 Original Research Article

Prevalence of Concomitant Onchocerciasis-Malaria Infection in Ahani-Achi Community, Enugu State, Nigeria.

Akujobi A.U.,[1] Nwakamma, G,I.,[2] Ejidike, G.E.,[3] Obioma-Elemba. J.E.,[1] Chinelo C. Vincent, C.C.,[4] and Akogu, O.A.[1]

1. Department of Optometry, Imo State University, Owerri, Nigeria.
2. Department of Optometry, Federal University of Technology, Owerri, Nigeria.
3. Department of Optometry, Madonna University, Elele, Nigeria.
4. Department of Nursing Science, Imo State University, Owerri, Nigeria.

Corresponding Author: Akujobi A.U. | Email: drakujobi@yahoo.com | Phone: M +2348033727967

Abstract

The preponderance of onchocerciasis and malaria infections in similar ecological settings is incidental to co-infection. Therefore, investigations into the mixed-infection pattern of these parasitic diseases are apt in order to maximize interventions and facilitate epidemiological mapping. A population-based cross-sectional study was conducted in Ahani-Achi community, a rain forest mosaic area of Enugu State, Nigeria from March 2012 to April 2014 to ascertain the prevalence of onchocerciasis-malaria co-infection. Four hundred and forty seven (447) persons aged 10 - ≥60 years were recruited into the study through a convenience sampling technique and examined using standard skin snip biopsy and smear microscopy techniques. Three hundred and nine (309) persons tested positive to concurrent O. volvulus and malaria parasitemia, representing a co-infection rate of 69.13%. In both males (53.62%) and females (70.83%), the ≥60 years age group was most infected and the infection rate was significantly ($p<0.05$) affected by age. On the overall, more females (77.50%) were infected than males (59.45%), although the difference was not statistically significant ($p>0.05$) while fishermen were significantly ($p<0.05$) more infected (81.08%) than other occupation groups. Although the burden of co-infection reported is worrisome, the study strongly posits that if periodic surveillance and integrated approach to control strategies are adopted and sustained, the morbidity, mortality and economic loss associated with these infections will be forestalled.

Keywords: Prevalence, Concomitant, Onchocerciasis, Malaria, Infection.

Introduction

Human onchocerciasis, commonly called river blindness is a chronic parasitic disease caused by Onchocerca volvulus-- a nematode that belongs to the family of Filaridae and the only Onchocerca with a human host, although an infected Spider-Monkey and Gorilla have been recorded[1]. In Nigeria and other West African Countries, the microfilaria are predominantly found in the lymphatic channels of the skin around the pelvis and upper arm and have caused blindness, visual impairments, general decrepitude and grave socio-economic problems[2]. Onchocerciasis is the second leading cause of blindness in the world[3] and it is estimated that between 18-37million people are infected, with up to 1-2 million people who are visually impaired and 270,000 people who are rendered completely blind[4].

1. Engelkirk PG, Williams JF, Schmidt GM, Leid RW. Zoonotic Onchocerciasis. Handbook Series in Zoonoses. 1982; (2): 225-250.
2. Nwoke BEB. Worms and Human Diseases (First Edition). Owerri. Alphabet Nigerian Publishers. 2009.
3. Deut AE, Kazura JW. Other Tissue Nematodes. Nelson Textbook of Pediatrics. 2011; 1225-1227.
4. Diemert DJ. Tissue Nematode Infections. Goldman's Cecil Medicine. 2011; 193-200.

Out of the 37 endemic countries, 30 are in Africa and 6 are in Latin America[5], however, more than 90 million people are estimated to be at risk in Africa, with Nigeria and Zaire being the most affected countries[6].

O. volvulus invades all ocular tissues from the eyelid through the conjunctiva to the deeper tissues of the eye. Predominantly, the ocular complications of onchocerciasis include conjunctivitis, keratitis, corneal opacity, angle closure glaucoma, chorioretinitis, Iridicyclitis, cataract and optic neuritis. Typically, dermatologic manifestations are the initial presenting features with ophthalmic signs often presenting several years later. The severity of these ocular complications depends on the duration of infection, the microfilarial load and the strain of microfilaria[7].

Malaria is a parasitic disease caused by *Plasmodium* parasites which are spread through the bites of infected female *Anopheles* mosquitoes. In Africa, the female *Anopheles gambiae* complex has been shown to be responsible for most transmission of the disease [8]. Among the different species, *Plasmodium falciparum* and *vivax* are the most common, while *Plasmodium falciparum* is the most deadly[9]. Recently, *Plasmodium knowlesi* has been identified as the fifth specie and has been prejudged to cause malaria among Monkeys and humans and occurs in certain forest areas of South-East Asia[10].

The prevalence, morbidity, mortality and associated economic loss due to malaria have been variously reported, for example, a worldwide prevalence of over 200 million cases and over 600 thousand deaths have been recorded by the World Malaria Report[11]. According to a report from the Federal Ministry of Health[12], malaria accounts for about 110 million diagnosed cases per year and an estimated 300,000 children die of malaria yearly. A study [13] had shown that 11% of maternal related mortality is associated with malaria in pregnancy. It has been implicated in 25% of infant mortality and 30% of childhood mortality while it's economic burden in Nigeria is estimated at about 132 billion Naira[14].

Malarial retinopathy stands out as a first line diagnostic feature in adults with severe malaria and children with cerebral malaria in endemic areas where parasitemia does not usually account for clinical malaria. It has been reported[15] that the visualization of malarial retinopathy is more reliable than clinical or laboratory features in distinguishing malarial from non-malarial coma.

Malarial retinopathy consists of four main components namely: retinal (macular) whitening, vessel changes, retinal haemorrhage and papilloedema. While retinal (macular) whitening and vessel changes are specific to malaria, retinal haemorrhage and papilloedema are incidental to other ocular or systemic conditions[15].

It is estimated that over a third of the world's population, mainly those individuals living in the tropics and sub-tropics, are infected with parasitic helminths (worms) and one or more of the species of Plasmodium[16,17]. Another study[18] had shown that school-age children are at the highest risk of co-infection of hookworm and malaria parasites.

5. Mcleod SD. Parasitic Keratitis. Yanoff and Duker: Ophthalmology. 2008; 274-278.
6. WHO Expert Committee on Onchocerciasis and Its Control (Geneva, 1995).
7. Keiser PB. Bacterial Endosymbiont of Onchocerca volvulus In the Pathogenesis of Post-treatment Reactions. Journal of Infectious Diseases. 2002; 185: 805-811.
8. White GB. Anopheles gambiae Complex and Disease Transmission in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1974; 63(4), 278-301.
9. WHO Malaria Fact sheet. World Malaria Report. 2014.
10. Kantele A, Jokiranta TS. Preview of Cases With the Emerging Fifth Human Malaria Parasite, Plasmodium knowlesi. Clinical Infectious Diseases. 2011; 52(11)1356-1362.
11. World Health Organization. World Malaria Report. 2012; http://www.who.int/entity/malaria/world_report_2010/en-40k (retrieved 18/04/2017)
12. Federal Ministry of Health. National malaria and vector control programme: Policy for the implementation of indoor residual spraying for malaria vector control in Nigeria. Prime Wonder Ltd Kaduna/Abuja. 2010; 13pp.
13. Aribodor DN, Nwaorgu OC, Eneanya CI, Okoli I, Pukkila-Worley R, Etaga HO. Association of low birth weight and placental malaria infection in Nigeria. Journal of Infectious Diseases in Developing Countries (USA). 2009; 3(8): 620-623.
14. FMOH & NMCP. Advocacy, Communication and Social Mobilization Strategic Framework and Implementation Plan. National Malaria Control Programme, Federal Ministry of Health, Abuja. 2010; 80pp.
15. Beare NAV, Taylor TE, Harding SP, Lewallen S, Molyneux ME. Malaria Retinopathy: A newly established diagnostic sign in severe malaria. American Journal of Tropical Medicine and Hygiene. 2006; 75(5): 790-797.
16. De Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil Transmitted Helminth Infections: Updating the Global Picture. Trends in Parasitology. 2003; 19: 547-551.
17. Snow RW, Guerra CA, Noor AM, Myint HV, Hay SI.The Global Distribution of Clinical Episodes of Plasmodium falciparum Malaria. Nature. 2005; 434, 214-217.
18. Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, Hotez PJ. Epidemiology of Plasmodium-Helminth Co-infection in Africa: Population at Risk, Potential Impact on Anemia, and Prospects for Combining Control. American Journal of Tropical Medicine and Hygiene: 2007; 77(6): 230-238.

While a recent study[19] reported a prevalence of 3.6% for concomitant malaria and lymphatic filariasis infection in Georgetown, Guyana, a previous one[20] recorded a prevalence rate of 8.4% for O. *volvulus* and Plasmodiasis co-infection in Garaha-Dutse community of Adamawa State, Nigeria.

Furthermore, studies[21,22] have also shown that helminth infections may alter susceptibility to clinical malaria and this has necessitated further investigations into the distributions and consequences of co-infection[23], for example, a study[24] suggested that infection with intestinal nematode, *Ascaris lumbricoides* was associated with the suppression of malaria symptoms and that anthelmintic treatment led to a recrudescence of malaria. Maizels et al.[25] postulated that the mechanism underlying this finding, and those of more recent studies are based on the assumption that helminth infections induce a potent and highly polarized immune response which has been proposed to modify the acquisition of immunity to malaria. In animal models, this evidence is suggestive of both synergism and antagonism in *Plasmodia* and helminth co-infections[26]. In mixed infections, the burden of one or both of the infectious agents may be increased, one or both may be suppressed or one may be increased and the other suppressed[27]. For example, in a case where onchocerciasis and malaria were co-infected, it was shown that the Th2 associated cytokines in microfilariae negative mice kept microfilariae at bay by impairing[26] or delaying[28] the control of malaria whereas Th1 cytokines (IFN-γ) in microfilariae positive mice promoted the clearance of malaria[29].

Many studies have assessed the distribution of separate infections of onchocerciasis and malaria, yet little has been done to appraise the burden of their concurrent infection irrespective of their similarity in ecological distribution which accounts for co-infection[30], likely heightened debility, ocular complications and immunological interactions in co-infected persons[31]. The study therefore evaluated the prevalence of mixed infection of onchocerciasis and malaria in Ahani-Achi, a co-endemic community in Enugu State, Nigeria.

Materials and methods:

Study-area:

Ahani-Achi is an autonomous community located in Oji River Local Government Area of Enugu State, Nigeria. It has a geographical coordinate of 6^0 8^0 North, 7^0 22^0 East. The climate of the area is marked with typical rain forest mosaic vegetation characterized by mainly rainy seasons.

The community is traversed by many streams and fast flowing rivers such as Iyibenze, Nwoka, Ogba, Iyi Owerri, Iyi Agwo and Ngene iyi agu drained by a principal tributary known as Oji River. These enhance the breeding of both *Simulium damnosum* and Anopheles mosquitoes, the insect vectors of onchocerciasis and malaria respectively.

There are two different climatic seasons in the area, the rainy season from March to October and dry season from November to February. Year round, rainfall of approximately 2,900-3,400 mm occurs, with maximum precipitation occurring from June to August.

19. Chadee DD, Rawlins SC, Tiwari TSB. Short Communication:Concomitant Malaria and Filariasis Infections in Georgetown, Guyana: Tropical Medicine and International Health. 2003; 8(2):140-143.
20. Rebecca SN, Akinboye DO, Abdulazeez AA. Onchocerciasis and Plasmodiasis: Concurrent Infection in Garaha-Dutse Community, Adamawa State, Nigeria. Biomedical Research. 2008; 19(2): 5-8.
21. Nacher M, Singhasivanon P, Silachomroon U, Treeprasertsuk S, Vannaphan S, Troare B, Gay F, Looareesuwan S. Helminth Infections are Associated with Protection From Malaria-related Acute Renal Failure and Jaundice in Thailand. American Journal of Tropical Medicine and Hygiene. 2001; 65: 834-836.
22. Druilhe P, Tall A. Worms Can Worsen Malaria: Towards a New Means to Roll Back Malaria?. Trends in Parasitology. 2005; 21: 359-362.
23. Nacher M, Gay F, Singhasivanon P, Krudsood S, Teeprasertsuk S, Mazier D, Vouloukis I, Looareesuwan S. Ascaris lumbricoids Infection is Associated with Protection from Cerebral Malaria. Parasite Immunology. 2000; 22: 107-113.
24. Murray MJ, Murray AB, Murray MB, Murray CJ. Parotid Enlargement, Forehead Edema and Suppression of Malaria as Nutritional Consequences of Ascariasis. American Journal of Clinical Nutrition. 1977; 30: 2117-2121.
25. Maizels RM, Balic A, Gormez-Escober N, Nair M, Taylor MD, Allen JE. Helminth Parasites- Masters of Regulation. Immunology Reviews. 2004; 201: 89-116.
26. Helmby H, Kullberg M, Troye-Blomberg M. Altered Immune Response in Mice with Concomitant Schistosoma mansoni and Plasmodium chabaudi infections. Infection and Immunity. 1998; 66: 5167-5174.
27. Cox, FE. Concomitancy infections, parameters and immune responses. Parasitology. 2001; 122: 23-38.
28. Von der Weid T, Koff M, Kohler G, Langhorne J. The Immune Response to Plasmodium chabaudi Malaria in IL-4 Deficient Mice. Europian Journal of Immunology. 1994; 1(24): 2285-2293.
29. Su Z, Stevenson MM. Central Role of Endogenous Gamma Interferon in Protective Immunity Against Blood-stage Plasmodium chabaudi Infection. Infect Immun. 2000; 68: 4399-4406.
30. Petney TN, Andrews RH. Multiparasite communities in animals and humans: Frequency, Structure and Pathogenetic Significance. Inter Journ of Parasitol. 1998; 28: 377-393.
31. Mwangi TW, Bethony J, Brooker S. Malaria and Helminth Interactions in Humans: An Epidemiological Viewpoint. Ann of Trop Med and Parasitol. 2006; 100(7): 554-570.

The main occupations of the people are farming and trading. The community is made up of 4 villages namely, Okpuno, Umuelebe, Mgbaragu and Oruchi. It is surrounded by some other towns such as, Isuochi (in Abia State), Inyi (also part of Oji-River LGA of Enugu State), Ugbo, and Obeagu (Awgu LGA of Enugu State) and some parts of Udi local government area of Enugu State.

Study population and sample size:

The population of the community was estimated at about 26,970 people based on the 2006 census exercise. A sample size of 447 persons aged 10 - ≥60 years was drawn using the convenience sampling technique; all subjects who satisfied the inclusion criteria were recruited into the study in no particular order.

The sample size was calculated using the Taro Yamane's formula for minimum sample size determination and reasonable allowance was made for attrition factors.

Ethical clearance:

The study complied with established protocols of the Helsinki declaration on human experiments. Appropriate ethical clearance from Enugu State Ministry of Health was obtained for the study, a written permission to conduct the study was obtained from the traditional ruler of the community and his cabinet, written informed consents of adult participants were procured and surrogate consents were given for participants below the age of 18 years.

Specimen collection and analysis:

The participants were examined for concomitant onchocerciasis-malaria parasitemia using the skin snip biopsy and smear microscopy techniques.

Skin snip biopsy:

The posterior iliac crest region was cleaned with a spirit swab and allowed to dry. One (1) mg of skin snip was extirpated using a sclero-corneal punch and the bloodless snip was incubated in a laboratory tube containing 0.2 ml of normal saline for 24 hours. The contents of the tube were transferred to a slide and examined microscopically using the gold standard technique[32] of 10x microscopy. Parasite quantification was done in all fields of the microscope.

Smear microscopy:

To obtain a thick (capillary) blood smear, the tip of the third finger was sterilized with a spirit swab and allowed to dry. The finger was pricked using a sterile and disposable lancet to obtain a drop of blood placed on a microscopic slide. The drop of blood was turned in a circular pattern 3-4 times with a glass rod to obtain a thick blood smear. The blood smear was air-dried and stained with field's stain A for 2 minutes. After 2 minutes, the stain was washed off with water sprayed from a wash bottle and a drop of field's stain B was placed on the smear for 3 minutes and washed off. The blood film was air-dried and a drop of oil immersion placed on the film and viewed under 100x objective microscope[32].

Statistical analysis:

Data were analysed using Chi-square (X2) to evaluate the associations between age, gender, occupation and disease frequency at 95% confidence level.

Results:

Four hundred and forty seven (447) participants comprising of 207 (46.31%)) males and 240 (53.69%)) females were examined for concomitant onchocerciasis-malaria parasitemia based on demographic predilections. In both genders, the ≥60 years age group had the highest number of participants (males n=177, females n=211). The predominance of this age group among the sample population may likely be predictive of retirement and attendant relocation to the villages at the age of 60 years. The least number of

32. Cheesbrough M. District Laboratory Practise in Tropical Countries, Part i. Cambridge University. 1998; 455pp.

male participants (n=20) was found within the 10-19 years and 30-39 years age groups while the least number of female participants was recorded in the 30-39 years age group (Table 1).

Table 1: Age and sex distribution of sample population in Ahani-Achi community.

Age (Years)	Sex		Total (%)
	Male	Female	
10-19	2 (0.97)	4 (1.67)	6 (1.34)
20-29	4 (1.93)	6 (2.50)	10 (2.24)
30-39	2 (0.97)	3 (1.25)	5 (1.12)
40-49	10 (4.83)	9 (3.75)	19 (4.25)
50-59	12 (5.80)	7 (2.92)	19 (4.25)
≥ 60	177 (85.5)	211 (87.92)	388 (86.80)
Total	**207 (46.31)**	**240 (53.69)**	**447**

On the overall, 309 (69.13%) participants tested positive to mixed onchocerciasis-malaria parasitemia and more females (77.50%) were infected than males (59.42%).

The ≥60 years age category was most infected in both males (53.62%) and females (70.83). There was no case of mixed onchocerciasis-malaria found among males and females within the age group of 10-19 as highlighted in Table 2. The association between age and infection rate was statistically significant ($p<0.05$) while infection rate did not vary significantly with gender ($p>0.05$).

Table 2: Age and sex distribution of concomitant onchocerciasis-malaria parasitemia.

Age (Years)	Male		Female	
	No.	No. infected (%)	No.	No. infected (%)
10-19	2	0 (0)	4	0 (0)
20-29	4	2 (0.97)	6	3 (1.25)
30-39	2	0 (0)	3	3 (1.25)
40-49	10	4 (1.93)	9	4 (1.67)
50-59	12	6 (2.90)	7	6 (2.50)
≥ 60	177	111(53.62)	211	170(70.83)
Total	**207**	**123(59.42)**	**240**	**186(77.50)**

Test statistics used: Chi-square (X^2) P-value (age) = 0.000122 P-value (gender) = 0.068195

The study identified seven occupation groups namely students, civil servants, fishermen, others, farmers, traders and the unemployed. Among these, the fishermen were most infected (81.08%) followed by farmers (77.50%) while the unemployed group was least infected (30%) as shown in Table 3. The relationship between occupation and prevalence of infection was statistically significant ($p<0.05$).

Table 3: Occupation-related distribution of concomitant onchocerciasis-malaria parasitemia in Ahani-Achi.

Occupation	No. Examined	No. Infected	% infected
Students	30	16	53.33
Civil service	40	29	72.50
Fishing	37	30	81.08
Others	30	15	50
Farming	200	155	77.50
Trading	70	52	74.29
Unemployed	40	12	30
Total	**447**	**309**	**447**

Test statistics used: Chi-square (X^2) P-value = 0.00001

Discussion:

The overall prevalence rate of 69.13% recorded by the study is burdensome, especially when compared with the findings of Rebecca et al.[20] where a prevalence rate of 8.4% was reported for onchocerciasis and plasmodiasis co-infection. Although the wide discrepancy could be as a result of extensive ecological variation between the study-settings which most likely affected the ferocity of causal vectors in both locations, it could also represent the level of success of intervention programmes in both areas. The gap in prevalence rate of both localities is huge and underscores the exigency for inter-agency partnership and exchange of resources between the advanced and upcoming intervention agencies and programmes.

Furthermore, the result of the present study does not compare with another one[19] observed in Georgetown, Guyana where a prevalence of 3.6% was reported for malaria and lymphatic filariasis co-infection. The gap between the results may be as a result of differences in parasite biology of lymphatic filariasis and onchocerciasis.

The ≥60 years age category was most infected in both males (53.62%) and females (70.83) followed by the 50-59 years age group with a prevalence of 2.90% and 2.50% for males and females respectively with a significant correlation ($p<0.05$) between age groups and infection rate. The infection burden among the ≥60 years age category is logical in view of the dense population of the age group in the area and their propensity for farming and fishing after retirement, which makes them vulnerable to the bites of causal vectors of onchocerciasis and malaria. Moreover, most persons within these age strata may have substantially inhabited in the area with attendant proneness to vector contacts.

In males (0%) and females (0%), the infection rate of the 10-19 years age group was the least and may be accounted for by the low population of this age group within the sample population, the lengthy time spent at school, parental care and non-involvement in predisposing activities such as farming and fishing. This result differs from that of another study[18] where school age children were observed to have the highest risk of co-infection, although for hookworm and malaria. The discrepancy may most likely be associated with variation in the general biology, life cycles, ecological preferences and vectoral ferocity of hookworm and onchocerciasis parasites.

On the overall, a higher co-infection burden was recorded among the females (77.50%) than the males (59.42%), although the difference was not statistically significant ($p>0.05$). The result is unprecedented irrespective of the greater number of females among the sample population and could therefore be inferred that more women were engaged in farming and riverside activities such as laundering, washing of farming tools, washing of harvested crops and bathing after exhaustive agricultural activities than men. These activities are predisposing factors to the bites of *Simulium damnosium* and female Anopheles mosquitoes, which are insect vectors of onchocerciasis and malaria respectively.

Among the occupation groups, the fishermen (81.08%) were most infected followed by farmers (77.50%) while the unemployed (30%) had the least infection burden, more so, the association between occupation groups and infection rate was statistically significant ($p<0.05$). On the contrary, Rebecca et al.[20] had shown that the prevalence of onchocerciasis was highest among the farmers (28.8%), malaria infection was highest among the fishermen (47.7%), while the highest concomitant infection rate (18.2%) was observed among the farmers. The occupation predilection of both studies could not have been a product of chance but a likely indication of the ecological diversity of the study-areas. The rain forest vegetation of the present study-area promotes fishing activities while the savannah vegetation of the previous study-area[20] does not, thereby accounting for less fishing enterprise.

The prevalence pattern among occupation groups in the present study is understandable for two reasons: first, the major occupations of the inhabitants are farming and fishing; second, the physical exertion associated with fishing and farming usually prompts the workers to go top-bare for better aeration thereby increasing the exposure rate, vector contact time and subsequent biting rates.

18. Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, Hotez PJ. Epidemiology of Plasmodium-Helminth Co-infection in Africa: Population at Risk, Potential Impact on Anemia, and Prospects for Combining Control. American Journal of Tropical Medicine and Hygiene: 2007; 77(6): 230-238.
19. Chadee DD, Rawlins SC, Tiwari TSB. Short Communication:Concomitant Malaria and Filariasis Infections in Georgetown, Guyana: Tropical Medicine and International Health. 2003; 8(2):140-143.
20. Rebecca SN, Akinboye DO, Abdulazeez AA. Onchocerciasis and Plasmodiasis: Concurrent Infection in Garaha-Dutse Community, Adamawa State, Nigeria. Biomedical Research. 2008; 19(2): 5-8.

Conclusion

Despite the decades of Ivermectin distribution, nodulectomy, environmental modifications, indoor residual spraying and free insecticide treated net distribution (ITN) programmes by various agencies aimed at mitigating the infections, the hyperendemicity of concomitant onchocerciasis-malaria infection in the study-area has remained unabated. The study emphasized the need for all stakeholders including relevant intervention agencies and inhabitants of the area to collaborate with the government of Enugu State, Nigeria in designing more radical approaches to the control of these diseases. If timely recession of the infections is not rapidly actualized, the general well-being, quality of life and economic prospects of the people may likely degenerate further.

Since significant success has not been recorded in curbing the rate of co- infection, the advocacy strategies, implementation approaches adopted by control agencies and individual compliance to recommended control techniques should be re-appraised to ascertain associated inconsistencies in the entire implementation process.

Furthermore, to forestall further morbidity, mortality and associated economic loss, more efforts should be channelled into health education, environmental sanitation, self-protective measures and periodic programme evaluation to ensure the attainment of set goals.

Acknowledgements:

The authors are very grateful to the traditional ruler of Ahani-Achi community, Igwe (Dr.) R.M. Nzekweh and members of his cabinet for their invaluable assistance during the study. The confidence reposed in the investigators by the study participants is sincerely appreciated.

Competing Interests:

The authors declare that there were no personal or financial interests which may have inappropriately affected the conduct or the results of the study.