian Medical Association Journal 31:376-381.
r-13. Malloy, H.T. and Evelyn, K.A. (1937). The determination of bilirubin with the photoelectric colorimeter. Journal of Biological Chemistry 119:481.
r-14. Mathieu, E.; Amann, J. and Lammie, P. Collecting baseline information for national morbidity alleviation programs: Different methods to estimate lymphatic filariasis morbidity prevalence. American Journal Tropical Medicine Hygiene, 78(1), 2008, pp. 153-158.
r-15. Nwobi, F.N. (2004). Personal communication from a prominent indigene of study area.
r-16. Nwoke, B.E.B., Edungbola, L.D.; Mencias, B.S.; Njoku, A.J.; Abanobi, O.C.; Nkwogu, F.; Nduka, F.O. and Oguariri, R.M. (1994). Human onchocerciasis in the rainforest zone of southeastern Nigeria. The Nigerian Journal of Parasitology, 15:7-18.
r-17. Ojiako O.A. and Nwanjo H.U. (2006). Is Vernonia amygdalina hepatotoxic or hepatoprotective? Response from toxicity and biochemical studies. African Journal of Biotechnology, 5(18),1648-1651.
r-18. Nutman, T. B., Zimmerman, P. A., Kubofcik, J. and Kostyu, D. D. (1994). A universally applicable diagnostic approach to filarial and other infections. Parasitology Today 10:239-243.
r-19. Okoye, I.C. and Onwuliri, C.O. (2007). Epidemiology and psycho-social aspects Of onchocercal skin diseases in northeastern Nigeria Filaria Journal, 6:15 doi:10.1186/1475-2883-6-15
r-20. Ottesen, E. A. (1984). Filariasis and tropical eosinophilia In: Tropical and Geographical Medicine. Warrenks and Mahmoud, A. A. F. (eds). McGraw-Hill Inc. New York. 390 – 422
r-21. Ottesen, E. A (1998). Towards elimination of lymphatic filariasis. In: Infectious diseases and public health. Angelico, M. and Rocchi, G. (eds), Balaban Publishers, Philadelphia, 58-64.
r-22. Peters, W. (1989). Liver enzymes and diagnosis, (6th edn), Golden Press, Philadelphia, 8-9.
r-23. Plaisier, A.P., Subramanian, S. and Das, P.K. (1998). The lymfasim simulation programme for modeling lymphatic filariasis and its control. Methods of information in medicine, 37:97-108.
r-24. Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of SGPT and SGOT, American journal of Clinical Pathology, 25: 56-62.
r-25. TDR (2001). Handbook of good laboratory practice (GLP): quality practices for regulated non-clinical research and development, TDR/PRG/GLP/01.2
r-26. TDR (2002). Guidelines for rapid assessment of Loa loa. UNDP/World Bank/WHO Special programme for research and training in tropical diseases, TDR/IDE/RAPLOA/02.1.
r-27. Tietz N. W. (1976). Fundamentals of Clinical Chemistry, W.B. Saunders Company, Philadelphia, 7.
r-28. Tietz N. W. (ed.) (1983). Study Group on Alkaline phosphatase: a reference method for measurement of alkaline phosphatase activity in human serum. Clinical Chemistry 29:751.
r-29. Udonsi, J.K. (1999). Parasites and Parasitic Diseases, A Textbook of General and Medical Parasitology in Tropical Africa, Josany Press, port Harcourt, 127.
r-30. Wanji, S. (ed) (2001).Rapid assessment procedures for loaiasis: report of a multi-centre study, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), (TDR/IDE/RP/RAPL/.01.1).
r-31. World Health Organisation (2004). Community participation and tropical disease control in resource-poor settings, TDR/STR/SEB/ST/04.1
r-32. World Health Organisation (2004). Report of the Scientific Working Group on Malaria, TDR/SWG/03, 7-8.
r-33. Zeibig, E.A. (1997). Clinical Parasitology: A Practical approach. W.B. Saunders Company, Philadelphia, 9ff.

Author Information

Okey A. Ojiako
Department of Biochemistry, Federal University of Technology Owerri, Nigeria

GOC Onyeze
Department of Biochemistry, Federal University of Technology Owerri, Nigeria