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Onchocerciasis in Ifeloju LGA, Oyo State, Nigeria and the Effect of some Anthelminthic Drugs and Nigerian Medicinal Plants on the Microfilariae of *Onchocerca volvulus in vitro*

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Abstract

A survey and experimental study on *Onchocerca volvulus* infection were carried out in four villages: Afefu, Ibele, Oba-Akintaro and Bale-Agbe, all in Ifeloju Local Government Area (LGA.) (presently constituting Central and North Ibarapa LGAs) of Oyo state, Nigeria in 1990. Infection was determined by finding characteristic microfilariae from skin snips. A total of 126 people were examined and nodule excisions were done on two people. The overall prevalence was 23.8% (30 of 126). Infection was seen in all the villages sampled. The prevalence in males was 25.7%, while that of females was 20.0%. Various clinical manifestations of the infection were seen in the area. Three anthelminthic drugs, viz., piperazine hydrate BPC (Antepar), pyrantel pamoate (Combantrin) and Levamisole hydrochloride (Ketrax) and aqueous extracts of three different Nigerian medicinal plants viz., *Albizia coriaria* (leaf), *Carica papaya* (seeds) and *Momordica charantia* (leaf), were tested for their micofilaricidal effects against the microfilariae of *O. volvulus*. Ketrax and *M. charantia* were most effective, while *A. coriaria* extract was least effective. All the drugs and plant extracts had microfilaricidal effects at the concentrations of 100µg/ml, 50µg/ml, 25µg/ml, 10µg/ml, and 1µg/ml, although the periods of their effectiveness differed. Further studies through *in vivo* screening may find the application of these drugs and herbal extracts in onchocerciasis chemotherapy effective. Their trials is recommended.

Keywords: Survey, experimental study, *Onchocerca volvulus*, Oyo state, Nigeria.

1.0 Introduction

Onchocerciasis is an infection by a parasitic filarial worm, *Onchocerca volvulus,* which is transmitted through the bite of an infected female blackfly of the species *Simulium damnosum* complex. As a result of the high disposition of the disease to cause blindness, and the fact that the foci of transmission are flanking the rapidly flowing rivers, the disease is often referred to as 'River Blindness'. (Faust *et al* 1958). Onchocerciasis is debilitating and incapacitating and in effect is a dehumanizing disease with effects on other organs apart from the eyes. Intense or severe itching, various skin changes, and in most cases nodules under the skin forming around the adult worms are some of the serious complications of the infection (WHO, 2018). Lymph edema of external genitalia, inguinal and femoral lymphadenopathy and hanging groin may occur. Although the disease is dramatic and disabling with tragic consequence of blindness, some of the complications are not as dramatic as to kill swiftly.

Epidemiological studies on onchocerciasis has shown that individuals and communities living under apparent similar conditions of transmission exhibit different manifestations. The factors which affect the clinical manifestations include human ecology, system of land use, environmental modification, economic organization of the area, sociocultural patterns and demographic dynamics (including migration) (Wyatt,1971). The disease is generally occupational, and the category of people affected are the farmers. The transmission foci are around swift, free-flowing river areas which are usually fertile and appropriate for cultivation of crops. The prevalent rates and the severity of the infection usually depend on the distance of the human settlement from the river course (WHO,1987).

1.1 Onchocerciasis in Nigeria

WHO, (1987), reported that Nigeria was the country with the largest number of people infected with onchocerciasis in the whole of Africa with five states, Kwara, Kaduna, Anambra, Oyo, and Ondo

State being the most affected. Acholonu (1989) reported that about five million people were infected in Nigeria and that the disease had been reported in 17 out of the then 19 states. Onchocerciasis was first reported in Nigeria in 1908 by Parson as quoted by Sasa (1976). According to Obidigbo (1982), in 1952, a survey team from Northern Nigeria Ministry of Health started surveillance programs which covered the towns and villages along River Niger and Benue and their tributaries extending to the border of Cameroon, and infection rate as high as 75% was found in some areas. The result of the survey led to the initiation of a project for vector control in the then Abuja emirate in 1956. Although onchocerciases is known to be prevalent in some other nations of Africa, it was realized that radically different situation existed in Nigeria. Even in the early stages in the knowledge of the distribution of the vector in the country, it was impossible to speak of foci in the same term as obtained in other parts of Africa (Gregory, 1982). Large areas of Nigeria were known to harbor closely connected breeding sites of *Simulium damnosum* complex.

1.2 Onchocerciasis in Ifeloju L.G.A., Oyo State.

Early information on the disease and its consequences came mainly from the northern parts of the country. Little was known about the infection in Western parts of Nigeria. Although Nnocheri (1964) and Ive *et al*. (1967) had reported serological, parasitological and clinical evidences of the infection in Ibadan, it was not until 1971 that a prevalence survey was conducted in the Western part of Nigeria at Ibarapa district (Wyatt 1971). Before then, even the WHO map of onchocerciasis areas excluded Western part of Nigeria in the zone of transmission (WHO, 1966). Wyatt (1971) conducted a prevalence survey of the infection in Ibarapa District of then Western State. Infections were found to be prevalent in the villages surveyed with infection rate as high as 76.2% obtained in some villages. Ogunba (1981) also conducted a prevalence survey in Idi-ata village, also in Ibarapa District and reported an infection rate of 48.8%. Acholonu and Nwanze (2018) studied onchocerciases infection in the same area (Igbo-Ora and Idere) and found the disease to be prevalent there at the rate of 25%. So, onchocerciasis is endemic in Ibarapa District and Ifeloju L.G.A. was in Ibarapa District (and is presently divided into Central and North Ibarapa LGAs (https:/en.wikipedia.org/wiki/ibarapa_people, 2018, Accessed on October 1, 2018). This area is therefore a transmission focus and the infection was most likely to be prevalent in all villages in the area. However, every village needs to be sampled to confirm this surmise. According to Acholonu (1982), the collection of prevalence data is necessary to assess the magnitude of infection and declare it as a major health problem in the country, and hence make those in the corridors of power see the imperative need to pursue attacks for the amelioration and control of the infection on communities most severely afflicted.

1.3 Chemotherapy

Until recently, the chemotherapy for onchocerciasis had not been satisfactory because drugs that were available were either inadequate (ineffective on all stages of parasite) or are toxic (either on their own or induce immunological reactions). Diethylcabamazine (Banocide) is only microfilaricidal and as such only suppressive and often aggravates or precipitates ocular lesions. It causes severe and sometimes dangerous systematic reactions usually referred to as Mazzotti reaction. Suramin which is known to be effective on the adult parasite could also not be used freely because of its toxicity especially on the kidneys. Also, Melarsonyl potassium, an arsenical drug could cause arsenical encephalopathy although it will kill adult parasites. Metrifonate also has partial effectiveness and would also precipitate Mazzotti reaction. Of all these, Banocide had been the drug of choice for a long time. Ivermectin is more promising as a single-dose oral agent that is effective and well tolerated in the treatment of onchocerciasis in man. However, it was not available for use until 1987. This drug is a synthetic derivative of a fermentative product of *Streptomyces averimitilis.* It is effective in elimination of microfilariae from skin and the eye and also may have effect on adult worms. It is effective. Its greatest potential is in preventing onchocercal blindness. Its side effects are mild, but it has to be used for a long time. WHO (2018) recommended treating onchocerciasis with Ivermectin at least once yearly for between 10-15 years.

1.4 Experimental Onchocerciasis and Drug Screening.

Important progress has been made in several major areas of research on *O. volvulus* and in experimental onchocerciasis. However, the parasite has been difficult to be cultivated in laboratory animals except chimpanzee. Chimpanzee is regarded as a protected animal and the use of it as laboratory animal is restricted. Moreover, the parasite, could be maintained alive in certain *in vitro* culture for various periods of time and both microfilariae and larvae can be successfully cryopreserved. The introduction of microfilariae of the parasite into rodent has been achieved and has been adapted to demonstration of drug efficacy.

According to Acholonu (1982), the experimental study is very important but lags behind in researches in Nigeria. The research for adequate drug for the treatment of this disease is of paramount importance. Most drugs now in use were initially devised for some other purpose in mind (WHO, 1987). Ivermectin now known to be efficacious for the treatment of ocular onchocerciasis had been an anthelminthic drug used in veterinary medicine for a long time before it found its use in the treatment of onchocerciasis. Anthelminthic drugs such as piperazine and its derivatives were all discovered by *in vitro* trials (Hawking and Cavier, 1973).

According to WHO (1984), the essential objective of experimental chemotherapy studies in onchocerciasis among others are as follows:
(i) To find new anti-microfilarial compounds that will eliminate microfilariae without causing serious reactions.
(ii) To find means of reducing the inflammatory reactions that occur in the human host in response to the presence and death of the parasite, and
(iii) To find and develop new compounds that will eliminate the adult but will not at the same time cause tissue changes as a result of rapid elimination of adult worms and microfilariae.

Laukamm-Josten (1987) carried out work to test the paralyzing effects of Midazolam on microfilariae of *O. volvulus in vitro* and found the drug to be efficacious. This type of work has in view, the reduction of the adverse effects of microfilaricidal drugs that are now available. Desowitz, *et al.* (1984) had also observed that the benzodiazepine derivative, diazepam inhibited the adverse reaction of Diethylcarbamazine (DEC).

Some Nigerian or native medicinal herbs are known to be efficacious against helminths especially intestinal nematodes. Oliver (1960) and Ayitey-Smith (1989) described various medicinal herbs that are active against intestinal worms. According to WHO (1989), choice of herbs for efficacy screening should take cognizance of ready availability of such herbs. In Nigeria and especially among the Yorubas, various herbs are usually used to treat worm infections. Drug and herbal screening experiments in this area might find a place in onchocercal chemotherapy.

1.5 Objectives

The existence of reliable baseline data is essential for effective surveillance of onchocerciasis (Edungbola, 1978a). Although studies on onchocerciasis has been conducted in parts of Nigeria, several communities where the health and socio-economic consequences of the disease are considerable remain unidentified and studied. Moreover, despite the fact that much research has been carried out, there is still only a limited number of drugs available for the treatment of onchocerciasis (WHO,1987). Literature review shows that a preponderance of studies done in Nigeria on onchocerciasis deal with prevalence (Adeoyeba and Adegoke (2002), Emukah *et al.* (2004), Wogu and Okala (2008), Nwaorgu *et al.* (2009), Abanobi *et al.* (2010), Siewe *et al.* (2019)) and drug control with Ivermectin (Richard, Jr. (1996), Gutman *et al.* (2010), Tekle *et al.* (2012), Olajide (2016)), or vector control (Davies, J, B. (1968), Crosskey, R.W (1981), Gemade and Dipeolu, 1983). On the contrary, minimal studies have been done on the development of herbal or alternative medicine or chemotherapy (Kuesel, 2016), hence this present report. It will augment the comparatively few studies done in Nigeria in the area of chemotherapy and new drug development for treatment of onchocerciasis. It is very relevant and needful. So, this study was carried out with the following objectives:
(i) To determine the prevalence of onchocerciases in four villages near Igbo-Ora in old Ifeloju L.G.A.

of Oyo State and also identify the important clinical manifestations of the disease in the area.
(ii) To contribute to the control of onchocerciasis through chemotherapy and herbal means by finding alternative drugs which will be adequate and safe for the treatment of the disease.

2.0 Materials and Methods

Ifeloju Local Government Area (LGA) is one of the former Local Government Areas in Oyo State (now divided into Central and North Ibarapa LGAs) (see Figure 1). Igbo-Ora was the headquarter of the Ifeloju L.G.A. The area belongs to the rain savanna forest and there are several rivers that cross the area. There are several hills and rocky mountains in the area (see Figure 2). The villages under this study are about fifteen kilometers from Igbo-Ora. The villages are essentially farm settlements with the inhabitants having dual residency in the nearby township of Idere. The villages are located between hills and with huts made of thatched roofs (Figure 3). Each hut contains family units comprising of the husband, wife and the children. Children attending schools however stayed back at Idere. This study was carried out in four villages. The villages are: Afefu, Ibele, Oba-Akintaro and Bale-Agbe. The villages are not very far from Ofiki River, one of the rivers in which Wyatt (1971) located the presence of *Simulium* larvae. The villagers were usually approached through a native of Idere who worked at Igbo-Ora. The fellow usually made an advance introductory trip to the village to seek the support and cooperation of the people and also enlighten the people of the nature of the study and the benefits of the study to the community. They always responded with interest and cooperation.

Figure 1a: Map of Nigeria showing Oyo State (study location).

Figure 1b: Map of Oyo State showin Ibarapa LGA.

Figure 2: Scenery and vegetation of the study area.

Figure 3. Huts of mud and thatched roots in one of the villages sampled.

Figure 4: Thickening and hyperpigmentation of skin with folds above the knee. (Skin snip positive).

2.1 Skin Snips

Skin snips were made using surgical razors and lancets. Methylated spirit and cotton wool were used

to sterilize the skin before each skin snip. The study was a field exercise and hence compound light microscope, clean slides and slide covers were always carried along. Normal saline was used for immediate examination and phosphate buffered saline (PBS) at pH 7.2 for short time culture. Skin snips were made at the hips of each subject and placed in saline on the microscope slide. Two skin snips were made on each person and left for 5 – 10 minutes to allow microfilariae to come out. The slides were examined under low power (X10) of the microscope and the result recorded on prepared form bearing the names and serial numbers of the people examined. Each person was asked questions regarding knowledge of the symptoms of the disease and were also physically examined for any visible clinical manifestations of the disease. Microfilariae obtained from positive skin snips and extra skin snips made on infected persons were collected into a sterile vial bottle containing phosphate buffered saline (PBS) for the drug.

2.2 Drug Screening

The *in vitro* study was carried out in the medical laboratory of the Igbo-Ora Comprehensive Hospital of the College of Medicine, University of Ibadan, Nigeria which is considerably near the study area. The drugs conventionally used against intestinal worms were used. They were: Piperazine hydrate BPC (Antepar), Pyrantel pamoate (Combantrin) and Levamisole hydrochloride (Ketrax). Also, three Nigerian medicinal plants were used. They are: *Albizia coriaria* (Ayunre-Yoruba), *Momordica charantia* (Ejirin-Yoruba) and *Carica papaya* (Ibepe-Yoruba, Popo - Igbo). The leaves of *A. coriaria* and *M. charantia* and the seed of *Carica papaya* were used. They were sun-dried until constant weights were obtained and were grounded into power and used in making stock solutions.

2.3 Stock solutions

The stock solutions of each of the drugs and herbs were made: This was done by weighing out 0.1mg of each of the crushed drug tablets and powered leaves and seeds. The weighed quantity of each was then put in sterile 6oz bottles containing 100ml sterile distilled water. The drugs were allowed to dissolve, and the herb also left overnight. They were each then filtered through Watman filter papers into another set of sterile bottles. The herbal extracts were thereafter sterilized in the autoclave. The stock solution thus obtained gave concentration of 1000µg/ml. Subsequent dilutions were made to obtain concentrations of 100µg/ml, 50µg/ml, 25µg/ml, 10µg/ml, 1µg/ml. To obtain concentrations of 100µg/ml from the stock solution, 0.1ml of stock solution was taken using sterile 1ml cc syringe and this was made up to 1ml in the test tubes. To make 50µg/ml, 0.1ml of the stock solution was made up to 2ml. To make the 25µg/ml concentration, 1ml of the 50µg/ml concentration was made up to 2ml by dilution. Then to make the 10µg/ml concentration, 0.25ml of the 25µg/ml concentration was made up to 1ml by dilution. And finally to make the 1µg/ml concentration, 0.1ml of the 10µg/ml concentration was made up to 1ml by dilution.Sterilised PBS was used in making each of the dilutions. About 1ml of each concentration of the different drugs and extracts was placed in sterile 5ml Pyrex test tubes and 8-10 microfilariae were put in each tube. A control experiment containing microfilariae on PBS only was also set up. The tubes were labeled and put in an incubator at 37°C. The content of each tube was examined every hour by pouring the contents one at a time in a clean small petri dish and motility of the microfilariae observed under the microscope. Three sets of trials were made on each drug and the aqueous extracts of herbs. The motility of the microfilariae was checked for highly motile (+++) according to motility as when it was checked from the skin snip in saline. Then just motile (++) when the motility was reduced and lastly weakly motile (+) when motility was weak and sluggish.

3.0 Results

A total of 126 individuals were examined and 86 were males while 40 were females. 30 (23.8%) of the people examined were infected by *Onchocerca volvulus*. Among the infected people, 22 (25.7%) of the males examined were infected while 8 (20.0%) of the females were infected (see Table 1). There was no significant difference between the infection in males and females, ($p > 0.05$). The infection rate was highest (61.5%) among age group 50-59 years. Infection was not found in people below the age of 20 years (see Table 2). Infection rate rose to 9.4% among age group 20-29 years to 61.5% among age group 50-59 years and declined from age group 60-69 (37.5%) to age group 70-79 years (25%) (see

Figure 3) The infection rates in each of the villages were as follows: Afefu- 19.4%; Ibele-25.9%; Oba-Akintaro – 25.0%; Bale-Agbe – 25.8%, (see Table 3). The infection rates in the villages were not significantly different, ($p>0.05$).

3.1 Clinical Manifestations

Various clinical manifestations of the infection were found in the villages (see Figures 4-7). Some people who were negative for skin snips also showed clinical manifestations of the disease (see Table 4). Eleven people had skin nodules, 3 had "Leopard skin", 35 had pruritus skin or (onchodermatitis), itching, 12, vision impairments and 2, "lizard skin". There was one person with velvet skin (see Table 4). Among those showing clinical manifestations, there were some that were skin snip negative. There were four people that were however skin snip positive but were not showing clinical manifestations. The number of those that were having clinical manifestations and were skin snip positive was 26, which is 86.7% of the infected persons. Thirty-eight (38) showed clinical manifestations but were skin snip negative. Statistical analysis using x^2 indicated that there was a relationship between skin snip positivity and clinical manifestations of onchocerciasis ($P<0.001$). No case of glandular involvement shown by hanging groin or lymphoedema was seen in the villages.

3.2 Nodule Excisions

Nodule excisions were done on two people by a medical doctor at the Igbo-ora Comprehensive Hospital. The initial aim of this exercise was to use the nodules as a source of obtaining microfilariae for the drug screening part of this study. This was however not successful as the microfilariae and adult worms had died before they could be used. However, two findings were obtained from the exercise that was worthy of note viz: i) One of the persons from whom a nodule was excised was initially skin snip negative. This person, an old man, also did not show any other clinical manifestations apart from the presence of skin nodule. The adult worm seemed to have died after the excision, (see Figure 8) (ii) The other person took banocide (Diethylcarbamazine) a week before the nodule excision (not at our instruction). He took two tablets (100mg) of the drug, thrice daily and took a total of 20 tablets (1000mg). The adult worms in this nodule were dead with depositions in the nodule and the surface of the adult worms (see Figure 9).

Table 1: Prevalence of *Onchocerciasis* by sex.

Sex	Number Examined	Number Infected	% of Infection
Male	86	22	25.7
Female	40	8	20.0
Total	126	30	23.8

Table 2: Distribution of Onchocerciasis in various age groups.

Age Groups	Number Examined	Number Infected	% Infected
A. 0-9	3	0	-
B. 10-19	13	0	-
C. 20-29	32	3	9.4
D. 30-39	36	10	27.8
E. 40-49	17	5	29.4
F. 50-59	13	8	61.5
G. 60-69	8	3	37.5
H. 70-79	4	1	25.0
TOTAL	126	30	23.8

Table 3: Prevalence of onchocerciasis by sex at different villages.

Name of village	Sex	Number examined	Number infected	% infection
	Male	22	5	22.7
Afefu	Female	14	2	14.3
	Total	36	7	19.4
Ibele	Male	18	4	22.2
	Female	9	3	33.3
	Total	27	7	26.0
Oba-Akin-taro	Male	27	7	26.0
	Female	5	1	20.0
	Total	32	8	25.0
Bale-Agbe	Male	27	6	22.2
	Female	4	2	50.0
	Total	31	8	25.8
Grand Total		126	30	23.8

Table 4: Clinical manifestation of onchocerciasis among the people examined.

Clinical Manifestations	Nodules	Leopard Skin	Pruritus/ itching	Vision Impairment	Lizard Skin	Velvet skin	Total
No. Skin Snip Positive	4	1	14	5	1	1	26
No. Skin Snip Negative	7	2	21	7	1	0	38
Total	11	3	35	12	2	1	64

Figure 5: Leopard skin on legs. Note left leg is thinner than right Leg. (skin snip positive)

Figure 6: Large nodule at the side of the knee. (Skin snip positive, 18 year old boy.)

Figure 7: Smooth shining skin around knee and velvet skin above left knee. With skin folding below knee and little appearance of leopard skin at right leg (Skin snip positive)

Figure 8: Skin nodule from a person (skin snip negative) Adult worms were normal in appearance.

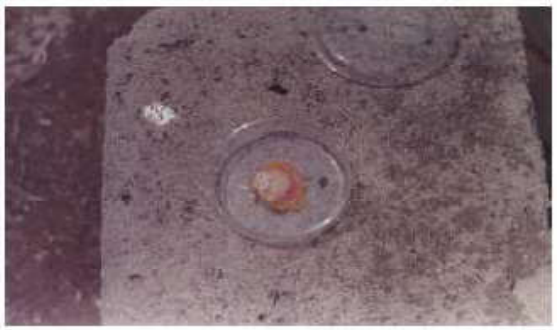

Figure 9. Skin snip from a person (skin snip positive) who took Banocide. Has worms with abnormal size and deposition of hard substance on the surface. No microfilariae in the nodule.

3.3 Drug Screening

All the drugs tested at all concentrations had microfilarcidal effects on the microfilariae of *O. volvulus.* However, the periods at which various drugs and at different concentrations were effective differed. At the concentration of 100µg/ml, each of the drugs and herbal extracts killed the microfilariae within one hour except *Albizia* which just weakened them but however killed them before the second hour. In none of the drugs and herbal extracts at no concentration did the microfilariae survive more than six hours. The periods at which the different concentrations were effective varied gradually along the concentration gradient. Among the three drugs tested, Levamisole (Ketrax) was the most effective. At the concentration of 50µg/ml, the microfilariae did not survive for more than one hour. Moreover, at the least concentration of 1µg/l, the mocrofilariae were killed by the fourth hour. There was only little difference in efficacy pattern of the pyrantel pamoate (Combantrin) and piperazine hydrochloride (Antepar). At the concentration of 10µg/ml however, pyrantel pamoate was effective by the third hour while the other drug (Antepar) was only effective at the fourth hour. Among the medicinal plants used, *Momordica charantia* was the most effective. It had the same pattern of efficacy as Levamisole. *Carica papaya* seeds extract was also more effective than *Albizia* leaves extract. With *Albizia,* at the concentration of 1µg/ml, the microfilariae were only killed at the sixth hour.

4.0 Discussion

The fact that onchocerciasis is prevalent in Nigeria is not in doubt, but according to Edungbola *et al.* (1987a), the implementation of a nation-wide control program can be handicapped by inadequate baseline data on the disease and its vectors that will help to plan, implement, monitor and evaluate effective interventions. Out if the 126 people examined in the four villages, 30 people were infected giving an infection rate of 23.8%. According to WHO (1966)'s indices of endemicity, this area falls into a region of low endemicity. This nevertheless identifies the area as a transmission site. Earlier work done in the area (Wyatt, 1971, Ogunba, 1982 and Acholonu & Nwanze, 1989), resulted in various rates of infection. The villages where this work was done are close to the site along River Ofiki where Wyatt (1971) found larvae of *Similium,* (See Figure.2). The work of Acholonu & Nwanze (1989) near these villages obtained an infection rate 25.0% which still places the area within the same indices of endemicity. Generally, prevalence survey conducted by different workers in the southern parts of the country were not as high as in the northern parts of the country (Engelbrecht *et al.* (2003), Braide *et al.* (1982) got 26.7% infection in Obudu, Cross River State. Wyatt (1971), obtained 39.7% and Ogunba (1982) obtained 48.8% both in the same area, Ibarapa District of Oyo State. Acholonu (1980) obtained 3.7% in Lagos.

This study gives different sex distribution infection rates from the result obtained by previous workers in the area. In this study there was no significant difference between the infection rates in males and females. This situation could be explained by the fact that the studies by the following workers (Wyatt, 1971, Ogunba,1982 and Acholonu & Nwanze, 1989) were done in the townships or relatively bigger villages where all their women did not all go to farm as a daily routine. The females in the villages in this study lived and worked on the farm and thus were as exposed to *Similium* bites as the males. Intensity of infection in females might however be different from that of males because males are likely to have longer and more frequent periods of exposure to infection than females.

The prevalence rate of infection of onchocerciasis among subjects in the age group 50-59 years was highest. This also was different from the infection patterns in age distribution obtained by Acholonu & Nwanze (1989). It was however noted that some (5 out of 8) within this age group who showed negative skin snips had clear symptoms of the infection. They had nodules and/or "leopard skin" and some had onchodermatitis and poor visual acuity in this onchocerciasis endemic area. In fact, a 72 years old man in this group who was skin snip negative but had a nodule was one of the two people whose nodules were excised, and adult parasites were found in them (see Figure 8).

Although infection rates could vary from village to village in an endemic area (WHO, 1987), it was not the case in this particular study. The villages were close to one another, within a radius of about one kilometer. The people in the different villages all had similar patterns of exposure to infection and the distances between them could not account for any factor that could bring varied infection rates.

The number of people showing different clinical manifestations was sixty-four and thirty-eight of these people were skin snip negative. If each of the clinical manifestations were used for identification of an infected person, then the infection rate would have increased. Some of these signs had been used by some workers as diagnostic criteria. Edungbola, *et al.* (1987b) used "leopard skin" as a rapid diagnostic index for estimation of the endemicity of onchocerciasis in Africa and concluded that it had a potential diagnostic value and also that other manifestations might be considered as alternative indicators of the infection.

More than half of the examined people had more than one clinical manifestations while a person (a woman) had all the clinical manifestations. Some of the clinical manifestations found in this study were age dependent. 'Leopard skin', 'Lizard skin', Velvet skin and vision impairments were found only in people of 50 years of age and above. However, onchodermatitis (skin rash), skin nodules and itching were found in people of all ages. Guinea-worm infection was common in the area as most of the people showed scars of the infection and blamed

guinea-worm infections for most of the onchocercal manifestations identified.

Wyatt (1971) stated that the most useful signs in the diagnosis of onchocerciasis are the presence of typical subcutaneous nodules, premature ageing of the skin and pretibial "leopard skin" depigmentation. Though the detection of nodules may have low sensitivity, yet because of its specificity and ease of notice and application, it may be of great use for the rapid preliminary assessment of infection at a community level (WHO, 1987). Therefore, the number of these people showing clinical manifestations could increase the infection rate to about 50%.

It is pertinent to note that these manifestations are late signs and some people may be infected and not show them as found in some cases in this study. Also, because some of the changes are irreversible, some people who had them may have lost the infection. This latter case may not be the situation in this area studied.

Generally, most of the people who had infection as indicated by positive skin snips also had clinical manifestations (86.7%). The clinical manifestations were disease related. The few cases that showed clinical manifestations and were skin snip negative, might be cases that were missed. According to Niklans & Marckaram (1989), the parasitological diagnosis is not sensitive in early infection and this could be so in exposed individuals living in an area with low transmission. This study area is a low transmission zone and it is possible that some cases might have been missed. Most of the manifestations here may likely be in the early development.

The findings here could not categorize the presence of nodules as late signs. About half of those people with nodules were negative for skin snips. Moreover, nodules were found in young people. There was the case of an eighteen years old boy with a large nodule below the knee, (See Figure 6). According to Buck (1974), nodule could be found in two years old chicken. Marked skin change like "leopard skin", "lizard skin" and velvet skin including vision impairments were age related. They occurred only in older people and might be late signs.

The two skin nodules excised had adult worms in them. One of the nodules had the worms dead during the course of transportation from the study area to our Lagos Laboratory. The worms in the other nodule died as a result of treatment with Banocide (DEC). The features on the worms in the first nodule differed from the second that had deposition on them, (See Figures 8 & 9). There was indication that the first worm died outside the host's body as there was no evidence of immunological reaction to the dead adult as was the case in the second worms.

It is commonly believed that Diethylcarbamazine (DEC), Banocide, does not kill adult *O. volvulus* especially at the low dosage normally recommended in order to minimize side reactions (WHO, 1966, 1984). In this particular study, it is believed that the adult worms were killed by DEC. The man on whom the nodule was excised had microfilariae in the skin snips a week before taking the drug and was skin snip negative just before undergoing the nodule excision. Moreover, the worms showed certain depositions on their surfaces. Plausible explanation for this effect of DEC is the high dosage of the drug. The fellow reported side reactions similar to the Mazzotti reactions, but there was no apparent ocular or any other complication as the fellow was in good condition and had the nodule excision barely seven days after the first dosage of the drug.

Many medicinal compounds now in use for the treatment of onchocerciasis. were originally designed with an entirely different purpose in mind (WHO, 1984). In this study, the drugs against intestinal nematodes had effect on the microfilariae of *O. volvulus in vitro*. The parasite is a helminthic nematode like the intestinal worms for which the drugs and herbal extracts were known to be efficacious. Based on the likely structural and physiological similarities that could exist between *O. volvulus* and the intestinal nematodes, the efficacy of these drugs on the microfilariae could not be far from being plausible or acceptable.

With respect to the plant leaves, according to Oliver (1960), the leaves of *Albizia*_are used as an anthelminthic drug. Ayitey-Smith (1989) also stated that extract of *Carica papaya* seeds is efficacious against intestinal worms. The plant, *Momodica*

charantia (Ejinrin – Yoruba) is commonly used among the Yorubas in Nigeria against intestinal worms. Their effects on *O. volvulus* could therefore be expected. Acholonu & Nwanze (1989) did similar work using *Albizia* leaves and had similar pattern of efficacy on *O. volvulus* microfilariae *in vitro*. They also worked on some anthelminthic drugs different from the ones used in this study and found them to be efficacious.

Although these drugs and herbal extracts remain to be tried *in vivo* against microfilariae and the adult worms of *O. volvulus,* it is likely that when done, they might be efficacious and reduce the side effects and ocular complications associated with DEC treatment. Laukamm-Josten (1987) did similar work using Midazolam and Desowitz *et al.* (1984) used diazepam with this motive in mind and found the diazepam to reduce the side reactions of DEC in Dog filariasis.

With the results thus obtained *in vitro* against microfilariae, of *O. volvulus*, these drugs and extracts could be tried against adult worms or both stages of the worm *in vivo*. The use of local herbs for treatment of *O. volvulus* could be remarkable in Nigeria especially as the country has sanctioned herbal medicine or alternative medicine and looks inward towards local sources of materials for industries. Also, the cost of treatment of onchocerciasis would be highly reduced if adopted.

5.0 Conclusion

The overall prevalence of onchocerciasis in the area surveyed was 23.8%. Levamisole hydrochloride (Ketrax) and *M. charantia* were most effective, while *A. coriaria* extract was least effective. All the drugs and plant extracts had microfilaricidal effects at the concentrations of 100g/ml, 50g/ml, 10g/ml, and 1g/ml, although the periods of their effectiveness differed. Further studies involving *in vivo* screening may prove the application of these drugs and herbal extract in onchocerciasis treatment effective and is recommended for future investigators.

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