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Comparative Analysis of the Diagnostic Methods Used in Detecting River Blindness in Selected Endemic Areas of Imo State, Nigeria

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ABSTRACT

Onchocerciasis also known as river blindness is a chronic parasitic disease caused by the filarial worm *Onchocerca volvulus.* This study was a cross sectional experimental study carried out to compare the diagnostic methods used in detecting river blindness in selected endemic areas of Imo state, Nigeria. The multistage sampling technique was adopted to select samples for the study. All subjects used for this study gave an informed consent to be part of the study. Bloodless skin snips were collected from the center of the nodule or other parts of the body with the assistance of a laboratory scientist and taken to the laboratory for analysis. A total of four hundred inhabitants of the studied communities (Umulolo, Amuro, Umuna, Umunumo, Onicha, Nzerem, Umuneke and Umulewe) were examined. Out of these, the number infected by *onchocerca volvulus* based on Skin-Snip Microscopy, Polymerase Chain Reaction (PCR), Mazzotti test, Dietylcarbamazine (DEC) patch test and Enzyme linked Immunosorbent Assay (ELISA) test were 59, 197, 50, 107, 201 respectively. SPSS analysis using the one way ANOVA showed a significance difference (P< 0.05) in the sensitivity of the PCR, Skin Snip Microscopy, Mazzotti, DEC Patch test and ELISA used for detecting *Onchocerca volvulus* in all the study areas. In conclusion, the diagnostic screening efficiency of ELISA and PCR were observed to be higher than that of the other diagnostic methods analyzed. It was recommended that further evidence-based, comparative research studies on current and conventional diagnostic methods should be done to ascertain reliability, reproducibility, sensitivity and accuracy of methods used for detecting River Blindness.

Keywords: River Blindness, Onchocerciasis, Skin-Snip Microscopy, Polymerase Chain Reaction (PCR), ELISA test

INTRODUCTION

River Blindness is a parasitic disease caused by the filarial nematode, *Onchocerca volvulus*.[1] This disease is also commonly called Onchocerciasis. The blindness component comes from its ocular involvement. It is the second leading infectious cause of blindness and ocular morbidity after Trachoma.[2] For centuries, *Onchocerca volvulus* has infected humans causing severe skin and eye disease. Transmitted through the bite of the *Simulium damnosum*, blackfly, the disease is prevalent in 19 African countries. In total, 37 million people are thought to have the active disease, with nearly all such cases in Africa where over 100 million people live at risk of new infections.[3] This old world disease originated in Africa and spread to New World via slave trade where it formally existed in 13 discreet geographical foci within Latin America.[4] Over 500,000 individuals live with a significant visual impairment from the disease, with an

additional 270,000 individuals who have suffered from complete vision loss. [5]

Onchocerca volvulus lives only in humans, making it a good candidate for elimination. The *Simulium* vector is infected when biting infected humans, and after maturation of larvae within the fly, can then re-infect others during subsequent blood meals. These flies breed within and live around fast-flowing rivers hence the name, *River Blindness,* and generally only persons living in or around these areas are at risk of infection. Once deposited within the skin, infective stage larvae mature and trigger the development of fibrous subcutaneous nodules in which they will mate and reproduce.[6] Annually, female adult worms can release hundreds of thousands of microfilariae (MF) that migrate freely through the skin with the potential for reaching and invading the eye. In the skin MF cause pruritus and dermatitis, and eventually can lead to skin atrophy and discoloration (leopard skin). In the eye, repeated microfilariae insults can lead to significant intraocular inflammation and subsequent eye damage.[7]The ocular pathology of the disease occurs in both anterior and posterior segments of the eye. Anteriorly, microfilariae travel through sclera and subconjuctival tissues to reach the cornea whereby they attempt to penetrate and migrate through the cornea. Within the cornea stroma, microfilariae can die and release *Wolbachia*sp, bacteria, an intracellular Rickettsia like bacterium that lives symbiotically with microfilariae and adult *Onchocerca volvulus* worms damage.[8]

Interestingly, these bacteria are extremely important to the life cycle and reproduction of the *Onchocerca volvulus* and without them, female adult worm cannot reproduce.[9] Onchocerciasis is a severe and debilitating parasitic infection of global concern. Its prevalence and the magnitude of associated social and economic effects vary widely in different geographical areas where the disease occurs. About 90 million people are at risk of which 17.6 million are already infected, including 326,000 people who have gone blind in 34 countries of the world. In Africa alone, close to over 60% of all global cases has been reported in 26 countries.[4] Onchocerciasis is perhaps the most studied filarial infection in Nigeria. The provisional estimates had suggested that 7-10 million Nigerians are infected with *Onchocerca volvulus,* approximately 40 million are at risk of the disease,[10] and 120,000 cases of onchocerciasis related blindness, with many thousands suffering from disabling complications of the disease. New foci of onchocerciasis are still being discovered and therefore its distribution could be far more expansive than has been earlier assumed. [11]

In South Eastern Nigeria, there are pockets of endemic foci as shown by some reported studies,[7,10] although there is gross under reporting of the scourge. Arguably, the most significant area in this sub-region as far as onchocerciasis is concerned is the hilly and undulating Udi-Enugu-Okigwe axis from where some rivers or their tributaries, supporting blackfly vector breeding have their origin. These include rivers such as Oji, Ajali, Mamu, Adada and Imo (the longest of them).[2]Consequent to the large socio-economic impact of the disease, several international programmes underway in Africa and America have the goal to eliminate onchocerciasis as a socio-economic and public problem. In any disease control programme, it is important to have safe and effective diagnostic methods available. The classical methods of detecting *Onchocerca volvulus* infection consists of microscopically examining small skin snips for the presence of the parasites' microfilariae. Although this method is a standard, it is invasive, painful and relatively insensitive.[12] Other possible methods of diagnosis include: molecular method (PCR), slit-lamp biomicroscopy, Enzyme-Linked Immunosorbent Assay (ELISA), Mazzotti test, Diethylcarbamazine (DEC) patch test, Rapid format antibody card test and current diagnostic technologies. [13] This study will compare

the diagnostic methods used in detecting river blindness in selected endemic areas of Imo state, Nigeria.

MATERIALS AND METHODS

The study was a cross sectional experimental study carried out in Riverine areas of Imo State, Nigeria where River Blindness is endemic. The multistage sampling technique was adopted to select samples for the study. All subjects used for this study gave an informed consent to be part of the study. Data was uploaded into the SPSS version 21 software and the one-way ANOVA was used to test for association at 5% level of significance.

Preliminary Tests

Before the collection of samples from the subjects, procedures for carrying out the tests were clearly explained and an informed consent obtained from each of the participants. Case history was taken to note subjects past and present ocular/medical histories if any. Visual acuity was assessed with the Snellen chart and external examination with the aid of a pen torch was carried out to detect any ocular surface abnormalities. Ophthalmoscopy was also done to check for pathologies.

Collection of Samples

Bloodless skin snips were collected from the center of the Nodule or other parts of the body (Shoulder or Upper arm) with the assistance of a laboratory scientist. The area of the skin was first disinfected with a gauze pad dipped in alcohol. A sterile needle is pushed into the skin 2.3 mm and lifted up. The cutting edge of a razor blade was placed at the top of the stretched skin. The pulled skin was then be cut off as close to the needle as possible. The cut specimen which is bloodless, 2.3 mm wide and attached to the tip of the needle was picked for parasitological examination. The samples were appropriately labeled and preserved in 80% ethanol.

Procedure for Skin Snip Microscopy

(i) A small drop of saline solution was dropped on a microscope slide which is clean and grease free.
(ii) The small piece of skin (skin snip biopsy) was placed in the drop of the solution on the slide.
(iii) It was then covered with cover slip.
(iv) The set up was allowed to stand for 30 minutes.
(v) It was thereafter examined under the microscope with x10 objectives for microfilariae.
(vi) For quantitative studies, 10mg of the skin specimen was weighed with a balance.
(vii) The specimen was transferred into one hole of a microtitration plate.
(viii) The plate was covered to avoid evaporation of water and left to incubate at ambient temperature for 24 hours.
(ix) To enhance digestion and sensitivity, collagenase enzyme was added.
(x) Microfilariae number was counted in the saline by microscopy using the x10 objective.

Polymerase Chain Reaction (PCR)

A molecular based Technique of PCR developed by Kary Mullis in 1983 involving DNA extraction from the prepared skin snip biopsies was followed by determination of DNA concentration primer, design and DNA sequencing for detection of the parasites identity. Real time quantitative PCR was used in determining parasite counts.

DNA Extraction from Skin Biopsies

DNA was extracted from skin biopsies and from three adult worm fragments using DNeasy Blood and Tissue Kit (QIAGEN, Germany) by adhering to the manufacturer's instructions. Briefly, samples were digested with 15µl of Proteinase K in 1.5 ml micro centrifuge tube, mixed by vortexing, incubating at 56^{o}C until completely lysed. Vortex was done during incubation and for 15seconds before 200µl Buffer AL was added. The mixture was incubated for 10 minutes at

56^{o}C and 200µl ethanol (96-100%) was added and mixed thoroughly by vortexing in between. The mixture was be transferred into the DNeasy spin column in a 2ml collection tube and centrifuged at ≥6000xg (8,000 rpm) for 1 min. Flow-through and collection tube were discarded. Spin column was placed in a new 2ml collection tube and 500µl Buffer AW1 added and centrifuged for 1 min at 6,000xg. Flow-through and collection tube was discarded. Spin column was placed in a new 2ml collection tube and 500µl Buffer AW2 added and centrifuged for 3 min. at 20,000xg (14,000 rpm). Flow-through and collection tube was discarded. Spin column was placed in a new 2ml micro centrifuge tube and DNA eluted by adding 200 or 100µl Buffer AE to center of spin membrane and incubated for 1 min. at room temperature (15-25^{o}C). It was centrifuged for 1 min. at ≥6,000xg and DNA extract in elution buffer was stored at -20^{o}C in deep freezer until used.

Determination of DNA Concentration

Each sample and positive control DNA extracts were subjected to spectrophotometric analysis at 260 and 280nm wavelength using NanoDrop 2000C (Thermo Scientific, England). To assess the purity of the DNA extract, the concentration in nanogram per microliter (ng/µl) was measured and the 260/280nm ratios of samples were obtained. Sample analysis was done as recommended by the manufacturer. Briefly, the software icon on desktop was double clicked and the application on menu bar was selected and the instrument was allowed to initialize. A blank was established using the distilled water in which the DNA sample was suspended. About 2µl was drawn with pipette onto the bottom pededstal and the upper arm was lowered and clicked on the black button. The Blank was wiped, the sample ID was entered in appropriate field and 2µl was pipette again and the measure on the menu bar was selected. Both pedestals were wiped after each sample with dry, lint-free laboratory wipe. Blanking cycle was completed with 4 repeats as above and obtained a spectrum readings not more than 0.04 A (10mm absorbance equivalent). After blanking, both pedestal surfaces was wiped and samples was analyzed. A new blank was taken every 30 minutes before the completion of sample analysis.

Onchocerciasis Primer Data Mining

GenBank database of National Centre of Biotechnology Information (NCBI), Atlanta, USA was searched for primer sequences specific for *Onchocerca volvulus*. The possibility of mis-match occurrence depends on the length of the primer, 18 was set as minimum, while 23 bp was taken as maximum to avoid to dimer-dimer formation.

Procedure for Mazzotti-Test

(i) The patients were given oral dose (50 mg) of Diethylcarbamazine.
(ii) The appearance of an acute pruritic rash within 2-24 hours (from death of microfilaria in the skin) confirms positive result.
(iii) Negative test does not produce any rash on the patient within 2-24 hours.

Procedure for Enzyme Linked Immunosorbent Assay (Elisa) Test

This is a serology-based rapid test that detects human IgG4 (immunoglobulin G4) antibodies to the *onchocerca volvulus* antigen OV-16.

(i) Capillary blood samples were collected by finger prick.
(ii) The immunochromatographic card test was used to detect the presence of immunoglobin G4 (IgG4) antibodies to recombinant OV-16 antigen.
(iii) A color change on the card surface indicates a positive test.

Diethylcarbamazine (Dec) Patch Test

(i) A local application of 10% DEC anhydrous Lanolin was applied to the skin and covered with dressing (OCP Patch).

(ii) The patch was then checked for local dermatitis caused by the dying microfilariae

(iii)The easy application and low risk of this test makes it ideal for testing for the re-emergence of the disease in a treatment area.

RESULTS

A total of four hundred (400) inhabitants of the studied communities (Umulolo, Amuro, Umuna, Umunumo, Onicha, Nzerem, Umuneke and Umulewe) were examined. Out of these, the number infected by *onchocerca volvulus* based on Skin-Snip Microscopy, Polymerase Chain Reaction (PCR), Mazzotti test, Diethylcarbamazine (DEC) patch test and Enzyme linked ImmunosorbentAssay (ELISA) test were 59, 197, 50, 107, 201 respectively. The diagnostic screening efficiency of ELISA and PCR were observed to be higher than that of the other diagnostic methods analyzed. Table 1 showed the prevalence of Onchocerciasis in the study area based on Skin Snip Microscopic test. Out of 400 subjects examined, 59 (14.75%) were infected. The prevalence of infection was highest 18(36%) at Umulolo, followed by Amuro 12 (24%), Umuna 9(18%), Umulewe 8(16%) Onicha 6 (12%), Umunumo 4(8%) and least at Nzerem and Umuneke 1(2%). Table 2 showedthe prevalence of Onchocerciasis in the study area based on PCR test. Out of 400 subjects examined, 197 (49.25%) were infected. The prevalence of infection was highest 49(98%) at Umulolo followed by Amuro 35(70%), Umuna and Umulewe 24(54%), Onicha 22(44%), Nzerem 15(30%), Umuneke 14(28%) and least at Umunumo 8(16%). Table 3 showed the prevalence of onchocerciasis infection in the study areas based on Mazzottitest. Out of 400 examined subjects, 50(12.5%) were infected. The prevalence of infection was highest in Umulolo 11(22%). Amuro and Umuna showed a prevalence rate of 10(20%). Umulewe had a prevalence rate of 7(14%), Onicha 4(8%), Umunumo and Umuneke 3(6%) and the least at Nzerem 2(4%). Table 4 showed the prevalence of Onchocerciasis infection in the study areas based on DEC patch test. The highest prevalence rate of infection was at Umulolo 30(60%). Followed by Amuro 27(54%), Umuna 17(34%), Onicha 12(24%), Umulewe 8(16%), Nzerem 5(10%) and the least at Umunumo and Umuneke 4(8%)

Table 5 showedthe prevalence of Onchocerciasis infection in the studied areas based on ELISA Test The prevalence of infection was highest at Umulolo 48(96%). This was followed by Amuro 40(80%), Umulewe 30(60%), Umuna 25(50%), Onicha and Nzerem 20(40%), Umuneke and the least at Umunumo 8(16%).Table 6 shows the distribution of onchocercal ocular findings in the studied areas. Out of the average number of subjects infected in Umulolo, 6 (30%) had itchy eyes, 8(40%) had impaired vision and 6 (30%) also were blind. In Amuro community, 8 (47.1%) had itchy eyes, 5 (29.4%) had impaired vision and 4 (23.5%) were blind. Umuna had 4 (36%) subjects with itchy eyes, 5 (45.5%) impaired vision and 2 (18.1%) were blind. Umunumo had 2 (50%) subjects with itchy eyes, 1(25%) impaired vision and 1 (25%) were blind. Onicha had 4 (36.4%) subjects with itchy eyes, 5 (45.5%) impaired vision and 2 (18.1%) were blind. Nzerem had 2 (22.2%) subjects with itchy eyes, 5 (55.6%) impaired vision and 2(22.2%) were blind. Umuneke had 1 (16.7%) subjects with itchy eyes, 4 (66.7%) impaired vision and 1 (16.7%) were blind. Umulewe had 3 (25%) subjects with itchy eyes, 8 (66%) impaired vision and 1 (8.3%) were blind. Out of the overall 124 (31%) of the study participants infected, about 30 (33.3% reported itching eyes, 41 (45.6%) reported vision impairment and only 19 (21.1%) were blind. Table 7 showed the comparative evaluation of the various diagnostic tests. The True Positive (TP) and False Negative values for Skin Snip, PCR, Mazzotti test, DEC Patch Test and ELISA were 59:85, 197:50, 50:87.5, 107:73.2 and 201:50 respectively.

Similarly, the False Positive (FP) and True Negative (TN) values for Skin Snip, PCR, Mazzotti test, DEC patch Test and ELISA were 15:341, 50:197, 12.5:350, 26.8:293 and 50:199 respectively. ELSIA and PCR indicated high level of Sensitivity and thus higher Accuracy in the disease diagnosis. Table 8 showed the comparative estimation of the diagnostic parameters used to assess the screening efficiency of the studied diagnostic tests. This revealed that the prevalence of Onchocerciasis in the studied areas were 14.75% for Skin Snip test, 49.25% for PCR, 12.50% for mazzotti, 26.75% for DEC Patch test and 50.25% for ELISA. Also the Sensitivity of Skin Snip tests was 40.97%, PCR had a Sensitivity of 80.24%, Mazzotti test had a Sensitivity as low as 36.40%, DEC Patch test had a Sensitivity of 59.40%, while the ELISA Test had a higher Sensitivity of 80.08%.

SPSS analysis using the one way ANOVA showed a significance difference ($P< 0.05$)in the sensitivity of the PCR, Skin Snip Microscopy, Mazzotti, DEC Patch test and ELISA used for detecting *Onchocerca volvulus* in all the study areas.

Table 1: Prevalence of Onchocercal Infection in the Study Area based on Skin Snip Microscopic Test

Study Communities	No. Examined	No. Uninfected	No. Infected	% Infection (Prevalence)
Umulolo	50	32	18	36.00
Amuro	50	38	12	24.00
Umuna	50	41	9	18.00
Umunumo	50	46	4	8.00
Onicha	50	44	6	12. 00
Nzerem	50	49	1	2. 00
Umuneke	50	49	1	2.00
Umulewe	50	42	8	16. 00
Total	**400**	**341**	**59**	**14.75**

Table 2: Prevalence of Onchocercal Infection in the Studied Areas based on PCR

Study Communities	No. Examined	No. Uninfected	No. Infected	% Infection (Prevalence)
Umulolo	50	01	49	98.00
Amuro	50	15	35	70.00
Umuna	50	23	27	54.00
Umunumo	50	42	08	16.00
Onicha	50	28	22	44.00
Nzerem	50	35	15	30.00
Umuneke	50	36	14	28.00
Umulewe	50	23	27	54.00
Total	**400**	**203**	**197**	**49.25**

Table 3: Prevalence of Onchocercal Infection in the Study Areas based on Mazzotti Test

Study Communities	No. Examined	No. Uninfected	No. Infected	% Infection (Prevalence)
Umulolo	50	39	11	22.00
Amuro	50	40	10	20.00
Umuna	50	40	10	20.00
Umunumo	50	47	3	6.00
Onicha	50	46	4	8.00
Nzerem	50	48	2	4.00
Umuneke	50	47	3	6.00
Umulewe	50	43	7	14.00
Total	**400**	**350**	**50**	**12.50**

Table 4: Prevalence of Onchocercal Infection in the Study Areas based on Diethylcarbamazine (DEC) patch test

Study Communities	No. Examined	No. Uninfected	No. Infected	% Infection (Prevalence)
Umulolo	50	20	30	60.00
Amuro	50	23	27	54.00
Umuna	50	33	17	34.00
Umunumo	50	46	04	08.00
Onicha	50	38	12	24.00
Nzerem	50	45	05	10.00
Umuneke	50	46	04	08.00
Umulewe	50	42	08	16.00
Total	**400**	**293**	**107**	**26.75**

Table 5: Prevalence of Onchocerciasis Infection in the Studied Areas based on ELISA Test

Study Communities	No. Examined	No. Uninfected	No. Infected	% Infection (Prevalence)
Umulolo	50	02	48	96.00
Amuro	50	05	40	80.00
Umuna	50	25	25	50.00
Umunumo	50	40	08	16.00
Onicha	50	30	20	40.00
Nzerem	50	30	20	40.00
Umuneke	50	40	10	20.00
Umulewe	50	20	30	60.00
Total	**400**	**199**	**201**	**50.25**

Table 6: Distribution of Onchocercal Ocular Findings in Studied Areas

Study Area	No. Examined	Average no (%) infected	Itchy Eyes	Impaired Vision	Blindness	Total (%) with Ocular Signs and Symptoms
Umulolo	50	30 (60%)	6 (30%)	8 (40%)	6 (30%)	20 (66.7%)
Amuro	50	25 (50%)	8(47.1%)	5 (29.4%)	4 (23.5%)	17 (68%)
Umuna	50	18 (36%)	4 (36.4%)	5 (45.5%)	2 (18.1%)	11 (61.1%)
Umunumo	50	5 (10%)	2 (50%)	1 (25%)	1 (25%)	4 (80%)
Onicha	50	13 (26%)	4 (36.4%)	5 (45.5%)	2 (18.1%)	11 (84.6%)
Nzerem	50	10 (20%)	2 (22.2%)	5 (55.6%)	2 (22.2%)	9 (90%)
Umuneke	50	7 (14%)	1 (16.7%)	4 (66.7%)	1 (16.7%)	6 (85.7%)
Umulewe	50	16 (32%)	3 (25%)	8 (66.7%)	1 (8.3%)	12 (75%)
Total	**400**	**124 (31%)**	**30 (33.3%)**	**41 (45.6%)**	**19 (21.1%)**	**90 (72.6%)**

Table 7: Comparative Evaluation of Skin Snip Microscopy, PCR, Mazzotti, DEC Patch and ELISA Tests for Onchocerciasis

Diagnostic Tests	TP	FN	FP	TN	Total Population (TP+TN)
SKIN SNIP	59.00	85.00	15.00	341.00	400
PCR	197.00	50.00	50.00	197.00	400
MAZZOTTI	50.00	87.50	12.50	350.00	400
DEC PATCH	107.00	73.20	26.80	293.00	400
ELISA	201.00	50.00	50.00	199.00	400

TP – True Positive (Disease Present, Detected by Test)
TN – True Negative (Disease not present, not detected by test).
FN – False Negative (Disease present, not detected by test)
FP – False Positive (Disease not present, detected by test)

Table 8: Comparative Estimation of Prevalence Rate and Sensitivity of the Studied Diagnostic Test

Diagnostic Parameters (%)	Skin snip	PCR	Mazzotti	DEC Patch	Elisa
Prevalence	14.75	49.25	12.50	26.75	50.25
Sensitivity	49.97	80.24	36.40	59.40	80.08

DISCUSSION

Diagnostic tools appropriate for undertaking interventions to control River Blindness (Onchocerciasis) infection are the key to a successful control and elimination of the disease. The accurate identification of infected populations at higher risk and with low infection is necessary to implement an effective control program for an endemic area which will improve the assessment of drug efficacy and patient management.[14] According to Zimmerman et al.[15] definitive diagnosis of onchocerciasis requires the identification of the parasites in either the skin or subcutaneous nodules. These parasitological approaches suffer from poor sensitivity. Much will be lost in local elimination programs if a significant number of low-level infections are not detected. The results from this research's diagnostic comparison showed that the test performances of Enzyme-Linked Immunosorbent Assay (ELISA) and the Polymerase Chain reaction (PCR) were in Conformity with other published studies. [16,17]

The results of this study revealed that onchocerciasis was endemic in eight areas of Imo State, Nigeria with total infection rate of 14.75%. More *onchocerca volvulus* infection was recorded in Umulolo of Okigwe. This observation is in line with the studies by Uttahet al.[2] and Anosikeet al.[3] This might be due to the geographical setting or more favorable ecological conditions for the breeding of Black flies that transmit the parasites. The Upper Imo Rivers and several streams and conditions

are favorable for transmission of River Blindness (Onchocerciasis). Eleven species of simulium including *S.damnosum* have thus been reported from the area.[18] Since microfilaria density is important in on-set of clinical manifestations, the high prevalence of onchocerciasis-related eye manifestations reported in the Universal Institution Review Board (UIRB) may be indicative of higher prevalence than reported here.[19] This area therefore should probably be regarded as the most severe onchocerciasis focus in the eastern part of Nigeria, forming a continuum of high onchocerciasis endemicity with the Udi Hill range, Oji River Basin and the Anambra River Basin. However, it takes approximately 1.5 years for adult worm to mature and release enough microfilaria to be detectable by Skin-Snip Microscopy, Mazzotti Test and DEC Patch Test.[18] Testing for cure in treated populations especially in those with a low prevalence of infection, presents a challenge.[20] It was observed in the results of this study that PCR and ELISA are more sensitive and thus more accurate in detecting *onchocerca volvulus* in tested specimen. However, it is important to note that PCR and ELISA both depended on the presence of *onchocerca volvulus* microfilaria in the skin and blood respectively. ELISA and PCR are clearly more sensitive for detecting Microfilaria (MF) than other diagnostic methods. While ELISA was performed using human serum, PCR was performed on recycled specimens. ELISA and PCR which are more sensitive than other methods can be used to determine whether MF that are transmitted by black flies vector are still present in humans prior to discontinuation of Community Directed Treatment with Ivermectin (CDTI).[14,16]

Although Ivermectin has little permanent effect on female adult worms, the drug clears MF from the skin for a period of months. For this reason, if PCR is used to verify the absence of MF in sentinel populations, it is important to delay sample collection for a period of months after the last Ivermectin distribution to provide time for MF from surviving adult worms to repopulate the skin. However, ELISA which is an antibody detection tests is more suitable, more reliable and more sensitivity as compared with the studies done by Rodriguez et al.[21] where the sensitivity of the ELISA to recombinant antigen to onchocerciasis and PCR were 97% and 86% respectively. This simply suggest that the PCR-based assay is significantly more sensitive than Skin Snip Microscopy and this overcomes many deficiencies of parasitologic and serologic methodologies in diagnosing active onchocerciasis.[22]

Onchocercal blindness is the most serious and perhaps feared clinical manifestation of onchocerciasis because of its irreversibility.[5] Results showed an overall blindness prevalence of 0.2% which by any known epidemiological index is low. This is not surprising since the prevalence of onchocercal ocular blindness in West Africa has been reported to be lower in the rainforest than in the Savanna zone.[5] The difference may be due to the reported greater invasiveness and pathogenicity of the cornea of the eye by savanna strains of parasite than the forest strains.[23] In addition, climatic factors may play a role in this difference especially with the intense sunlight combined with Harmattan dust and dryness in the savanna which could influence/alter corneal metabolism, making it more susceptible to microbial invasion. Furthermore, from the statistical analysis using the one way ANOVA, a comparative analysis between the different diagnostic methods among the selected endemic communities showed that there was a significant difference in the diagnostic parameters of the different diagnostic methods. The results showed a very strong difference between the diagnostic methods used in detecting *Onchocerca Volvulus*. The result of this study is in line with the results of other studies. [16,24]

CONCLUSION

In conclusion, the most important concept to understand when comparing

diagnostic modalities is the need for a reliable gold standard. This research work has shown from results and analysis that the antibody detection serological ELISA test and the molecular based PCR technique are the most sensitive and most accurate diagnostic methods in detecting onchocerciasis (River Blindness). It was recommended that further evidence-based, comparative research studies on current and conventional diagnostic methods should be done to ascertain reliability, reproducibility, sensitivity and accuracy of methods used for detecting River Blindness.

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