COMPARISON OF CLINICAL, PARASITOLOGICAL AND SEROLOGICAL DIAGNOSTIC METHODS FOR THE DEFINITIVE DIAGNOSIS OF ONCHOCERCIASIS IN NSUKKA SENATORIAL ZONE

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ABSTRACT

Clinical, parasitological and serological diagnostic methods were compared for definitive diagnosis of human onchocerciasis in three endemic communities of Nkpologu, Ukpabi and Obimo; located at differing distances from the bank of Adada river in Nsukka senatorial zone of Enugu State, Nigeria. The results revealed that 43.98%, 2.78%, 57.60% and 76.55% of the total number of volunteers tested were positive by most common and rare clinical symptoms, skin biopsy and Enzyme-linked immunosorbent assay (ELISA) respectively. Of those seropositive, 86.02% had microfilariae in their skin. Similarly, 67.28% and 91.91% of those who were positive by ELISA and skin biopsy respectively, displayed onchocercal nodules either on the head, trunk, groin, laps or near the knee. However, 96.76% of those with nodules had microfilariae in their skin. The results further showed that the incidence of onchocerciasis and worm burden in the three communities vary inversely with their respective distances from the river. Considering the relative significance of these methods in the diagnosis of onchocerciasis, we recommend the use of a combination of the most common clinical manifestations, skin biopsy and ELISA in the diagnosis of onchocerciasis, at least for epidemiological studies, until a single definitive diagnostic method is developed.

Keywords: Human onchocerciasis, Clinical symptoms, Skin biopsy, ELISA

INTRODUCTION

Human onchocerciasis is a major blinding disease in equatorial Africa, Central and South America (Guderian *et al.*, 1997), Yemen and Asia (Morroquin, 1981). The disease is also associated with other morbid and debilitating presentations such as visual impairment, blindness, dys-pigmentation, itching-skin rashes, skin keratin-ization, onchocercal nodules, elephantiasis, genital hydrocoele and muscular skeletal pains (Nwoke, 1992; Nwoke *et al.*, 1993; Abanobi *et al.,* 1994) and renal impairment (Ngu and Blackett, 1976).

Although skin biopsy is the main diagnostic method for human onchocerciasis (Toel *et al.*, 1998) and nodule palpation rated indicator of choice for clinical diagnosis, on account of its close correlation with the result of skin biopsy (TDR News, 1992), they cannot be used to detect low and prepatent infections (Anya, 1981). Apart from not being very sensitive, skin biopsy has an additional defect such as being painful and its high risk of blood-borne infections such as human immunodeficiency virus (Hagan, 1998). The development of a single, sensitive and specific diagnostic test for onchocerciasis has remained a priority of the World Health Organisation (WHO). Previous unsuccessful attempts have been made to resolve the problem associated with onchocerciasis diagnosis. Such attempts were the assessment of two or more clinical manifestations of the disease (Gemade and Utsalo, 1990; Kelly and Akogun, 1997), provision of large quantities of onchocercal antigens by cloning for serodiagnosis (Chandrashaker *et al*., 1991, 1996), the use of polymerase chain reaction method to identify onchocercal specific complement DNA (Zimmerman *et al.*, 1994) especially in superficial bloodless skin scrapping (Toel *et al*., 1998) and the monitoring of the elevated serum angiotensin (Ronday *et al.,* 1996). However, the search for a sensitive diagnostic tool has hitherto been rendered illusive by many varying clinical manifestations of the disease in patients (Cohen and Warren, 1982). The need therefore to solve the problems of the existing diagnostic tools and the dire need for a single more definitive diagnostic method for onchocerciasis informed this study. Consequently, the existing clinical manifestations for onchocerciasis, skin biopsy and ELISA were therefore assessed and compared for the definitive diagnosis of the disease in three onchocerciasis – endemic contiguous communities located at differing distances from Adada river in Nsukka Senatorial Zone of Enugu State, Nigeria.

MATERIALS AND METHODS

Study Area and Population: This study was carried out in Nkpologu, Ukpabi and Obimo communities, located between longitude 7°08' and 7°20' East and latitude 6°46' and 6°49' North. The communities lie at the northern bank of Adada river, a well aerated fast-flowing river that flows south-westwards into Obinna river which, in turn empties into Anambra river, a tributary of river Niger. While Nkpologu community is located one (1) kilometer, Ukpabi and Obimo communities are located five (5) and six (6) kilometers from Adada river respectively. According to the Nigerian census of 1991, the total population of the three communities was 33,630 (males = 15938 and female = 17,692). The inhabitants of these communities are predominantly crop - farmers whose farmlands are as close as possible to the Adada river for purposes of improved farm yields. Many young male and female children are either in primary or secondary schools but actively take part in their seasonal farm work.

Sample Size: A total of 4120 inhabitants of the three communities (2834 males and 1286 females) were randomly tested in this study. A total of 1906 persons (1311 males and 595 females) were tested in Nkpologu, 886 (609 males and 277 females) were sampled in Ukpabi and 1328 volunteers (914 males and 414 females) were tested in Obimo communities. A total of 103 negative control subjects (77 males and 26 females) were selected from the University of Nigeria Nsukka community. These negative control subjects had no history of onchocerciasis nor had they any onchocercal infection by the time the samples were taken from them.

Clinical Diagnosis: Proforma was completed by or for each of the volunteers to obtain their sex, age, and information on intake of "banocide" (diethylcarbamazine, DEC), discovered to be widely administered to the onchocerciasis victims by patent medicine dealers in the area. The subjects were then palpated for the presence of onchocercal nodules and examined for both most common and rare clinical manifestations such as tygroid-leopard skin, tough lizard skin, itching-skin rashes, blindness, elephantiasis, genital hydrocoel and impaired vision, by the medical officers attached to the Cottage Hospital, Nkpologu, Uzouwani Local Government Area of Enugu State. No detailed ophthalmologic examination was performed. However, visual impairment was determined by counting fingers at varying distances. Inability to count fingers at 3 meters or less was regarded as blindness (WHO, 1996). An auxiliary measure for the clinical diagnosis was done by watching out for the development of itching-rashes within a few hours after 2mg DEC per kilogramme body weight was administered, Mazzotti test (Mazzotti, 1951).

Parasitological Diagnosis: Skin snips were taken from iliac crest (Buck, 1974), calves and the back shoulder with a 1.5 mm corneo-sclerectomy punch (Storz, Instrument Comp, St Louis, USA). The sclerectomy punch was sterilized in absolute alcohol and then flamed over a spirit burner before using it on another subject. The operation was bloodless and painless. The skin snips, relatively of uniform size were weighed with torsion balance and then suspended in 150 μl physiological saline solution (0.85 % $^{w}/_{v}$), contained in 1.5 ml volume screw-stopped cryotube. The suspensions were incubated for six hours to achieve maximum migration of the microfilariae (Mf) out of the skin snips (Tada *et al.*, 1973). The presence of Mf in the solution was identified under low power magnification of Olympus microscope (Japan). Positivity for *Onchocerca volvulus* microfilariae was based on the presence of at least one Mf in the suspension (Figure 1). The total number of Mf was then counted. Worm density (worm burden) was expressed as the mean number of Mf per milligramme skin (Mf/mg Skin^{-1}).

Figure 1: Identified *Onchocera volvulus* microfilaria

Preparation of Sera: Blood (5 ml) was drawn from each of the volunteers by venipuncture and poured gently into 10 ml plastic centrifuge tube to clot. Serum was then prepared by centrifuging the blood at 3000 x g for 10 minutes using Hettich universal bench centrifuge (model 1200 Tutilingen). The upper layer (serum) was collected and stored frozen in 1.5 ml plastic vials in aliquots of 200 μl until used.

Preparation of Microfilarial Antigen Homogenate: Onchocercal nodules, excised from onchocerciasis patients by medical health officers (Figure 2) working at Cottage Hospital, Nkpologu, were freed of connective tissues and fatty materials, weighed and then immersed in normal physiological saline (0.85 % $^{w}/_{v}$) for washing. The nodules, contained in cold compartment, were transported to the Biochemistry Laboratory of the University of Nigeria, Nsukka, and stored frozen until used. Adult worms were isolated from the nodules according to the collagenase-enzyme digestion method of Schulzkey *et al.* (1977), and

then washed three times in RMPI buffer solution (Biolab) reconstituted as directed in the manufacturer's manual. Onchocercal antigen homogenate was then prepared from washed adult worms according to the modified method of Lobos *et al.* (1991) as summarized below. The worms were homogenized thoroughly, using glass homogenizer with Teflon piston in some quantity of extraction buffer. The extraction buffer contained 20 mM Tris-HCl, 2 % Deoxycholic Acid, ImM ethylenediamine tetracetic acid (EDTA) and I mM phenylmethyl sulphonyl fluoride (PMSF, Sigma) in a ratio of 20:1:20:20 respectively. 2.5 mg each of L-5-amino-L-(P-toluenesulphonyl)-amidopentyl-chloro-methyl ketone (TLCK) and L-I-(toluenesulphonyl) – amido-2-phenyl ethyl-chloromethyl ketone (TPCK, Sigma USA) were added and the solution centrifuged at 26,000 x g for 30 minutes at 4 ^{o}C. The supernatant containing the crude soluble antigen was collected, the protein concentration determined according to the modified micro-lowry method of Sachaterle and Pollack (1973) as reported by Beechey *et al.* (1975) and then stored in 200µl aliquots at –20 ^{o}C until used.

Figure 2: Excision of onchocercal nodules from onchocerciasis patient by medical health officer

Enzyme-Linked Immonosorbent Assay Procedure: Enzyme-linked immonosorbent assay (ELISA) was performed according to the method described by Voller *et al.* (1977) as modified by Speiser (1980).

RESULTS

Clinical Symptoms: Clinical examination of all the volunteers showed that, itching-skin rashes (57.94 %, 2387/4120), nodular presence (52.52 %, 2164/4120), tygroid-leopard skin (dyspigmentation) (36.84 %, 1518/4120) and tough, rough lizard skin (28.64 %, 1180/4120) were the most observable common symptoms, while blindness (0.02 %) elephantiasis (0.02 %), visual impairment (7.96 %) and hydrococle (0.02 %) were the less frequently encountered symptoms (Rare symptoms) among the people of all the communities. Although, goiter was observed in 15.68 % (646/4120) of the whole population, only 0.29 % (12/4120) was positive for microfilariae by skin biopsy (Table 1). While onchocercal nodules (Onchocercomas) were predominantly located at the forehead, legs, trunk, groin and near the knee of the victims (Figures 3 and 4). Skin dyspigmentation was located mainly on the hands and legs (Figures 5, 6 and 7).

Figure 3: Onchocercomas located at the forehead and trunk of a twelve year old onchocerciasis patient

Figure 4: Multiple onchocercal nodules located on the leg of a 67 year old farmer from Nkpologu community

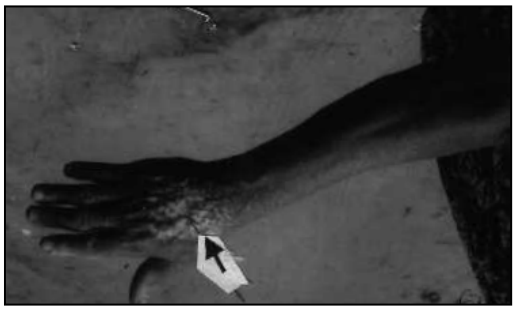

Figure 5: Leopard skin on the hand of an onchocerciasis patient from Nkpologu community

Table I: Prevalence of most common and rare clinical features of onchocerciasis in Nkpologu, Upkabi and Obimo Communities

	No. Positive	*Percentage Incidence (%)*
Observable Most Common Clinical Symptoms		
Communities	Nkpologu	
Total No. Tested	1906	
Itching of skin rashes	1290	67.68
Nodular Presence	1364	71.56
Tygroid-Leopard Skin		
Tough, Rough Lizard	904	47.43
Skin	698	36.62
(AVERAGE)	1064	25.82
Communities	Ukpabi	
Total No. Tested	886	
Itching of skin rashes	530	59.82
Nodular Presence	400	45.15
Tygroid-Leopard Skin		
Tough, Rough Lizard	250	28.22
Skin	160	18.06
(AVERAGE)	335	37.81
Communities	Obimo	
Total No. Tested	1328	
Itching of skin rashes	567	42.70
Nodular Presence	400	30.12
Tygroid-Leopard Skin		
Tough, Rough Lizard	364	27.41
Skin	322	24.25
(AVERAGE)	413	31.10
All Communities	Total	
Total No. Tested	4120	
Itching of skin rashes	57..94% (2397/4120)	
Nodular Presence	52.52% (2164/4120)	
Tygroid-Leopard Skin		
Tough, Rough Lizard	36.84% (1518/4120)	
Skin	28.64% (1180/4120)	
(AVERAGE)	43.98 (1812/4120)	
Less frequently encountered (Rare) Symptoms		
Communities	Nkpologu	
Total No. Tested	1906	
Blindness	-	-
Elephantiasis	1	0.05
Hydrocoel	-	-
Visual impaired	103	5.40
Goiter	4	0.21
AVERAGE	36	5.66
Communities	Ukpabi	
Total No. Tested	886	
Blindness	-	-
Elephantiasis	-	-
Hydrocoel	1	0.11
Visual impaired	164	18.51
Goiter	2	0.23
AVERAGE	57	6.32
Communities	Obimo	
Total No. Tested	1328	
Blindness	1	0.08
Elephantiasis	-	-
Hydrocoel	-	-
Visual impaired	61	4.59
Goiter	6	0.45
AVERAGE	23	1.73
All Communities	Total	
Total No. Tested	4120	
Blindness	0.02%(1/4120)	
Elephantiasis	0.02%(1/4120)	
Hydrocoel	0.02%(1/4120)	
Visual impaired	7.96%(328/4120)	
Goiter	0.29%(12/4120)	
AVERAGE	1.63%(67/4120)	

Figures 6 and 7: Depigmentation on the legs of a 66 and 70 year old onchocerciasis patients from Ukpabi and Obimo communities respectively. The 66 year old farmer carries one onchocercal nodule on one side of each knees (Figure 6)

Ninety percent (90 %) of those with onchocercomas were positive for microfilariae by skin biopsy, while 62 % of those with tygroid, leopard skin were positive by skin biopsy. Tough-rough lizard skin (Figure 8) was mainly observed in older patients, 93 % of whom were positive by skin biopsy.

Figure 8: Rough scaly legs of a 79-year old onchocerciasis patient from Nkpologu community

Rashes, accompanied by itching were located at all parts of the body (Figures 9 and 10). Ninety-six percent (96 %) of those with rashes were positive for microfilariae by skin biopsy. Elephantiasis (Figure 11) and genital hydrocoele were observed in one patient each and these patients were positive by skin biopsy and ELISA. Visual impairment was common in the residents of all the communities but only 15.20 % of them were positive by skin biopsy. Blindness was observed in only one of the subjects tested. Detailed investigation of the cause of the blindness at the University of Nigeria Teaching Hospital, Enugu, revealed accumulation of microfilariae in the eye, indicating that the microfilarae could be a possible cause of the blindness.

Incidence of Onchocerciasis in Relation to Proximity to River Adada: Tables 2 (A and B), show the incidence of onchocerciasis by skin

biopsy in the three communities, their relative distances from the river and the average worm-burden of the patients in each community. They indicate that the prevalence and the worm-burden increase as the distances of the communities from the river decrease.

Figures 9 and 10: Skin rashes on the hands and body of female onchocerciasis patients from Ukpabi community

Figure 11: Elephantiasis affecting the left leg of an onchocercal microfilariae-infected patient from Obimo community

Table 2: Prevalence of onchocerciasis in Nkpologu, Ukpabi and Obimo communities by skin snips (A) and ELISA (B)

(A) Skin Biopsy	Nkpologu	Ukpabi	Obimo	Total
No. Tested	1906	886	1328	4120
No. Positive	1270	542	561	2373
Percentage Positive (%)	66.63	61.17	42.24	57.60
(B) ELISA				
No. Tested	1906	886	1328	4120
No. Positive	1725	634	795	3154
Percentage Positive (%)	90.53	71.56	59.86	76.55
Distance From River (km)	1	5	6	4
Worm Burden (Mf/mg skin)	3.90	1.92	1.25	2.38

Comparison of Incidences of Onchocerciasis Obtained from Different Diagnostic Methods: Table 3 summaries the onchocerciasis diagnostic results of the communities.

Table 3: Prevalence of onchocerciasis based on clinical, parasitological and serological diagnostic methods

Diagnostic Methods	No. Tested	No. Positive	No. Positive Rate (%)
Most Common Clinical Symptoms	4120	1812	43.98
Less Frequently Observed Clinical Symptoms	4120	115	2.79
Skin Biopsy	4120	2373	57.60
ELISA	4120	3154	76.55

DISCUSSION

A combination of onchocercal clinical manifestations, parasitological and serological diagnostic methods were evaluated and then the results were accessed and compared for their onchocercasis diagnostic potentials in the three onchocerciasis endemic communities of Nkpology, Ukpabi and Obimo in Nsukka senatorial zone of Enugu State, Nigeria. The results identified positive correlation between the most common clinical features (nodular presence, skin dyspigmentation, itching-skin rashes and tough, rough scaly lizard skin) on one hand and skin biopsy and ELISA on the other hand. Although significant positive correlation ($p < 0.05$) exists between the most common clinical features, and ELISA, that between skin biopsy and ELISA was not significant ($P > 0.05$) (Table 4).

Table 4: Correlation coefficient of prevalence of onchocerciasis based on clinical pathological and serological diagnoses

		Most common	Less frequent	Skin biopsy	ELISA
Most common	Pearson Correlation	1.000	-.173	-.919	-.478
	Sig. (2-tail)	.	.781	.028	.416
	N	5	5	5	5
Less frequent	Pearson Correlation	-.173	1.000	-.089	-.368
	Sig. (2-tail)	.781	.	.886	.542
	N	5	5	5	5
Skin biopsy	Pearson Correlation	-.919*	-.089	1.000	.375
	Sig. (2-tail)	.028	.886	.	.534
	N	5	5	5	5
ELISA	Pearson Correlation	-.478	-.368	.375	1.000
	Sig. (2-tail)	.416	.542	.534	.
	N	5	5	5	5

Correlation is significant at the 0.05 level (2-tailed)

The most common clinical symptoms and skin biopsy diagnostic methods could therefore be relied upon for the diagnosis of the disease

particularly where laboratory facility for ELISA is not available. The average incidence rate of the less frequently observed clinical symptoms (1.63%) is too low to be considered for use in the clinical diagnosis of onchocerciasis.

The average prevalence of 43.98 %, 57.60% and 76.55% for the most common clinical features, skin biopsy and ELISA respectively, classify the communities as being mesoendemic for onchocerciasis according to the classification adopted for onchocerciasis endemicity (WHO, 1973). This result is consistent with a similar one reported by Edingbola and Asaolu (1984), in their parasitological survey for onchocerciasis in the Babara district of Kwara State of Nigeria. The study also identified low rate of blindness 0.02 % (1/4120) caused by onchocerciasis, perhaps because blindness rate seldom exceeds 3 % in onchocerciasis patients in the forest zone of Africa, but is responsible for high blindness rates up to 10% in the Sudan Savanna zone (Sasa, 1976, Ogurinade, *et al,* 1999). The study area is situated at the forest zone of Nigeria. The apparent causes of the low blindness rate, visual impairment and low incidence of worm-burden among the people of the study communities were not investigated. However, one could implicate large consumption of diethylcarbamazine (DEC) by the inhabitants of the communities as revealed by the patients during the study. Massive DEC consumption could reduce blindness and visual impairment rates by killing microfilariae that could migrate into the eyeball where they cause opacity of the eye or often damage the optic nerve (Sasa, 1976). In addition, microfilariae could invade the anterior chamber of the eye, their death causes chronic iritis which is one of the consistent and common ocular involvements of onchocerciasis (Sasa, 1976). Microfilarial worm burden could be reduced by the death of onchocercal microfilariae. Dead microfilarial constituents could trigger off allergic reaction (Hensan, *et al.,* 1979) which could be responsible for the high incidence of itching-skin-rashes (56.75 %) observed in this study.

The observation of skin dyspigmentation (leopard, tygroid skin), in 34.35% of the total volunteers tested, mainly among onchocerciasis patients over 30 years of age (Figs. 5, 6 and 7) is similar to that reported by Edungbola *et al.* (1987) who observed the symptom in only patients over the age of 20 years. The age-dependence of this manifestation could implicate the duration and intensity of onchocercal infection. However the occurrence of this manifestation in some people not positive for onchocerciasis (Figure 12) could implicate other factors such as leprosy (Browne, 1960), treponematoses (Buck, 1974) in the generation of the manifestation. Tough, rough scaly lizard skin observed mainly in older onchocerciasis victims positive for *O. volvulus* infection by skin biopsy and ELISA (Figure 7) could implicate long-standing onchocercal infection in the generation of the symptoms.

Figure 12: Depigmentation caused by another infection on a 48 year old woman from Nkpologu community. The woman was not positive for *Onchocerca volulus* by skin biopsy or by ELISA

The highest incidence of onchocercal nodules (71.56 %) and worm-burden (3.90 mg/mg skin) were apparent in Nkpologu community among older onchocerciasis victims than among younger ones, except in a 13 year old boy with multiple onchocercal nodules located in many parts of his body (Fig. 3). This selective occurrence of nodules in these communities could implicate duration of infection and degree of exposure to the bites of infected *Similium* vector among others, in the generation of onchocercal nodules.

Our results also revealed that the average incidence of onchocerciasis in all the communities as detected by ELISA (76.55%) is significantly higher ($p < 0.05$) than the overall average incidence (57.60%) evaluated for skin biopsy. Going by this result, it is probably reasonable to speculate that ELISA method of onchocerciasis diagnosis is more sensitive than the skin biopsy method. This speculation is consistent with the observation of Voller *et al.* (1977). The higher sensitivity by ELISA is not surprising given that ELISA method is principally based on the detection of serum antibodies produced against soluble or surface onchocercal antigen and thereby can detect prepatent or occult onchocercal infection. However, cross-reactions with the antibodies against antigens of some other common African parasitic worms (Roffi *et al.*, 1982) could possibly

exaggerate the ELISA incidence. In addition, consumption of DEC, evident in these communities, could as well contribute to this sensitivity difference existing between these two diagnostic methods. Diethylcarbamazine can flush microfilariae from the skin into the urine or sputum within few hours of treatment (Bryceson *et al.*, 1977), thereby reducing the number in the skin and precipitating false lower incidence by skin biopsy method.

Despite close proximity of the communities, similar climatic conditions and local topography, there still existed wide differences in onchocerciasis prevalences in the three communities as manifested by the different diagnostic methods in this study (Table 3). These differences could be attributed to variations arising from the frequency, duration and degree of exposure to the bites of infected blackflies, the vector of O*nchocerca volvulus*, possibly imposed by differences in the distances of the communities from the breeding site of the vector. The role played by proximity is supported by the fact that differences in prevalence rates of onchocerciasis obtained by different diagnostic methods show inverse relationship with the respective distances of the communities from Adada river, the breeding site of *Similium damnosum* as well as the worm-burden results in which Nkpologu, the nearest community to the river exhibited the highest worm-burden of 3.90 Mf/mg skin, while Obimo, the farthest community showed the least worm-burden of 1.25 Mf per mg skin. Alternatively, these differences could reflect the variations in the susceptibility of the residents to the infecting organism or the variations in the amount and regularity of drug treatment.

Going by the classification adopted for onchocerciasis endemicity by WHO (1973) and Tada *et al.* (1973), ELISA results depict hyperendemicity in the three communities while those of skin biopsy depict hyperendemicity in Nkpologu and Ukpabi and mesoendemicity in Obimo. This result can also support the higher sensitivity of ELISA when compared with skin biopsy. The most common clinical manifestation results exhibited mesoendemicity for Nkpologu and Ukpabi but hypoendemicity for Obimo, while those of minor clinical manifestations showed only hypoendemicity for all the communities. Although the incidences of the most common clinical manifestations considered in this study varied widely in the communities, they are still significant enough to be considered as relevant diagnostic tools for onchocerciasis as indicated by Nelson (1981). However, the incidences of the less frequently observed clinical manifestations obtained in these communities are too low to be considered relevant diagnostic tools.

Based on the high prevalence rate of this disease manifested by the most common clinical symptoms, parasitological and serological diagnostic methods in this study, we recommend, in support of other workers (Nwoke, 1992; Ovuga, *et al.*, 1992 and Abanobi, 1994) that a combination of the methods be employed in the diagnosis of the disease at least for epidemiological purposes, until a single, more definitive method is developed. The development of such a single definitive method is our priority.

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REFERENCES

ABANOBI C, EDUNGBOLA, L. D., NWOKE, B. E., MONICIAS, B. S. NKWOGU, F. U. and NJOKU, A. J. (1994). Validity of leopard skin manifestation in community diagnosis of onchocerciasis infection. *Applied Parasitology. 35(1):* 8 – 11.

ANYA, A. O. (1981). Nemathelminthes (Round Worms). Pages 704 – 726. *In:* Brande, A. T. (ed). *Medical Microbiology and Infections Diseases*. W.B Saunders Coy. USA.

BEECHEY, B. R., HUBBARD, A., LINNETT, P. E., MITCHELL, A. D. and MUNN, E. A. (1975). A simple and rapid method for the preparation of Adenosine Triphosphatase from submitschondrial particles. *Biochemistry Journal*, *148:* 533 – 537.

BROWNE, S. G. (1960). Onchocercal depigmentation. *Transactions of Royal Society of Tropical Medicine and Hygiene, 54:* 325 – 334.

BRYCESON, A. D. M., WARREL, D. A. and POPE, H. M. (1977). Dangerous reactions to treatment of onchocerciasis with dielthylcarbamazine. *British Journal, I:* 742 – 744.

BUCK, A. A. (1974). Onchocerciasis: symptomatology, pathogen and diagnosis. WHO Geneva.

CHANDRASHAKAR, R., MOSHOOD, K., ALVAREZ, R.M., OGUNRINADE, A. F., LIJAN, R. RICHARDS, F. O. and WEIL, G. J. (1991). Molecular cloning and characterization of recombinant parasite antigens for immunodiagnosis of onchocerciasis. *Journal of Clinical Investigation, 88(5):* 1460 – 1466.

CHANDRASHEKAR, R., OGUNRINADE, A. F. and WEIL, G. J. (1996). Use of recombinant Onchocerca volvulus antigens for diagnosis

and surveillance of human onchocerciasis. *Tropical Medicine and Health, 1(5):* 575 – 560.

COHEN, S. and WARREN, S. K. (1982). *Immunology of parasitic infections*, 2nd ed. Blackwell Publications, pp 622 – 653.

EDUNGBOLA, L. D. and ASAOLU, S. O. (1984). Parasitologic survey of onchocerciasis (River blindness) in Babana District, Kwara State, Nigeria. *American Journal of Tropical Medicine and Hygiene, 33:* 1147 – 1154.

EDUNGBOLA, L. D., WATTS, S. J. and KAYOLE, O. O. (1987). Endemicity and striking manifestation of onchocerciasis in Shao, Kwara State, Nigeria. *African Journal of Medical Sciences, 16:* 147 – 156.

GEMADE, E. J. and UTSALO, S. J. (1990). Onchocerciasis in Benue State of Nigeria VI: The prevalence and distribution of the disease among the human population in Sati-Ikpov village. *Acta-Leiden, 59(1-2):* 51 – 58.

GUDERIAN, R. H., ANSELMI, M., ESPINEL, M., MANCERO, T., RWADENEISA, G., PROANO, R., CALVOPINA, H. M. VIEIRA, J. C. and COOPER, P. J. (1977). Successful control of onchocerciasis with community-based invermectin distribution in the Rio Santiago focus in Ecuador. *Tropical Medicine International Health, 2(1):* 982 – 988.

HAGAN, M. (1998). Onchocercal dermatitis: Clinical impact. *Annals of Tropical Medicine and Parasitology, 92 Supplementary I:* 585 – 596.

HENSEN, P. M., MACKENZIE, D. S. and SPECTOR, W. G. (1979). Inflammatory reactions in Onchocerciasis: A report of current knowledge and recommendations for further study. *Bulletin World Health Organization, 57(5):* 667 – 682.

KELLY, M. G. and AKOGUN, O. B. (1997). Rapid assessment of onchocerciasis prevalence and model for selecting communities for ivermertin distribution in West Africa. *Zentralble – Baketeriology, 28(1):* 146 – 154.

LOBOS, E., WEISS, N., KARAM, M., TAYLOR, H. R., OTTENSEN, E. A. and NUTMAN, T. B. (1991). An immunodiagnostic *Onchocerca volvulus* antigen: A specific and early marker of infection. *Science, 251(5001):* 1603 – 1605.

MAZZOTTI, L. (1951). Observations on the use of hatrazan and diethylearbamazine in Mexico. *American Journal of Tropical Medicine, 31:* 628 – 632.

MARROQUIN, F. H. (1981). Cutaneous, onchocerciasis. Pages 1628 – 1638. *In:* Brande, A. J. (eds). *Medical Microbiology and Infectious Diseases.* W. B. Saunders Coy, USA.

NELSON, G. S. (1981). Issues in filariasis – a century of enquiry and a century of failure. *Acta-Tropica, 38:* 197 – 204.

NGU, J. L. and BLACKETT, K. (1976). Immunological studies on onchocerciasis in Cameroon. *Tropical Geographical Medicine, 28:* 110 – 120.

NWOKE, B. E. B. (1992). Musculo-skeletal pain (MSP) in onchocerciasis: A potential in the low-cost epidemiological survey and in the assessment of impact of ivermectin treatment on a community. *Angew-Parasitology, 33(3):* 133 – 138.

NWOKE, B. E. B., ONWULIRI, C. O. and UFOMADU, G. O. (1993). Onchocerciasis in Benue State, Nigeria: Ecological background, local disease perception and treatment; vector/parasite dynamics. *Journal of Hygiene Epidemology, Microbiology and Immunology, 36(2):* 153 – 160.

OGUNRINADE, A., BOAKYE, D., MERRIWEALTHER, A. and UNNASH, T. R. (1999). Distribution of the blinding and non-blinding strains of Onchocerca volvulus in Nigeria. *Journal of Infectious Diseases. 179(6):* 1577 – 1579.

OVUGA, E., KIPP, W., MUNGHERERA, M. and KASARO, S. (1992). Epilepsy and retarded growth in hyperendemic focus of onchocerciasis in rural western Uganda. *East African Medical Journal, 69(10):* 554 – 556.

ROFFI, J., LAFABRIE, B., CHOVET, M., VIALE, H. and DIGOUTTE, J. P. (1982). Application du test ELISA an despistage de l'onchocercose. *Medica Tropica*, *42:* 297 – 306.

RONDAY, M. J., VAN-DER-LELIJ, A., WIENESEN, M., ROTHOVA, A. STILMA, J. S. and KIJLSTRA, A. (1996). Elevated serum angiotension – converting enzyme activity in onchocerciasis. *Lung, 174(6):* 393 – 397.

SACHATERLE, G. R. and POLLACK, R. L. (1973). A simplified method for the quantitative assay of small amounts of protein in biological material. *Analytical Biochemistry, 51:* 654 – 655.

SCHULZ-KEY, H., ALBIEZ, E. J. and BUTTNER, D. W. (1977). Isolation of living Onchocerca volvulus from nodules. *Tropenmedical Parasitology, 28*: 428 – 430.

SASA, M. (1967). *Human filariasis: A global survey of epidemiology and control.* University Park Press, Baltimove, p. 39.

SPEISER, F. (1980). Application of the Enzyme linked Immunoassay (ELISA) for the Diagnosis of filariasis and Echinococcosis.

Tropenmedicine and Parasitology, 31: 459 – 466.

TADA, I., IWAMOTO, T. and WONDE, T. (1973). Quantitative studies on the emergency of *Onchocera volvolus* microfilariae from skin. *Japan Journal of Tropical Medicine and Hygiene, 1:* 13 – 24.

TDRN (1992). A publication of UNDP/World Bank/WHO Special Research and Training in Tropical Diseases. *Tropical Diseases Research News, No. 4*, August (1992), p.5.

TOEL, L., BOATIN, B. A., ADJAMI, A., BLACK, C., MERRIWEATHER, A. and UNNASCH, T. R. (1998). Detection of *onchocerca volvulus* infection by 0-150 polymerase chain reaction analysis of skin scratches. *Journal of Infectious Diseases. 189(1):* 282 – 285.

VOLLER, A., BIDWELL, D. E., BARTLETT, A. and EDWARDS, R. (1977). A comparison of isotopic and enzyme-immunoassays for tropical parasitic diseases. Transactions of Royal Society of Tropical Medicine and Hygiene. 70, 98-106.

WHO (1973). Report of the preparatory assistance mission to the government of Dahomey Ghana, Ivory Coast, Mali, Niger, Togo and Upper Volta. Onchocerciasis control in the Volta River Basin Area. *World Health Organization OCPA,* 73: 1.

WHO (1996). WHO expert committee on onchocerciasis second report. *World Health Organization Lecture Report Series*, No. 334, 1996.

ZIMMERMAN, P. A., GUDERIAN, R. H., ARUAJO, E., ELISON, L., PHADKE, P., KUBOFAIK, J. and NUTMAN, T. P. (1994). Polymerase chain reaction based diagnosis of Onchocerca volvulus infection. *Journal of Infectious Diseases, 169(3):* 686 – 689.