



Effect of community treatment initiative on antiretroviral therapy uptake among linkage-resistant people living with HIV in Northern Nigeria



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ABSTRACT

Background: Community Treatment Initiative (CTI) was developed in northern Nigeria as an intervention to link a cohort of people living with HIV (PLHIV) who refused antiretroviral treatment through a conventional linkage method to care and treatment. The CTI attempted to take treatment to PLHIV in the community.

Methods: This was a non-control interventional study that evaluated the proportion of linkage-resistant PLHIV linked to treatment through the CTI in nine geographical areas. Data were collected between October and December 2015. Linkage-resistant PLHIV were identified and linked to treatment using the CTI. Data were analyzed using Excel and IBM SPSS version 20.0. The simple proportion was used to estimate the linkage-resistant PLHIV who were eventually linked and retained in care and who ultimately achieved virological suppression (viral load <1000 copies/ml). The Chi-square test was used and the level of significance set at a p -value of <0.05.

Results: An estimated 541 (20%) PLHIV (239 (44.2%) male, 302 (55.8%) female) seen from October to December 2015 refused linkage to treatment. This was statistically significant at a p -value of <0.0001. Three hundred and seventy-seven (69.7%) of the PLHIV who refused linkage to treatment eventually accepted treatment using an alternative community treatment method; this was significant ($p < 0.0001$). The 6-month retention rate for PLHIV who accepted the alternative treatment method was 88.1% ($n = 332$); this was significant ($p < 0.0001$). Seventy-eight percent of those retained in care attained virological suppression.

Conclusions: The CTI improved linkage to care and treatment for a cohort of linkage-resistant PLHIV. Focus on this cohort of linkage-resistant positive clients is required to achieve HIV epidemic control. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

In order for Nigeria to control the HIV/AIDS epidemic in line with the United Nations (UN) vision, the cohort of positive clients who are resistant to commencing antiretroviral therapy (ART) must be reached and offered an alternative method of care and treatment. Nigeria is among the six nations facing the triple threat of a high HIV burden, low ART coverage, and unsatisfactory decline in new HIV infections and poor viral suppression (Awofala and Ogundele, 2018). Globally, about 3.8 million Nigerians are estimated to be living with HIV (Odimegwu et al., 2017). Together

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with South Africa and Uganda, Nigeria accounted for half of the new HIV infections in Sub-Saharan Africa in 2017 (Avert, 2018).

Even though the number of people placed on ART in Nigeria has slowly improved over the years to 970 000 in 2016 (UNAIDS, 2017), only 34% of adult positive clients are said to be receiving treatment (Avert, 2018). This figure is way below the number required to achieve epidemic control. According to the World Health Organization (2017), countries will make less progress in ART when the epidemic is predominantly concentrated in populations where there is poor access and service utilization (WHO, 2017). To increase treatment coverage, community outreach programs must be implemented in high prevalence geographic areas. The uptake of HIV testing services (HTS) and immediate treatment of HIV-positive clients could lead to cost-effective epidemic control over time (Aliyu et al., 2014).

Immediate initiation of ART irrespective of CD4 cell count has been found to be more beneficial when compared to treatment initiation after the CD4+ cell count has declined to <500 cells/ μ l (Simms et al., 2018). Additionally, evidence has shown that there is no significant increase in the rate of adverse effects in individuals commenced on treatment irrespective of CD4 levels (AIDSinfo, 2017). As a result of this, the World Health Organization, in 2015, recommended that people infected with HIV be initiated on ART irrespective of their CD4 cell count (World Health Organization, 2017). Therefore it has been recommended that CD4 cell counts as well as other baseline laboratory tests may no longer be required for ART initiation (Eholié et al., 2016). Nevertheless, patients on ART may be monitored using CD4 cell counts or viral load estimation, with other baseline tests often determined on an 'as needed' basis.

Early initiation of ART will increase the number of people living with HIV (PLHIV) receiving treatment, lead to better clinical outcomes and a suppressed viral load, and reduce the transmission of new infections, which will ultimately change the course of the HIV epidemic (Kasaie et al., 2018). Commencement of ART without baseline laboratory tests will expand access to HIV treatment, which could ensure the achievement of UNAIDS 95–95–95 goals by 2030, thereby averting over 21 million HIV-related deaths and 28 million new HIV infections (Stover et al., 2016).

Access barriers to HIV treatment and retention include fear of stigma and denial exhibited by linkage-resistant PLHIV who refuse treatment outright, a long distance to ART facilities, competing social priorities such as work, and poor health-seeking behavior (Yehia et al., 2015).

In order to overcome these barriers and improve ART uptake, the Institute of Human Virology Nigeria (IHVN) through the President's Emergency Plan for AIDS Relief (PEPFAR) has proposed a number of community-based ART (cART) models. One of these models, the community treatment initiative (CTI), is a community-oriented intervention developed to remove barriers to the commencement of ART and to improve ART uptake among PLHIV who have refused ART through conventional linkage methods. The CTI aims to take treatment to PLHIV in the community and within the confines of their homes.

This article describes an outreach-based CTI model in which treatment was initiated within the community for PLHIV who refused linkage (linkage-resistant) to care and treatment services at the facility (conventional treatment method). The study determined the proportion of clients who refused linkage to treatment using the conventional treatment method. It also evaluated the impact of the CTI on ART uptake by examining the proportion of linkage-resistant PLHIV linked to treatment through the CTI in nine geographical program areas. The study also evaluated the 6-month retention rate among clients linked to ART through the CTI across program areas and also determined the virological suppression rate among clients linked to ART through the CTI across program areas (Figure 1).

Research in context

Over the last couple of years, several studies have explored new interventions aimed at increasing HIV care and treatment in affected populations (Ridgeway et al., 2018). Although a few studies may have explored the benefits of alternative treatment methods, it appears that no study performed in Nigeria has assessed the impact of alternative treatment methods such as a CTI on ART uptake.

Added value of this study

It is expected that this study will contribute to the body of knowledge by providing evidence on how a community intervention such as a CTI can ensure that PLHIV who refuse treatment at the facility for one reason or another are placed on treatment in the community (Busza et al., 2018; Oladele et al., 2018). Additionally, this study presents the CTI as an alternative treatment method in a resource-poor setting such as Nigeria.

Implications of all the available evidence

The body of available evidence shows that care and treatment has to be decentralized in order to achieve HIV epidemic control. Decentralization can be done by engaging different approaches such as community treatment methods to increase access to HIV treatment and care (UNAIDS, 2015; Intrahealth, 2017).

Methods

Study design

This was a non-control interventional study that evaluated the impact of a CTI on ART uptake by examining the proportion of linkage-resistant PLHIV linked to treatment through the CTI in nine geographical program areas across three states in Northern Nigeria.

Study setting

The study was conducted in nine program local government areas (LGAs): Karu, Nasarawa, Lafia, Doma, AMAC, Bwari, Ushongo, and Buruku. These LGAs were projected to have a high HIV burden from program-level and spectrum data and were therefore selected to demonstrate HIV epidemic control.

Study population

Data on PLHIV were collected from the National HIV registers based at program facilities between October and December 2015. A cohort of PLHIV who resisted linkage to treatment using conventional linkage methods (in clinics and facilities) was selected from HTS registers and offered alternative treatment via the CTI.

Inclusion criteria

All PLHIV who refused linkage to facility level care (conventional treatment method) between October–December 2015 were included.

Exclusion criteria

Patients who were already linked to treatment at the facility were excluded.

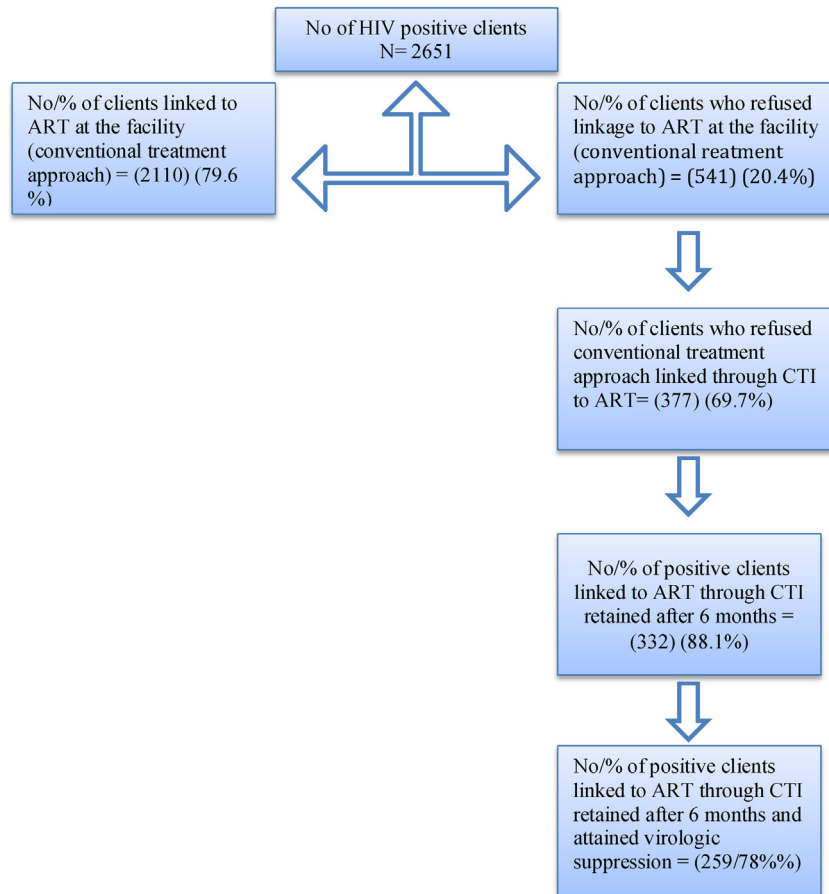


Figure 1. CTI model chart.

Intervention—community treatment initiative

The CTI is a differentiated ART model of care (DMC) utilizing community outreach to improve treatment uptake among linkage-resistant PLHIV. The CTI ensured effective linkage to an alternative model of care in nine LGAs located in the three Nigerian states of Benue, Nasarawa, and the Federal Capital Territory (FCT). This intervention targeted PLHIV who had refused to commence ART through the widely used conventional treatment method. The conventional method involves using volunteers to escort newly diagnosed PLHIV to a healthcare facility and the use of a coupon service where PLHIV claim an incentive on reaching the healthcare facility in order to receive care and treatment services.

A community team consisting of adherence counselors, clinicians, nurses, pharmacists, and laboratory technicians worked with LGA HIV coordinators, primary healthcare (PHC) center managers, and the village ward health committee to plan and implement the community HTS. Following HTS in the community, identified PLHIV were conventionally linked to a healthcare facility. PLHIV identified were either escorted to the facility or given a redeemable coupon that entitled them to claim the sum of N2000 (\$5) as transport incentive when they reported to the healthcare facility. Those who failed to show up despite collecting the redeemable coupons or who were perceived as unlikely to show up were approached by linkage coordinators who offered to escort them to the facility to commence ART. However, some clients did not show up even with the aid of the linkage coordinators. These clients formed the cohort of linkage-resistant PLHIV. This cohort of PLHIV was targeted with the CTI.

The CTI involved offering treatment-resistant PLHIV an alternative to facility care in the community where they live. It began with adherence counseling, where clients were educated on the need to commence and continue their ART. Following adherence counseling, PLHIV were initiated on a 4-week course of ART, with the aim of seeing them at the healthcare facility of their choice for continuation of care within this 4-week period. PLHIV were linked to a comprehensive facility. Physician contact details were shared with the clients for use in the case of any emergencies. Weekly call-in to clients was instituted to reassure clients and ensure treatment adherence. The service of support group members (other PLHIV on treatment) was utilized during counseling to encourage the treatment-resistant clients to commence and adhere to treatment. A line record of all treatment-resistant PLHIV placed on treatment using the CTI was generated to ensure proper documentation and prevent client loss to follow-up (LTFU). An ART care card was completed in the community for all CTI clients and held in the comprehensive healthcare facility of their choice for safe keeping in line with a data confidentiality agreement.

Treatment stabilization and follow-up

There was complete documentation of all clients tested in the community. Data collected included name, telephone number, address, test date, facility of reference, date commenced on ART, date due for viral load assay, etc. A letter 'c' was indicated on the intake forms of clients recruited from the community to enable the team to differentiate between PLHIV recruited in the facility from

those recruited in the community. It is planned that in the future, stable PLHIV linked through the CTI will form the critical mass of clients who will be migrated to other DMC such as community pharmacy and community ART groups (CAG), to ensure continuation of community-level treatment through the DMC.

Ethical approval

The Government of Nigeria and local research accreditation body approved this project for implementation by the IHVN. Written informed consent was obtained from the patients. Participants were made to understand that all information obtained would be treated with the utmost confidentiality, and the benefit of doing this towards ending the AIDS epidemic was made clear to them.

Role of the funding source

The funder of the study, PEPFAR, had no role in the study design, data collection, data analysis, and data interpretation, or in the writing of the report. The corresponding author had full access to the data as a member of staff of IHVN at the time of the study and had final responsibility to submit it for publication.

Statistical analysis

Data were analyzed using Excel and IBM SPSS version 20. Simple proportion was used to estimate the proportion of linkage-resistant PLHIV, the proportion of linkage-resistant PLHIV linked to ART using the CTI, the proportion of PLHIV retained in care at 6 months after commencing ART through the CTI, and the proportion of those on treatment who attained virological suppression. The Chi-square test was used to assess the association between categorical variables, and the statistical significance of the proportions was set at a p -value of <0.05 .

Results

ART uptake and the CTI

IHVN was funded by the US Government through the Center for Disease Control and Prevention (CDC) in Nigeria to implement a large-scale project on HIV epidemic control in line with the UN 90-90-90 vision. IHVN commenced the implementation of an aggressive large-scale case-finding strategy using the house-to-house model for test and treat, with a focus on hotspots in the community. This large-scale HIV testing led to the identification of a number of PLHIV. However, program review revealed inadequate linkage of PLHIV to care and treatment in the first 3 months of implementation (October–December 2015) of fiscal year 2016,

with resultant low ART uptake. ART uptake was found to be 79% (Table 1), which is less than the 90% required to achieve epidemic control in line with the UN 90-90-90 vision. There was thus an urgent need for a course correction strategy, and this led to the development of a community level intervention aimed at taking treatment to the people in the community. The CTI, a community ART model, was developed and implemented within the following 4–6 months (January–March 2016) of implementation to address gaps in ART uptake. With the CTI, a cohort of unlinked positive clients (linkage-resistant) who had refused to commence ART using the conventional linkage methods (linkage coordination/escort services and redeemable coupon) were commenced on ART in the community and subsequently linked to the nearest comprehensive healthcare facility of their choice for continuation of treatment within 4 weeks of community ART.

An estimated 541 (20%) PLHIV (239 (44.2%) male, 302 (55.8%) female) identified during the first 3 months of program implementation (October–December 2015) could not be linked using the conventional facility treatment model (Table 2). All 541 treatment-resistant PLHIV were interviewed and three major reasons for non-linkage to treatment were elicited: long distance to healthcare facility ($n=333$, 61.6%), outright refusal for personal reasons ($n=367$, 67.8%), and fear of stigma and discrimination at the facility by acquaintances ($n=102$, 18.9%). Out of the 541 linkage-resistant PLHIV, 377 (69.7%) (139 male, 238 female) were linked to care and commenced on ART using the CTI treatment model as compared to 164 (30.3%) who were not linked to ART. This was found to be highly significant ($p < 0.0001$).

Retention rates

PLHIV linked to treatment through the CTI were followed up for 6 months and the treatment retention rate was assessed. It was found that the 6-month retention on ART among clients linked through the CTI was 88.1% (Table 3) and that this varied across the nine study areas, with urban LGAs of AMAC, Karu, and Lafia with higher retention rates of 88.9%, 90.8%, and 94.5%.

In order to achieve epidemic control for HIV, PLHIV must adhere to treatment and be retained in care to achieve the desired virological suppression (Matsumoto et al., 2015). To achieve epidemic control, 90% of clients on treatment must attain a viral load of less than 1000 copies/ml (Ameyan et al., 2017). Retention on treatment is key to achieving this (Babatunde et al., 2015). The 6-month retention rate was found to be better in clients linked through the CTI when compared to clients linked through the conventional facility treatment method (Table 4). This was attributed to excellent record keeping, efforts of peer support groups, and constant reassurance of clients by physicians who called them on a weekly basis to ensure the PLHIV adhered to treatment.

Table 1
Pre-intervention program cascade analysis for Q1 (October–December 2015).

LGA	HTS, n	Number of PLHIV identified	Number of PLHIV linked to ART	Number of linkage-resistant PLHIV	% ART uptake (column G/F \times 100%)	Level of significance
AMAC	228 186	464	390	74	84.1	
Bwari	116 178	310	238	72	76.8	
Karu	6445	432	340	92	78.7	
Nasarawa	16 588	196	161	35	82.1	
Obi	17 759	163	121	42	74.2	
Doma	12 693	228	193	35	84.6	
Lafia	9901	440	370	70	84.1	
Buruku	13 912	216	143	73	66.2	
Ushongo	15 516	202	154	48	76.2	
Total	437 178	2651	2110	541	79.6	$p < 0.0001$

LGA, local government area; HTS, HIV testing services; PLHIV, people living with HIV; ART, antiretroviral therapy. The proportion of linkage-resistant clients was found to be significant at $p < 0.0001$ as compared to the number linked to ART via the conventional method.

Table 2

Cascade analysis for CTI model Q2 intervention period (January–March 2016).

LGA	Number of PLHIV identified	Number of PLHIV linked to ART	Number of linkage-resistant PLHIV	Number of linkage-resistant PLHIV linked through CTI	Percentage linkage-resistant PLHIV linked through CTI
AMAC	464	390	74	45	60.8
Bwari	310	238	72	39	54.2
Karu	432	340	92	65	70.7
Nasarawa	196	161	35	28	80.0
Obi	163	121	42	30	71.4
Doma	228	193	35	27	77.1
Lafia	440	370	70	55	78.6
Buruku	216	143	73	48	65.8
Ushongo	202	154	48	40	83.3
Total	2651	2110	541	377	69.7

CTI, community treatment initiative; LGA, local government area; PLHIV, people living with HIV; ART, antiretroviral therapy.

Table 3

Six-month retention in care for linkage-resistant PLHIV placed on treatment through the CTI.

LGA	Number of linkage-resistant PLHIV linked through CTI	Number of linkage-resistant PLHIV linked through CTI retained on treatment after 6 months	Number of linkage-resistant PLHIV linked through CTI lost to follow-up after 6 months	Retention rate %	Level of significance
AMAC	45	40	5	88.9	
Bwari	39	34	5	87.2	
Karu	65	59	6	90.8	
Nasarawa	28	21	7	75.0	
Obi	30	26	4	86.7	
Doma	27	21	6	77.8	
Lafia	55	52	3	94.5	
Buruku	48	42	6	87.5	
Ushongo	40	37	3	92.5	
Total	377	332	45	88.1	$p < 0.0001$

PLHIV, people living with HIV; CTI, community treatment initiative; LGA, local government area; ART, antiretroviral therapy. The number of linkage-resistant clients linked to ART through the CTI and retained in care was found to be significant at $p < 0.0001$.

Virological suppression rates

Out of the 377 PLHIV placed on ART, 332 were retained on treatment (88% retention rate). From those retained on treatment after 6 months, 259 (78%) attained virological suppression with a viral load count of <1000 copies/ml (Table 5). Seventy-three PLHIV on CTI did not attain virological suppression.

In order to achieve HIV epidemic control, there is a need to counsel those PLHIV who do not attain virological suppression on adherence (Hart et al., 2010). Following adherence counseling, the viral load level of this group of unsuppressed PLHIV will be re-assessed after a 6-month period. This group of patients should not be treated with a one-size fits all approach, but should instead have tailored interventions that will address the specific reasons why they did not attain virological suppression. They should be linked to peer support groups (PLHIV who have

already attained virological suppression) for mentorship and to improve adherence towards achieving viral suppression (Yu et al., 2018). Linking clients to support groups has been shown to foster adherence to medication among PLHIV, which can improve virological suppression rates (Shah et al., 2007). Some of the reasons why the PLHIV in the present study did not attain virological suppression were as follows: skipping medication due to perceived side effects such as nightmares, religious perceptions about healing, and missing appointments due to one reason or another. Young people are known to forget to take their medications on weekends after a night out (Achappa et al., 2013; Kim et al., 2018).

There is need to integrate CTI into other differentiated models of care such as community pharmacy and community ART groups in order to improve client retention and ultimately achieve virological suppression.

Table 4

Six-month retention rate among clients linked through the CTI vs. conventional approach.

LGA	Number placed on ART (CTI)	Number placed on ART (conventional)	Number retained on treatment (CTI)	Number retained on treatment (conventional)	6-month treatment retention rate	
					CTI	Conventional approach
AMAC	45	390	40	297	88.9	76.2
Bwari	39	238	34	194	87.2	81.4
Karu	65	340	59	255	90.8	75
Nasarawa	28	161	21	109	75	67.9
Obi	30	121	26	97	86.7	80.1
Doma	27	193	21	147	77.8	76.2
Lafia	55	370	52	332	94.5	89.7
Buruku	48	143	42	100	87.5	69.8
Ushongo	40	154	37	126	92.5	81.8
Total	377	2110	332	1657	88.1	78.5

CTI, community treatment initiative; LGA, local government area; ART, antiretroviral therapy. The number of linkage-resistant clients linked to ART through the CTI and retained in care was found to be significant at $p < 0.0001$ as compared to the number retained through the conventional approach. Chi-square = 34.974; DF = 8; significance level, $p < 0.0001$.

Table 5
Virological suppression rate among linkage-resistant PLHIV linked to ART through the CTI retained on treatment after 6 months.

LGA	Number of linkage-resistant PLHIV linked through the CTI retained on treatment after 6 months	Number of linkage-resistant PLHIV linked through the CTI retained on treatment after 6 months who attained virological suppression ^a	Number of linkage-resistant PLHIV linked through the CTI retained on treatment after 6 months who did not attain virological suppression ^a	Virological suppression rate among linkage-resistant PLHIV linked through the CTI	Level of significance
AMAC	40	30	10	75.0	
Bwari	34	25	9	73.5	
Karu	59	47	12	79.7	
Nasarawa	21	15	6	71.4	
Obi	26	20	6	76.9	
Doma	21	14	7	66.7	
Lafia	52	45	7	86.5	
Buruku	42	36	6	85.7	
Ushongo	37	27	10	73.0	
Total	332	259	73	78.0	$p < 0.0001$

PLHIV, people living with HIV; ART, antiretroviral therapy; CTI, community treatment initiative; LGA, local government area.

^a Viral load <1000 copies/ml.

Sex and CTI uptake

There were more females linked to ART through the CTI (63.1%) compared to males (36.9%) (Table 6). This may be due to cultural practices: males in Nigeria are more likely to seek care in a facility than females, who often need the permission of their husband/a male relative to receive treatment in the clinic. Therefore, female clients were likely more comfortable with the community treatment idea, as they comprise a larger number of the linkage-resistant PLHIV.

Discussion

The significance of the study results is the fact that the CTI, as an intervention, improved ART uptake in the community and could therefore be used in rural communities. Low- and middle-income countries that often battle with poor health infrastructure underpinned by poor human resource for health (HRH) will benefit from community interventions in order to control the HIV epidemic (Oladele et al., 2018). The conventional method for ART includes self/community referrals to the facility, the use of linkage coordinators/escort services, and redeemable coupons as an incentive for linkage. Forty-three percent of clients commenced on ART during quarter 2 (months 4–6) of the program did so through the conventional facility method. The average HIV seroprevalence observed across all LGAs in quarter 1 (months 1–3) of the program was found to be 0.6% (range 0.2–1.7%). This is way below the national average of 3.1%. This variation could be due to the fact that we estimated our prevalence from population/program-based testing as compared to the national prevalence, which was estimated from an antenatal clinic (ANC) (see Figure 2) sentinel survey (Bashorun et al., 2014). The implication of this is that the prevalence of HIV may be less than estimations from ANC surveys.

Table 6
Sex distribution of HIV-positive clients linked to ART through the CTI.

LGA	Number placed on ART (CTI)	Male	Female	% Male	% Female
AMAC	45	17	28	37.8	62.2
Bwari	39	15	24	38.5	61.5
Karu	65	24	41	36.9	63.1
Nasarawa	28	6	22	21.4	78.6
Obi	30	10	20	33.3	66.7
Doma	27	11	16	40.7	59.3
Lafia	55	24	31	43.6	56.4
Buruku	48	11	37	22.9	77.1
Ushongo	40	14	26	35.0	65.0
Total	377	139	238	36.9	63.1

ART, antiretroviral therapy; CTI, community treatment initiative; LGA, local government area.

The CTI has been shown to be effective in improving client linkage to ART in low-resource settings like Nigeria and may therefore be adopted as a way of increasing ART uptake following community testing. This has positive implications for ending the HIV epidemic in low- and middle-income countries with poor health systems and limited numbers of facilities, which culminate in positive clients experiencing delays in accessing care for reasons such as the long distance from home to the facility, access barriers (financial and physical), and perceived level of stigma and discrimination against PLHIV by health workers in health facilities (Ankomah et al., 2016). Healthcare facilities will be overstretched with the introduction of 'test and start' due to the increase in number of positive clients seeking care (Takarinda et al., 2016). CTI as a community ART model will significantly help in reducing the initial health worker burden associated with the enrollment of clients into care at the facility by taking care of PLHIV in the community and also enrolling into care the critical mass of clients who are resistant to commencing ART (Kharsany and Karim, 2016). With this method, it is possible to reduce congestion at the facility. It will not be possible to end HIV without focusing on this cohort of positive individuals who have refused to commence ART for one reason or another. The CTI ensures that the initial 4-week period of community ART allows the client to come to terms with their status and get the support they need from health workers and groups directly linked to them within the community. A community approach also helps to reduce stigma and discrimination, which most clients avoid in the facility.

The observed prevalence in this study, which was conducted among the general population, is not as high as the national prevalence from the ANC sentinel survey; therefore, there is a need for Nigeria to conduct a National HIV/AIDS survey among the general population. A population-based survey, the Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS), is currently ongoing to ascertain the dynamics and prevalence of the HIV/AIDS epidemic in Nigeria (NAIIS, 2018). The results from this survey should indicate the prevalence and burden of the epidemic in Nigeria and allow the better use of resources to achieve results.

A significant number of clients were linked to ART through the CTI, with a higher retention rate compared to clients linked through the conventional facility method. This supports the fact that the CTI is effective in improving client linkage to ART in low-resource settings and should therefore be adopted.

This study did not collect data on the socio-economic status of the participants; the assumption of a poor socio-economic status for people in the rural areas in Nigeria has been widely appraised and publicized.

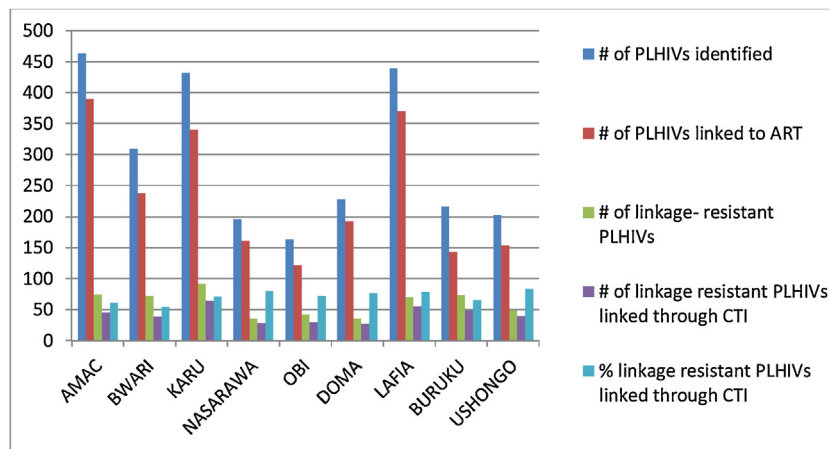


Figure 2. CTI cascade analysis.

Conflict of interest

The authors declare that they have no conflicts of interest in this study.

Author contributions

The first two authors contributed equally to the development of this manuscript. The contributions of other authors varied; however they contributed in significant proportions.

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