Full Length Research Paper

Epidemiological and biochemical studies of human lymphatic filariasis and associated parasitoses in Oguta, South-Eastern, Nigeria

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Possible organ infections associated with human filariasis, helminthiasis and malaria in Oguta Local Government Area of Imo State, South-Eastern 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*, 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 hematological parameters relative to 'normal' respondents. Comparative biochemical analyses showed significant ($p < 0.05$) 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 co infection) 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.

Key words: Filariasis, co-parasitoses, Nigeria, prevalence patterns, liver dysfunction.

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). 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 *W. bancrofti* is the most common (Anosike et al., 2005) 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 house-holds, costs to government-funded healthcare systems, lost productivity of infected individuals, and reduced pro-

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ductivity 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 filarialsis 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 South-eastern 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 from over 1000 volunteers. Informed consent of the volunteers was obtained as demanded by WHO (TDR, 2001). With the assistance of individuals recognised by WHO (2004a) 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 of filariasis like presence of eye worm, skin depigmentation, itching, diarrhoea, fever, hydrocoele, lympho-edema 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 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 5 ml portion of blood was collected from each respondent using a disposable syringe and a needle. A 2 ml portion of the blood sample was put into an EDTA bottle to prevent clotting while the remaining 3 ml was allowed to clot in the syringe. Sera from the clotted samples were used for liver function tests after centrifuging for 10 min at 3000 rpm 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 Medi-Test Combi 9[TM] (Macheray-Nagel, Duren) 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 Randox[TM] 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).

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

- = Not detected.

All the biochemical parameters were also obtained for a set of persons (matched for age and gender) in the same area that tested negative to all the parasitic infections investigated (n = 10).

Statistical analysis

Chi-square test of independence was used to test the association 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. Differences at P < 0.05 were considered significant (Bailey, 1994). Sign test was used for comparing liver function parameters between infected and non-infected volunteers.

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 (*Ascaris 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. The difference in prevalence of filarial infection between the female (26.1% of total sample) and the male respondents

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
	female	88	4	4.55
	Total	200	13	6.5

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

(15.2% of total sample) was at the limits of statistical significance (P = 0.054). What about the gender differences in other parasitc infections?

Results of the liver function tests (Table 4) show that of the 40 filaria-positive cases 23 (57.5%) 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.

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 (Mectizan[R]) and prevalence of disease has already been confirmed in different parts of the State (Nwoke, et al., 1994; Dozie, 2003, Unpublished PhD thesis). The respondents were recruited from a group of volunteers with clinical symptoms of filariasis as well as other parasitic diseases, who were already feeling sick and responded to invitation of health personnel with the hope of receiving free medication.

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 aminotransferases 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

Table 4. Results of liver diagnostic parameters.

Age (Years)	ALP (IU/L)	ALAT (IU/L)	ASAT (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	ND	ND	ND	ND	ND
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

Values are means ± S.D.
ALP = Alkaline phosphatase, ALAT = Alanine aminotransferase, ASAT = Aspartate aminotransferase and ND = Not Determined.

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	ND	ND
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

Values are means ± S.D
ND = Not Determined, PCV = Packed cell volume.

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 micro-scopy. This will improve the clinical diagnosis and ma-nagement of filariasis especially as most fever pa-tients that report for treatment assume that they had malaria (as we found out through our questionnaire) and it is a

it is a common medical practice to treat malaria in almost all patients that present symptoms of fever.

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