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Editorial

*Corresponding author
Moreen Kamateeka, MBChB, MPH
African Field Epidemiology Network (AFENET) Nigeria
50 Hallie Selassie Street
Asokoro, Abuja, Nigeria
E-mail: mkamateeka@nfeltp.org

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It is Time to Explore the Potential Benefit of Routine Micronutrient Supplementation in Optimizing Bone Health and Growth in HIV Exposed Uninfected Children in the Context of Early Antiretroviral Exposure in Resource Limited Settings

Moreen Kamateeka, MBChB, MPH[1,2*]; Dorothy Sebikari, MBChB, MPH[2]

[1]*African Field Epidemiology Network (AFENET) Nigeria, 50 Hallie Selassie St, Asokoro, Abuja, Nigeria*

[2]*Makerere University - Johns Hopkins University Research Collaboration, Upper Mulago Hill Road, P.O. Box 23491, Kampala, Uganda*

There is evidence that human immunodeficiency virus (HIV) infected children have decreased bone mineral density (BMD) compared to population norms.[1-4] Similarly, several studies have shown impaired growth among HIV infected children with several patterns of disrupted growth.[5-7] Among other factors, micronutrient deficiencies are believed to contribute significantly to growth failure in HIV infected children, particularly in resource constrained settings with high background rates of micronutrient deficiencies.[5] Some of the major risk factors of decreased BMD in HIV infected children include antiretroviral therapy (ART) related toxic effects particularly with tenofovir disoproxil fumarate (TDF) containing regimens.[2,8]

There is some evidence that micronutrient supplementation in HIV infected children improves growth and BMD. In a longitudinal study involving 37 perinatally HIV infected children done in USA, multivitamin use was independently associated with higher BMD *Z*-scores among multivitamin users compared to non-users.[2] The provision of multiple micronutrient supplements to ART naïve HIV-infected South African children resulted in improvement in weight-for-height *Z*-score compared with a placebo.[9] A systematic review of 11 trials mostly conducted in Africa concluded that multiple micronutrient supplements offer some clinical benefit in HIV infected children.[10] These results have provided a strong rationale for the World Health Organization (WHO) recommendation for multiple micronutrient supplementation for HIV infected children, especially in settings where micronutrient deficiencies are prevalent.[11]

Whereas, WHO currently recommends multiple micronutrient supplementation for all HIV infected children, supplementation in HIV exposed uninfected children is recommended if they are malnourished and yet this growing population of children in resource limited settings is similarly at risk of impaired growth and bone health.[12] As a result of accelerated scale-up of use of triple ARV combinations for preventing mother-to-child HIV transmission (PMTCT), worldwide, a large number of HIV exposed Uninfected (HEU) infants are exposed to triple ARVs early in life. In resource limited settings where extended breastfeeding for at least 12 months is recommended for infant survival among HIV infected women, these infants are exposed to ARVs (predominantly TDF and Efavirenz (EFV) containing regimens, the current WHO preferred first line triple ART combination for HIV infected pregnant women in PMTCT programmes) for up to 2 years. While lifelong ART greatly minimizes HIV transmission to the baby, prolonged ART exposure both *in utero* and through extended breastfeeding raises safety concerns for the baby, including potential growth impairment and adverse effects on bone health. Additionally, children born to HIV infected women are already prone to undernutrition related to maternal factors yet nutrition plays a critical role in bone mass formation

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and mineralization during the fetal and infancy periods,[13,14] Some studies have indicated lower Bone Mineral Content (BMC) and lower height-for-age *Z*-score (HAZ) as well as lower head circumference-for-age *Z*-score (HCAZ) in infants exposed to TDF *in-utero*.[15-17] More recently, a study that was designed to evaluate the potential bone and kidney toxic effects of TDF among HIV-infected pregnant and breastfeeding women and their infants reported that there were significant decreases in BMC among newborns whose mothers received Protease Inhibitor-based ART during pregnancy compared to those who received only zidovudine (ZDV) during pregnancy.[18] Another first line antiretroviral, EFV is associated with vitamin D deficiency through multiple postulated mechanisms.[19,20] Vitamin D is critical for calcium absorption and bone mineralization and its deficiency is associated with rickets. Therefore, EFV associated vitamin D deficiency may have potential adverse effects on bone health.[21-24] The growing number of HIV infected pregnant and lactating women on TDF/EFV containing regimens for PMTCT has the potential to have a negative impact on bone health of their infants and young children. Consequently, failure to achieve adequate bone mass during early infancy may predispose these infants to increased risk of childhood fractures and osteoporosis in adulthood.

Although, there is conflicting data on the effect of ART on growth in HIV exposed uninfected infants, a number of studies conducted in Africa have shown that *in utero* ART exposure is associated with lower birth Weight for Age z-scores (WAZ) and length for age z-score (LAZ).[25,26] Coupled with high background rates of micronutrient deficiencies, prolonged ART exposure in HEU infants raises potential risk for growth impairment in this population. Given these concerns, and background rates of stunting among a third of children in many African settings, it is important to take a closer look at interventions that might counter any added negative effects on bone mineralization and growth caused by prolonged ARV exposure of up to 24-30 months during gestation and extended breast milk ingestion of ARVs among HEU children in resource limited settings.

A number of minerals including calcium, phosphorous, sodium as well as magnesium and vitamins A, B6, B12, C, D, and K, directly or indirectly affect bone mineralization.[27] Several studies in both resource rich and resource limited settings have demonstrated some health benefit from micronutrient supplementation in children. In a blinded placebo controlled cluster randomized trial done in India involving 268 HIV uninfected children aged 6-16 years, supplementation with a micronutrient enriched beverage was associated with significantly greater increments for height, weight, whole-body bone mineral content (BMC), whole-body bone area, and BMD at the neck of the femur after 14 months in the supplemented group than in the placebo group($p<0.05$).[28] A recent randomized placebo controlled trial done in Tanzania revealed small but significant improvements in WAZ with zinc and multivitamin supplements among HIV unexposed infants aged 6 weeks-84 weeks.[29]

The growing population of HEU may benefit from early multiple micronutrient supplementation given the concerns of effects of prolonged ART exposure on BMD and growth; however, to date, there is very limited published data on effect of multiple micronutrient supplementation on growth and BMC in this population. It is therefore important to evaluate the use of interventions like micronutrient supplementation in HEU infants that could potentially boost bone health and growth in this pediatric population at risk of TDF/EFV adverse drug effects, particularly in Sub-Sahara Africa where micronutrient deficiencies are prevalent. This will build on evidence that would be critical in determining the applicability of such interventions in routine care of the growing population of HEU.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interet.

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Technical Report

Linkage-To-Care: A Model for Success

Ashley King, MPH[1*]; Caitlin Canfield, MPH[2]; Joseph Olsen, MPH[3]

[1]Patient Navigator, CrescentCare-NO/AIDS Task Force, 2601 Tulane Ave. Suite 500, New Orleans, LA, USA

[2]Evaluation Coordinator, Louisiana Public Health Institute, New Orleans, LA, USA

[3]Counseling Testing and Referrals Manager, Crescentcare-NO/AIDS Task Force, New Orleans, LA, USA

Corresponding author
Ashley King, MPH
Patient Navigator
CrescentCare-NO/AIDS Task Force
2601 Tulane Ave. Suite 500
New Orleans, LA 70119, USA
Tel. 6092343765
E-mail: ashleyking059@gmail.com

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ABSTRACT

On July 13th 2010 the National HIV/AIDS Strategy (NHAS) set forth a goal of increasing national linkage to care rates from 65% to 85%. A local AIDS Services Organization (ASO) that recently transitioned to a Federally Qualified Health Center (FQHC) in New Orleans, LA, USA had a testing cohort of 2,785 with a consistent 2% positivity rate. Prior to hiring a dedicated Patient Navigator in 2012, agency linkage to care rates hovered around 50%. This article charts how the agency increased its linkage to care rate by 40% over the course of three years and will examine inputs, strategies and best practices of a successful linkage-to-care model implemented in New Orleans, LA, USA.

KEYWORDS: HIV/AIDS, Linkage to Care.

ABBREVIATIONS: NHAS: National HIV/AIDS Strategy; ASO: AIDS Services Organization; FQHC: Federally Qualified Health Center; PLWHA: People living with HIV/AIDS; HHS: Health and Human Services; HRSA: Health Resources and Services Administration; HAB: HIV/AIDS Bureau; LTC: Linkage-to-care; PN: Patient Navigators; ARTAS: Antiretroviral Treatment Access Study; CTR: Counseling, Testing and Referral; LOPH: Louisiana Office of Public Health; ROI: Release of Information; PMC: Primary Medical Care.

HISTORY OF PATIENT NAVIGATION

The concept of patient navigation was first initiated by Harold P. Freeman in 1990 at a public hospital in Harlem, New York following the *American Cancer Society National Hearings on Cancer in the Poor in 1989*.[1,2] Intended to bridge the gap between the point of suspicious cancer findings, diagnosis and entry to care, the patient navigation model was focused on the critical window of opportunity to save lives from cancer by eliminating barriers to timely care.[2] Since then, this model has received national recognition, and has been adapted for various chronic and infectious disease continuums of care.[2]

In July 2010, the White House released the National HIV/AIDS Strategy (NHAS), a comprehensive roadmap for reducing the impact of HIV by 2015.[3] As the nation's first-ever comprehensive, coordinated HIV/AIDS effort, one of the goals set forth was to increase access to care and improve health outcomes for People living with HIV/AIDS (PLWHA).[1] As a result of this initiative, the U.S Department of Health and Human Services (HHS), Health Resources and Services Administration (HRSA), HIV/AIDS Bureau (HAB) and Special Project of National Significance funded the Systems Linkages and Access to Care for Populations at High Risk of HIV Infection Initiative (the Systems Linkages initiative) aimed to improve linkages of PLWHA to necessary testing, treatment, and care services.[4]

HHS, HAB and HRSA also administer the Ryan White HIV/AIDS Program, a national program that provides financial support to primary medical care and support services for PLWHA.[5] Under this program, the Ryan White administration created different programs to service the need of various communities and populations affected by HIV/AIDS.[3] Part C of

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the Ryan White program was created to provide comprehensive medical care in outpatient settings to PLWHA. Part C includes an Early Intervention Services component, which helps fund Linkage-to-care (LTC) initiatives for agencies providing care to PLWHA.[6]

THE IMPORTANCE OF PATIENT NAVIGATION

Patient Navigation is an approach used to improve health care delivery by identifying and eliminating barriers to care that impede equitable, quality care.[1,2] Common barriers that exist for people entering into care are transportation, appointment attendance, and competing priorities. Additionally, poverty, unemployment, intimate partner violence, unstable housing including homelessness, hunger, and other issues can prevent people from accessing health care.[7] To better address these barriers, implementation and models vary by setting disease, but the overall goal is to help eliminate barriers to care, provide a system of support, and ensure a seamless transition into treatment.[8] LTC plays an important role in the HIV continuum of care in that it bridges the gap between diagnosis and engagement in care.[1] In 2010 when the NHAS was released, an action step along the continuum of care was to increase LTC from 65% to 85% within in three months of diagnosis by 2015.[3] In July 2015, the White House challenged Patient Navigators (PN) to achieve an 85% LTC rate within 30 days of diagnosis.[6] After the release of the NHAS 2020 strategy, a local community-based organization sought to meet that challenge, using a model that had proven successful since 2012.

This article will detail a successful patient navigation model currently used in New Orleans, Louisiana, USA.

STREAMLINING THE PROCESS FROM A TO Z – BUILDING A SUCCESSFUL LINKAGE-TO-CARE MODEL

Overview of a Model for Success

Many HIV LTC models are designed following the Centers for Disease Control's Antiretroviral Treatment Access Study (ARTAS), which used a two-armed randomized controlled study comparing brief strengths-based case management intervention with standard-of-care referrals.[9] The results of this study established best practices for implementation of LTC programming for HIV clinics. In calendar year 2011 this agency administered 2,785 HIV tests with over 2% positivity rate. While the organization's linkage-to-care rates hovered around 50% in 2011, the National LTC standard was 66% and Louisiana reported an overall LTC rate of 56%.[10] In June 2012, a community based AIDS Services Organization (turned Federally Qualified Health Center, 2013) in New Orleans, LA, USA adapted the ARTAS model by streamlining the LTC process through one PN which resulted in an increased LTC rate of 33% within the first few months of model implementation. By December 2012, this LTC program had an average rate of 83%. Building on best practices, during the 2014 calendar year the model resulted in an average quarterly LTC rate of 90% (N=108 clients) within 90 days for HIV-positive individuals tested through the agency's Counseling, Testing and Referral (CTR) program. For PLWHA that were newly diagnosed, a quarterly average LTC rate of 90% (N=68 clients) was achieved within 90 days of their diagnosis. (Table 1)

Detailing a Successful Model for Linkage-to-care: From Testing to Linkage

Under this model, the LTC process begins when a client receives two consecutive positive HIV test results on rapid test kits, followed by same-day post-counseling from a state-certified HIV counselor (see Figure 1). When the client is ready to continue, the counselor fills out paperwork mandated both by the Louisiana Office of Public Health (LOPH) and the agency's CTR program. Included in this paperwork is a Release of Information (ROI) allowing the PN to contact the client. The paperwork also requests the client's preferred method of contact (email, phone, facebook, etc.), documents the client's preference on leaving voicemails, and clarifies whether texting is acceptable. In cases where a client is too emotional to proceed through all of the paperwork, the ROI is the only additional information collected and the client is scheduled to come in at a later date to complete the intake paperwork.

Once the CTR session concludes, the counselor immediately notifies the PN and CTR Manager *via* phone, text or email, alerting them of a positive result. If the PN is on-site or nearby, the PN makes contact with and/or meets the client before they depart from the office. If this immediate meeting is not feasible, the client is assured that the PN will reach out to them within 24 hours and given multiple ways to contact the PN themselves. Paperwork is distributed to the PN, who then becomes responsible for ensuring that it is received by the LOPH. Using this model and Continuous Quality Improvement measures, LTC data are effectively tracked by the PN as well as the agency's electronic health record system and LOPH.

	Test Performed	CTR Confirmed HIV+	Agency LTC Rate
HIV tests performed by case-study agency in calendar year 2011	2785	N/A*	**50%
HIV tests performed by case-study agency in calendar year 2012	3131	69	83%
HIV tests performed by case-study agency in calendar year 2013	3407	94	91%
HIV tests performed by case-study agency in calendar year 2014	3864	108	90.5%

*Data not available for full year. **50% represents Sept.-Dec, 2011 where 8 of 16 newly diagnosed clients testing positive linked to care in less than 90 days.

Table 1: Overview of model of diagnosis.

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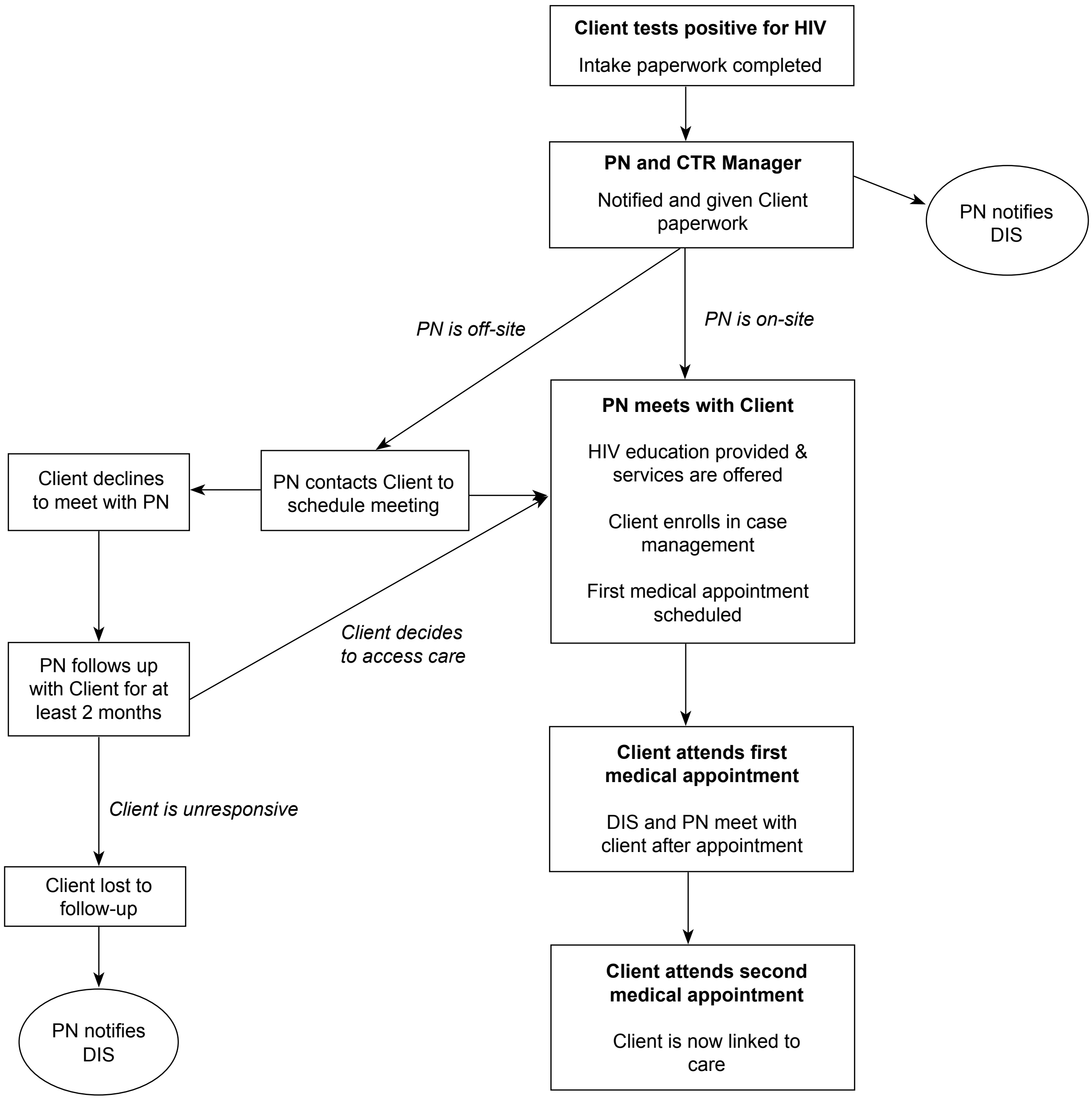

PN: Patient Navigator; CTR: Counseling, Testing & Referrals; DIS: Disease Intervention Specialist with the Office of Public Health

Detailing a Successful Model for Linkage-to-Care

The PN calls the client within 24 hours of being notified of a positive result in order to initiate the LTC process. During the call, the PN provides emotional and mental support to the client, and assesses their readiness to link-to-care. If the client indicates that they are ready to move forward, the PN sets up an appointment time for the client to come to the office. Should the client decide that she/he is not ready to engage in care, the PN requests permission to follow-up with the client in the upcoming weeks. If permission is granted, the PN will contact the client at least once a week to maintain rapport until they're ready to move along the continuum of care.

Depending on the client, calls and text messages may be placed multiple times a day, week or month until the client is linked-to-care.

Once the client decides they are ready to engage in medical care, the PN informs the client of the documentation they will need to bring (proof of Louisiana residency and proof of income) in order to enroll in case management as well as medical services, and schedules a time to meet. If the client does not possess the necessary documents, legal services are provided for them during their first meeting where they can acquire affidavits. During the first in-person meeting with the client, the PN provides an overview of the LTC process and its key components, and discusses any concerns the client may have regarding HIV transmission, health implications, medication adherence, disclosure laws, and other issues as necessary. The PN also provides a

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detailed description of medical services offered in the New Orleans metropolitan area, including but not limited to those provided by the host agency, and helps the client identify a clinic or hospital that will meet their needs. This provides the client with more autonomy regarding their medical care.

If the client chooses to receive medical care at the host agency, the PN accesses the clinic's scheduling software and schedules the first medical appointment with the Primary Medical Care (PMC) department. Outside of core PMC staff, the PN is the only other agency staff member allowed to make medical appointments. Immediately after the appointment is scheduled, the client is enrolled into case management services and any additional services that may be needed.

Throughout these linkage steps, the PN provides personalized messages *via* phone, email and/or text message to the client in order to remind them of upcoming medical and social service appointments. This continuous follow-up is meant to provide "red carpet" service and an inviting experience for the client across the continuum of care. At the client's first medical appointment, it is standard to collect an extensive medical history as well as a CD4/viral load in addition to other tests. Once a client has received a blood draw for CD4/viral load within three months of diagnosis, they are considered linked to care by LOPH standards.

In the case that a client does not return initial calls/texts OR ceases to communicate after initial contact, the PN turns the case fully over to the state Disease Intervention Specialists (DIS) and assists them with LTC for the client as needed.

Detailing a Successful Model for Linkage-To-Care: Follow-Up

After the client attends their first two medical appointments, personalized reminders of appointments from the PN cease, allowing the client to assume greater responsibility for their care. However, the PN monitors appointment attendance and reaches to out the client as needed. The PN may recommend that the client be enrolled in a more intensive tier of case management if they require additional supports. The PN continues to act as a personal advocate and support system for the client, as well as an essential part of their care team.

If a client falls out of care, the PN will reach out to the client to help identify barriers to accessing care. The PN may suggest additional resources to help the client overcome these barriers and ultimately re-engage them in care.

METHODS

After a client tests positive for HIV, their paperwork is transported to a locked filing cabinet maintained by the PN. The PN transfers client demographic information from their file into an encrypted, password protected excel spreadsheet located on the PN's work computer. As the PN begins to track their progress through the continuum of care, encounters with the client are documented, along with appointment attendance, initial CD4 and viral load, risk factors and where the client decides to link to care. While preparing data for this paper, the agency was in the process of transitioning to a more secure data management system, Client Track, Inc.©

Information on LTC rates necessary for reporting purposes are pulled from the excel spreadsheet and analyzed manually.

Limitations

Though data was reviewed at least three times before being incorporated into this paper by two individuals, there is always a small risk of human error.

RESULTS

The above-mentioned model resulted in an average quarterly LTC rate for newly and previously diagnosed clients. In calendar year 2014, there were 108 confirmed positive HIV tests through this agency's CTR program. Of those, 96 were successfully linked to care within 90 days of diagnosis. Of the 108 confirmed positives, 4 additional clients were linked within greater than 90 days. The 2014 LTC rate of <90 days by quarter was 89%, 100%, 88%, and 85%, respectively, for an average rate of 90.5%. Of the 108 confirmed positives, 68 reported being newly diagnosed; of these, an average quarterly rate of 90% were linked to care (N=60) in less than 90 days. LTC rates for sub-populations such as Men who have sex with men (MSM) and young Black men who have sex with men (YBMSM), data showed MSM (N=52) and YBMSM (N=34) were linked-to-care at a rate of 90% and 88%, respectively.

In July 2015, this agency adjusted reporting protocol to meet updated NHAS goals. For July (N=10) and August (N=4) 2015, 86% of clients were LTC within 30 days of diagnosis. Conversely, independent LTC rates for MSM (N=5) and YBMSM (N=8) LTC <30 days were 60% and 100%, respectively.

DISCUSSION

The Importance of Collecting and Analyzing Data for Successful Patient Navigation

Agency employees report that streamlining the LTC process through one person created a simpler approach for HIV counselors, case managers, and providers, and resulted in more complete and accurate data collection. The agency's high LTC rates for 2014 appear to support this conclusion. Prior to hiring a dedicated PN in 2012, multiple employees shared the role of LTC and data tracking. During that time, LTC rates were around 50% and only increased to 83% when LTC was streamlined through one person. In analyzing data, this program found that examining linkage rates for sub-populations is useful in identifying groups that may be underserved or need special attention. For

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example, MSM and YBMSM are often referred to in literature as "hard-to-reach populations".[11] However, this model only saw a 2% difference between the populations.

This agency found it important for partners and funders to have a shared definition and understanding of LTC. For example, it was found that that one grantor considered individuals linked-to-care if a CD4/viral load was drawn within 90 days of diagnosis, while another required clients to have had CD4/viral load drawn *and* have attended at least one appointment with a primary care provider to be considered linked to care. In order to be accountable to different funders and partners, our agency has found that counting, labeling and reporting data according to the requirements of different funding sources while keeping track of linkage numbers beyond the minimum requirements is critical. Finally, it was found that the best LTC is achieved when the PN continues to follow up with clients that did not link-to-care within the 90 period. The data showed that 4 individuals in 2014 were linked to care beyond the 90 day period. Though linkages beyond 90 days may not count towards funding requirements, experience demonstrates the importance of being sensitive to client needs and mindful that clients will move along the continuum of care at their own pace.

The Importance of a Streamlined PN Model, Both for Staff and Clients

Feedback from clients and agency employees demonstrated that keeping the patient navigation flow simple and concise positively influences clients' experiences and the efficiency of the PN and reduces clients' perceived barriers in accessing care. A 2011 study found/recommended increased engagement (patient navigation) at each step in the continuum of care after finding low LTC rates nationally.[12] This agency showed that extended or multiple meetings with a client will often compound barriers to accessing care. Oftentimes, clients become frustrated or lose interest if they are required to return multiple times before receiving medical care. Therefore it is important for providers to realize that many clients may have competing priorities and decreasing the number of visits is likely to augment entry to care. For example, the time it takes to travel to and from multiple enrollment appointments may conflict with clients' work and familial responsibilities, affect involuntary disclosure, or are prohibitively expensive for the client.

This patient navigation model works to ensure that clients complete the following steps during their first meeting with the PN: completion of all intake paperwork, enrollment in case management services, and scheduling of the first medical appointment. While this results in a lengthy (normally half-day) appointment for the client, feedback from past clients is positive; one long day is preferable to multiple appointments spread out over time. Having the client prepared with all the necessary documents, managing expectations around the time necessary to complete the process, and having snacks on hand are also helpful.

Staff Training and Communication

This patient navigation program found that training and retraining of HIV counseling staff and volunteers is essential to a achieving a successful LTC model.[13] Testing technologies and protocols change frequently and front line staff must remain up to date in effort to effectively counsel and refer clients to the proper resources. Additionally, allowing the PN to create intuitive intake paperwork influences the success of this model. Experience has shown that convoluted forms and instructions create barriers for both the client and volunteers who provide HIV testing and counseling, and that clear communication from the PN helps staff and volunteers provide clients who have tested positive the most efficient and streamlined transition into care. Furthermore, communication between the PN and the state DIS has proven to be an indispensable tool for this program; in some cases the DIS were able to reach out to clients or share certain data with the PN that assisted in LTC for especially hard-to-reach clients.

Data-Sharing Between Agency Departments & Improved Linkage Outcomes

Data sharing between prevention staff and primary care staff has been critical to the success of this agency's patient navigation model, as well as culturally appropriate communication with clients. Information sharing agreements are obtained from the client immediately after they test positive, which enables different actors within the continuum of care to contact the client and offer their services in a timely manner. In addition, beyond the sharing of formal data, there is also the sharing of "soft" data, aka conversations and emails, between prevention and primary care providers. This allows for a more individualized client experience.

A critical component to the success of this patient navigation model is the PN cell phone. The PN maintains a "smart" cell phone purchased by the agency used in all communication with the client. The cell phone is critical to this program's success for multiple reasons. First off, the cell phone does not show up as a particular agency when it calls a client, which reduces stigma and anxiety for the client. Secondly, this model has demonstrated that clients of all ages prefer communication *via* text message to phone calls and voice mails. Text messages are perceived as less intrusive and intimidating than phone calls and allow the client to respond at their own pace and comfort. Critical to successful client text messaging is the sending of culturally and age appropriate text messages to clients. In particular, "code switching," or changing the vernacular and delivery of verbal and written communication, is critical to building trust with the client and maintaining their confidence. A randomized control study from March 2015 found that a group of HIV positive clients receiving personalized case-management around linking-to-care were more likely to link-to-care than the control group of HIV positive clients who received standard of care communication.[14] Furthermore, the study found that increased communication (5 interactions) was associated with higher LTC. The cell

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phone used at the agency in Louisiana allows for more of this interaction at the client's leisure.

Addressing Challenges to Successful Linkage-To-Care

Major challenges to LTC for this patient navigation model align with those found nationally. In particular, clients' individual-level and situational variables can present barriers to successful LTC. Financial instability, age, and emotional distress are three of the most challenging barriers to LTC that this agency has found using this model. A lack of finances may result in housing instability or poor access to regular cell phone or internet communication, both of which can impede the ability of the PN to be in regular contact with the client. Age also plays a major factor in the successful linkage of clients to primary care following diagnosis. Staff has found that younger clients are more likely to have unstable living situations and/or be less likely to divulge their status to family members. Youth may have little to no experience navigating the healthcare system as an adult, and are more likely to face challenges understanding the medical implications of their diagnosis and the importance of being proactive about health. Lastly, extreme emotional distress in the form of anxiety, depression, and denial can be an impediment to timely LTC, especially for newly diagnosed clients. This is one reason for the continued long-term follow-up used by this model.

By streamlining the patient navigation model and maintaining close ties with case management and primary care programs at the agency, this model has been successful at addressing the various barriers to LTC faced by clients. Many services are physically housed at the same location as the PN, which enables the client to enroll in and begin receiving services on the same day that they meet with the PN. Early in the program it was noted that clients became frustrated if they had to come back for multiple appointments before seeing a doctor and receiving medication. By limiting the number of initial appointments before seeing a provider, and extending the length of the initial appointment, staff have seen greater LTC when clients enroll in the services the same day as their first appointment with the PN. Furthermore, data sharing agreements allow for fluid communication between programs and with the client. As soon as a client's needs are identified, they can be referred to and access a given program, such as behavioral health, housing or food pantry support. In addition, the agency maintains programs and providers that specialize in working with adolescents and young adults, including a primary care clinic and behavioral health providers that solely serve women and adolescents.

Health Implications for Patient Navigation

Efficient and patient-centered LTC results in improved physical and mental health outcomes for clients testing HIV-positive, especially those who are experiencing medical complications or mental health issues. Co-housing the PN within the same location as PMC and case management allows for strong professional and social ties between the PN and other agency employees. The strength of these relationships means that in the event that a client with severe medical complications needs to see a provider more quickly than the normal scheduling would allow, the PN is able to coordinate with providers to expedite their medical care. The trust and respect developed and sustained between the PN and staff within PMC and case management departments is critical to the success of the patient navigation model, and ultimately to the timely and patient-centered care that the model provides to clients.

Adaptability of this Linkage-to-Care Model for other agencies/organizations

When adapting any service delivery model from one agency to another, undoubtedly there will be a need to deviate from the original model and customize it for different service approaches and protocols. While the overall model presented has a very high LTC rate, different components of the model presented in this article contribute to the agency's rate. Organizations possess varying levels of capacity and each element of this model's approach to LTC will not be necessary or appropriate for every agency. For example, a PN cell phone that doesn't register as a clinic on the caller ID may not be possible if an agency does not have the funds for a dedicated cell phone. In this case, it may prove worthwhile to investigate other forms of communication that allow for that anonymity and a client-centered approach to communication: a dedicated PN Facebook account, a secured PN Gmail/Gchat account for messaging, or a program that allows for FaceTime are all forms of communication that clients may be willing to consent to. For those agencies working to achieve a higher LTC rate, the authors recommended looking at each piece of this model individually to see what may be feasible and useful, instead of trying to implement the full model.

LIMITATIONS/DISCUSSION

Complete data for 2011 is unavailable due to new program implementation. Data tracking did not become consistent until late 2011. Furthermore, because it was not possible to prepare a control group for the purposes of this paper, it is important to note that other factors could contribute to high LTC rates. During the period in which the PN program was being implemented the agency providing this data was one of the larger and well-known clinics in New Orleans, LA, which may have influenced some of the results. For example, advertisements and targeted outreach during high volume seasons (i.e. Decadence, Mardi Gras, etc.) increases the visibility of the clinic. As a result, individuals that engage in high-risk behaviors during these seasons may have a heightened awareness of the risks and consequences associated with their behaviors. On the other hand, up until 11/11/14 the Louisiana based agency was using a rapid/conventional test (western blot) algorithm for providing a proof of diagnosis. One study from New Jersey found that "many persons who receive preliminary positive rapid test results do not return for their confirmatory test results and thus might not access necessary medical care."[15,16]

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This finding from New Jersey did not include a patient navigation model that could help a client navigate the system between tests like the model discussed in this paper. Furthermore, the New Orleans based agency began using a rapid/rapid model for LTC on 11/11/14 and since then has continued to see the same high LTC rates that were achieved prior to the switch. It is recognized that this model's success could have been influenced by the change in testing algorithms.[17-19]

CONCLUSION

The above technical review provides a detailed description and analysis of the LTC model used at a local health clinic in New Orleans, LA, USA which exceeded the 2015 National HIV/AIDS Strategy (NHAS) goal of reaching an 85% LTC rate, ahead of schedule. This model effectively identifies and decreases traditional barriers to care through the use of innovative algorithms and intradepartmental collaborations. Identifying a PN that is relentless in their approach and culturally sensitive, tech savvy, and self-motivated can help provide equitable access to LTC and medical services. This model is not intended to be used as a one-size-fits-all solution, rather a reference for agencies working to enhance their LTC experience and achieve the updated NHAS goals.

DISCLOSURE

Ashley King, Caitlin Canfield and Joseph Olsen are all current or former employees of the Federally Qualified Health Center in New Orleans, LA, USA presented in this article.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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*Corresponding author
Taisuke Togari, PhD, RN, PHN
Department of Liberal Arts
The Open University of Japan
2-11 Wakaba, Mihama-ku
Chiba 261-8586, Japan
E-mail: ttogari@ouj.ac.jp

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Virtual and Real Social Support Networks in Mental Health of Japanese HIV-Positive Men: Nationwide HIV/AIDS Web Research

Taisuke Togari, PhD, RN, PHN[1*]; Yoji Inoue, PhD, RN, PHN[1]; Yosuke Takaku, BEc[2]; Sakurako Abe, PhD, RN, PHN[3]; Rikuya Hosokawa, MHSc, RN, PHN[4]; Takashi Itagaki, BHSc[5]; Sachiko Oki, PhD, RN, PHN[6]; Naoko Katakura, PhD, RN, PHN[7]; Chihiro Wakabayashi, MHEc[8]; Takashi Yajima, BEc[2,9]

[1]*Department of Liberal Arts, The Open University of Japan, 2-11 Wakaba, Mihama-ku, Chiba 261-8586, Japan*

[2]*Japan Network of People Living with HIV/AIDS, 1-21-12-103, Hyakunincho, Shinjyuku-ku, Tokyo 169-0073, Japan*

[3]*Division of Health Administration, TIS Inc., 2-11-1, Nagatacho, Chiyoda-ku, Tokyo 100-6150, Japan*

[4]*School of Nursing, Nagoya City University, 1 Kawasumi, Mizuhocho, Mizuho-ku, Nagoya 467-8601, Japan*

[5]*accelight Inc., 4-1-5-5, Hongo, Bunkyo-ku, Tokyo, Japan*

[6]*Faculty, Department of Nursing, Kyorin University, 6-20-2, Shinkawa, Mitaka 181-8611, Japan*

[7]*Kobe City College of Nursing, 3-4, Gakuen-nishimachi, Nishi-ku, Kobe 651-2103, Japan*

[8]*Department of Health Sciences, Saitama Prefectural University, 820, Sannomiya, Koshigaya 343-8540, Japan*

[9]*Place Tokyo, 4-11-5-403, Takadano-baba, Shinjuku-ku, Tokyo 169-0075, Japan*

ABSTRACT

Background: This cross-sectional study examined the effects of face-to-face (real) and virtual social support networks on the mental health status of Japanese HIV-positive males.
Method: A nationwide online cross-sectional survey was conducted by HIV Futures Japan project from July 2013 to February 2014: 1,095 responses were obtained, and we analyzed those of 879 not females. Two subscales of the Hospital Anxiety and Depression Scale (HADS) were measured in depressive and anxiety tendencies.
Results: The mean (SD) scores for depressive and anxiety tendencies were 8.1 (4.2) and 8.6 (4.8), respectively (the range was 0-21). Hierarchical regression analysis showed that virtual-support networks did not display direct effects on depressive and anxiety tendency. "No one", "only one" and "two to three" in the real-support network showed significant relations compared to "20 or more" (B=2.76, 1.86, 1.46, respectively. 95% CIs [1.35, 4.17], [0.28, 3.44], [0.10, 2.82], respectively) to the depressive tendency. With anxiety tendency, "no one", "only one" and "two to three" in the real-support network showed a significant relation compared to "20 or more" (B=2.42, 2.04, 1.61, respectively. 95% CIs [0.83, 4.00], [0.27, 3.81], [0.08, 3.14], respectively).
Conclusions: It is necessary to develop face-to-face social support systems to promote mental health among people living with HIV/AIDS.

KEYWORDS: Social support; Mental health; Virtual-support network; HIV/AIDS, Japan.

ABBREVIATIONS: HADS: Hospital Anxiety and Depression Scale; PLHIV: People living with HIV; WHO: World Health Organization; QoL: Quality of Life.

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INTRODUCTION

Since 2007, around 1,500 new HIV/AIDS patients have been reported annually in Japan. According to the National Institute of Infectious Disease, the number of HIV/AIDS patients in the country was 23,000 in 2013.

People living with HIV (PLHIV) have to suffer internalized stigma.[1] This stigma causes stress, affects their mental health,[2] and presents a long-standing problem for care of PLHIV. Gay PLHIV are subject to social segregation, rejection in their relationships, and social withdrawal.[3] The World Health Organization (WHO) quality of life (QoL) group has underlined the importance of social inclusion for PLHIV.[4] Positive social support for PLHIV is reflected in a good mental health status,[5] medication adherence,[6] and appropriate risk behavior.[7]

In recent years, personal connections *via* the Internet have grown alongside the development of social networking services. Social support has increasingly adopted the form of virtual communities and electronic support groups.[8] Social support in online communities currently has a similar level of acceptance to that in face-to-face situations.[9]

The aim of the present study was to examine how face-to-face (real) and virtual social support networks for HIV/AIDS are related to mental health status in Japanese HIV-positive males.

METHODS

Subjects and Methods

In this cross-sectional study, we used data from a national online survey conducted by HIV Futures Japan from July 2013 to February 2014. The HIV Futures Japan national survey was performed as part of the HIV Futures Japan project, which is intended to help realize healthy, independent lifestyles and create a liveable social community for HIV-positive individuals.[10] In Japan, there have been many surveys that measured medicinal and curative aspects. Moreover, there have not developed adequately HIV sero-positive registration system in Japan. However, that research did not include aspects of members of the general population living with HIV/AIDS. Therefore, a multidimensional and multidisciplinary survey for HIV-positive individuals was needed.

The questionnaire in this survey was composed of the following large categories: (1) about yourself (socio-demographic items), (2) health status, (3) medicine, (4) sexual health, (5) substance use and addiction, (6) having children, (7) social relations, (8) mental health, (9) social welfare/health management and (10) others. The survey posted online based on self-reported HIV infection, and subjects were recruited in 2 ways. First, we set up online links and banners to promote the survey site. We began by placing links on general sites directed at HIV-positive individuals; we also included links to sites for HIV-prevention groups, sites for HIV-positive groups, social networking sites for HIV-positive participants, and so on. Second, we distributed the fliers at medical facilities, HIV-related events, and meetings of HIV-prevention groups and support groups.

After excluding incomplete answers, we obtained responses from 1095 individuals. We determined 913 responses to be valid, and we subjected these to analysis. The survey participants were resident in 46 of Japan's 47 prefectures (the exception was Tottori).

Of the valid responses received, 879 subjects remained for analysis after we excluded females. Descriptive statistics of subjects were shown in Table 1. The mean age of the participants was 38.2 years (standard deviation [SD], 8.1 years); the average number of years since testing positive for HIV was 5.9 years (SD, 4.9 years).

Variables

Virtual- and real-support network for HIV/AIDS: In the survey, we inquired whether participants were able to talk about HIV/AIDS online (virtual support) and whether they were able to speak about it directly (real support). We defined each 6 categories: (1) no one; (2) only 1; (3) 2-3; (4) 4-9; (5) 10-19; and (6) 20 or more.

Mental health status: We assessed mental health status by means of the HADS.[11] This scale comprises 2 subscales-depression and anxiety. Each subscale is made up of 7 items assessed on a five-point Likert scale. Cronbach's alpha coefficients are 0.75 for the depression subscale and 0.85 for the anxiety subscale.

Demographics and time since HIV infection: In the survey, we inquired about age, sexual orientation (gay, bisexual, heterosexual, other) and number of years since HIV infection.

Analysis

We used the depression and anxiety subscales as continuous variables for depressive and anxiety tendencies. We performed a multiple regression analysis estimated by restricted maximum-likelihood method, in which the dependent variables were depressive tendency and anxiety tendency; the independent variables were age, sexual orientation, number of years since HIV infection, virtual-support network, and real-support network. In the hierarchical regression model (model 1), the independent variables were age, sexual orientation, number of years since HIV infection and the virtual-support network. In model 2, the real-support network was added to model 1. All statistical analysis were performed using IBMSPSS, version 19.0 (IBM Corporation).

RESULTS

The mean (SD) scores for depressive and anxiety tendencies were 8.1 (4.2) and 8.6 (4.8), respectively (the range was 0-21). Score distributions by categories were shown in Table 1.

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	n	(%)	Depression score			Anxiety score		
			Mean	(SD)	p^a	Mean	(SD)	p^a
Sexual orientation					.896			.201
gay	717	(81.6)	8.1	(4.3)		8.5	(4.8)	
bisexual	50	(5.7)	8.5	(4.3)		8.9	(4.6)	
heterosexual	95	(10.8)	7.9	(3.7)		8.9	(4.4)	
other	17	(1.9)	8.1	(4.4)		10.0	(6.4)	
Virtual-support network on HIV/AIDS					.048			.239
no one	441	(50.2)	8.4	(4.4)		8.6	(4.8)	
only one	40	(4.6)	8.0	(4.0)		8.9	(4.8)	
two to three	182	(20.7)	8.1	(4.2)		8.9	(4.6)	
four to nine	85	(9.7)	8.0	(4.0)		9.0	(4.8)	
10–19	44	(5.0)	7.4	(3.4)		8.0	(4.5)	
20 or more	82	(9.3)	6.9	(4.2)		7.4	(4.8)	
Real-support network on HIV/AIDS					<.001			.001
no one	199	(22.6)	9.3	(4.3)		9.3	(4.6)	
only one	93	(10.6)	8.3	(4.7)		9.0	(4.9)	
two to three	309	(35.2)	8.0	(3.9)		8.7	(4.6)	
four to nine	143	(16.3)	7.8	(3.9)		8.4	(4.8)	
10–19	65	(7.4)	7.2	(4.5)		7.5	(5.2)	
20 or more	66	(7.5)	6.5	(4.3)		6.6	(4.8)	
Age	38.2	(8.1)[b]	-0.015[c]			-0.187[c]		
Number of years since HIV infection	6.9	(4.9)[b]	-0.045[c]			-0.133[c]		

[a]F-test in one-way ANOVA
[b]mean (SD)
[c]Pearson's product-moment correlation coefficient

Table1: Descriptive statistics and destribution of depression and anxiety score (n=879).

Table 2 presents the results of hierarchical regression analysis. In terms of depressive tendency, "no one" and "two to three" in the virtual-support network showed significant relations in model 1, (B=1.65, 95% CI [0.63, 2.67], B=1.29, 95% CI [0.16, 2.42]), though there was no significant relation in model 2. "No one", "only one" and "two to three" in the real-support network showed significant relations (B=2.76, 1.86, 1.46, respectively. 95% CIs [1.35, 4.17], [0.28, 3.44], [0.10, 2.82], respectively) to the depressive tendency in model 2.

With anxiety tendency, however, the virtual-support network evidenced no significant relation in models 1 and 2. "No one", "only one" and "two to three" in the real-support network showed a significant relation (B=2.42, 2.04, 1.61, respectively. 95% CIs [0.83, 4.00], [0.27, 3.81], [0.08, 3.14], respectively) to depressive tendency in model 2.

DISCUSSION

We found that the mechanisms related to the social support network for HIV/AIDS differed with respect to depression and anxiety. In the case of depression, virtual social support was indirectly related to depression mediation by the real-support network. However, the virtual-support network showed no relation in the case of anxiety; only the real-support network displayed a relation with respect to anxiety.

This study shows that virtual support did not have a direct effect on mental health. In contrast to online support groups, Setoyama et al[9] identified a high degree of emotional insight as part of the peer support function in face-to-face support groups. The authors believed this to be possibly the result of participants being able to establish a closer relationship with other group members in face-to-face support. As elsewhere in the world,[12] Japanese PLHIV are subject to severe mental health problems. In this research conducted by HIV Futures Japan, we found high proportion of high-risk depressive (28.7%) and anxiety (33.1%) disorders which are higher than those in the general Japanese population.

Also, a social phenomenon called 'AIDS panic' occurred in 1990's Japanese society. Research which was conducted to find quality of life of medically induced PLHIV in Japan reported a lot of felt stigmas were in Japanese PLHIV at that time, and these were related to social isolation and negative mental health.[13] In this HIV Futures Japan survey, we found almost Japanese PLHIV still remain in having felt stigma. For example, 35% of participants answered "*Since I am HIV+, I hardly interact with others*". It is therefore necessary to provide emotional support to promote the mental health status of PLHIV.

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	Depression tendency						Anxiety tendency					
	Model 1			Model 2			Model 1			Model 2		
	B	(95% CI)	*p*	B	(95% CI)	*p*	B	(95% CI)	*p*	B	(95% CI)	*p*
Intercept	6.32	(3.58 9.06)	<.001	5.25	(2.41 8.09)	<.001	12.46	(9.41 15.52)	<.001	11.45	(8.26 14.64)	<.001
Virtual-support network on HIV/AIDS												
no one	1.65	(0.63 2.67)	.002	0.52	(-0.68 1.73)	.395	0.79	(-0.34 1.93)	.171	-0.35	(-1.70 1.01)	.616
only one	1.24	(-0.39 2.88)	.136	0.20	(-1.58 1.97)	.828	0.53	(-1.29 2.35)	.568	-0.62	(-2.62 1.37)	.539
two to three	1.29	(0.16 2.42)	.025	0.41	(-0.90 1.72)	.540	1.01	(-0.24 2.27)	.114	0.06	(-1.41 1.54)	.934
four to nine	1.24	(-0.06 2.54)	.062	0.57	(-0.86 1.99)	.437	1.14	(-0.32 2.59)	.125	0.39	(-1.22 1.99)	.635
10–19	0.61	(-0.95 2.17)	.442	0.12	(-1.55 1.79)	.889	0.24	(-1.49 1.98)	.782	-0.29	(-2.17 1.59)	.764
20 or more	ref.			ref.			ref.			ref.		
Real-support network on HIV/AIDS												
no one				2.76	(1.35 4.17)	<.001				2.42	(0.83 4.00)	.003
only one				1.86	(0.28 3.44)	.021				2.04	(0.27 3.81)	.024
two to three				1.46	(0.10 2.82)	.035				1.61	(0.08 3.14)	.039
four to nine				1.28	(-0.14 2.70)	.077				1.29	(-0.30 2.89)	.113
10–19				0.77	(-0.76 2.30)	.322				0.51	(-1.20 2.23)	.558
20 or more				ref.						ref.		
-2 Restricted Log Likelihood		4957.56			4913.74			5140.82			5109.56	

All models were adjusted for age, number of years since HIV infection and sexual orientation.
CI: confidential interval
[a]fixed effect model estimated by restricted maximum-likelihood method

Table 2: Hierarchical multiple regression analysis about effects of virtual and real support networks on mental health.

This study has the following implications. It is necessary to establish face-to-face support systems to promote mental health among PLHIV. To reduce depressive tendencies, it is important that virtual support systems for HIV/AIDS be designed to facilitate the creation of face-to-face support networks.

This study has a number of limitations. First, we relied on online survey data, which limited participants to those with access to the Internet, such as individuals with smart phones or personal computers, and that may have led to selection bias. It would be appropriate to confirm the generalizability of our findings by comparing our online survey results with those acquired by other means, such as from mailed surveys. Second, this was a cross-sectional study. It is therefore necessary to clarify the relationships among the studied factors using a vertical study design through follow-up surveys. Finally, we did not examine the various kinds of social support, such as instrumental and emotional: such aspects should be measured and analyzed in future research.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

CONSENT

This study was approved by Open University of Japan Research Ethics Committee (No. 2013-5) and Osaka National Hospital Institutional Review Board (IRB) (No. 13-037).

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*Corresponding author
Martin Marufu Gazimbi, PhD Student
School of Education and Social Sciences, University of Hull
Cottingham Road, Hull
HU6 7RX, UK
Tel. +44 (0)1482462032
E-mail: m.gazimbi@2013.hull.ac.uk

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A Multilevel Analysis of the Determinants of HIV Testing in Zimbabwe: Evidence from the Demographic and Health Surveys

Martin Marufu Gazimbi, PhD Student*; Monica Akinyi Magadi, PhD

School of Education and Social Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, UK

ABSTRACT

Introduction: Zimbabwe is still burdened with HIV epidemic and the government has an ambitious aim in the post-2015 era to end the AIDS epidemic by 2030. To achieve this, the government has set up the 90-90-90 strategic milestones to be achieved by 2020. It is a daunting task to increase HIV testing uptake from the current estimate of 56% to 90% to meet these targets. The current government's initiative requires an understanding of determinants of HIV testing.
Objectives: The specific objectives of this study are to: (i) identify the individual and community-level determinants of HIV testing, focussing on predisposing, enabling and perceived need factors (PREP); and (ii) establish gender differences.
Materials and Methods: We applied multilevel logistic regression models to nationally-representative samples of 17,797 women and 14,587 men from the 2005/2006 and 2010/2011 Zimbabwe Demographic and Health Surveys (ZDHS) to examine the determinants of HIV testing.
Results: HIV testing uptake increased significantly between 2005/2006 and 2010/2011, especially for women (females OR=5.60; males OR=2.57). Most PREP factors associated with HIV testing are largely consistent with patterns in Southern Africa (e.g., higher uptake by women and those who are wealthier), but unique patterns have also emerged. In particular, results reveal important gender differences: rural residence is associated with lower uptake of HIV testing for women (OR=0.74) but higher for men (OR=1.16); community wealth is a more important factor in enabling HIV testing than household wealth for women, but the converse is true for men; and individual-level, rather than community-level stigma is important for women, while for men, it is community-level stigma that is important.
Conclusion: Observed gender disparities in determinants of HIV testing calls for gender specific response. Couple-oriented HIV counselling and testing services where men accompany their spouse to HIV screening during pregnancy may help increase HIV testing uptake for males and reduce gender disparities.

KEY WORDS: HIV testing; Community-level determinants; Gender disparity; Multilevel analysis; Zimbabwe demographic and health surveys (ZDHS).

ABBREVIATIONS: MQL: Marginalized Quasi Likelihood; PQL: Penalized Quasi Likelihood; VPC Variance Partition Coefficient; ZDHS: Zimbabwe Demographic and Health Surveys; STD Sexually Transmitted Diseases.

INTRODUCTION

A national HIV testing program was implemented a decade ago in Zimbabwe, providing HIV treatment to millions of HIV-positive individuals, many of whom previously struggled with the illness due to unknown HIV status. By 2015, Zimbabwe sought to half new HIV infection prescribed by the World Health Organisation's (WHO's) strategic plan.[1] Although, it has been a daunting task, adult HIV prevalence in Zimbabwe has almost halved from its peak of nearly 30% around 1997 to around 13.7% in 2011.[2] HIV related deaths have also been reduced by over 60% as a result of a successful HIV testing, treatment and support programme.[2] However,

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despite government's efforts to stem HIV scourge, Zimbabwe remains one of the countries in Sub-Saharan African countries still burdened with HIV epidemic.[3] The current aim in the post-2015 era is to end the AIDS epidemic by 2030. To achieve this, the government, in collaboration with its partners have set up the 90-90-90 strategic milestones to be achieved by 2020. The milestones means that by 2020, 90% of all people living with HIV will know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of all people receiving antiretroviral therapy will have viral load suppression.[4] HIV testing has been used as an entry point for both HIV prevention and treatment; and an early detection of HIV can add 15 years to a person's life span.[5] People have to get tested and know their HIV status to enable them to seek treatment and to choose preventative strategies. For this reason, HIV testing services need to be available and accessible to all people to enable easy utilisation.[6]

To ensure targets are met, Zimbabwe needs to improve HIV testing coverage, but scaling up HIV testing coverage; currently estimated at 56% can be daunting, given that HIV testing is voluntary. The success of the HIV self-testing programme will require a better understanding of the factors that influence people's HIV testing behaviour, which this study seeks to achieve. Existing studies have identified a range of factors to be associated with HIV testing, including: place of residence, gender, marital status, and socio-economic status.[7,8] Extant literature from Sub-Saharan African countries indicates that gender has been an important determinant of HIV testing behaviour. Specifically, the studies found that females were more likely to get tested for HIV than males,[9,10] due to reasons such as frequent access to health care services through maternal care.[11]

Besides gender, marital status has been found to be strongly associated with HIV testing and findings have been consistent across gender. Individuals who are divorced, widowed and married tend to have higher odds of HIV testing than never married individuals.[12-14] For example, married individuals were found to be 2.54 times more likely to be tested for HIV than never married individuals in South Africa.[15] Similarly, a study in Ivory Coast[16] found that never married individuals were 66% less likely to be tested than married people.

HIV testing is also influenced by enabling factors such as wealth status, education, HIV awareness and media exposure. Several studies have found that poorer individuals were less likely to test for HIV than those from wealthy households.[10,14,16] It has been noted that poverty is likely to be associated with lack of education, and lack of education implies that messages regarding HIV testing are often inaccessible.[7,17] Education is a key indicator of socio-economic status and more educated individuals are more likely to have better health[5] due their ability to have better access to health information and to understand and respond to such information. Socio-economic challenges may also decrease the likelihood of HIV testing, due to lack of financial resources that would enable individuals to have access to health care services.[18-21]

The effect of HIV/AIDS factors (e.g., stigma and discrimination, risk perception of HIV infection, risky behaviour, HIV awareness, or knowing someone who died of HIV/AIDS) on HIV testing may depend on an individual's predisposing factors such as age, education, marital status, social and economic status.[5,22] These interactions may lead to an increased risk perception of HIV infection which may in turn decrease the willingness to utilise health care services that are linked to HIV.[23] The risk perception of contracting HIV may either increases or decreases the likelihood of using health care services.[24] An example of this phenomenon could be that, since HIV prevalence is reported to be positively associated with higher socio-economic status and education,[25] individuals with higher educational attainment and those who are wealthier may perceive themselves to be at risk of HIV infection, and therefore have recourse for HIV testing, compared to those who are less well-off.[16]

There is also evidence of a positive association between HIV awareness and HIV testing,[14] and awareness and education are highly correlated.[26] However, Lepine et al[12] have argued that HIV awareness may be negatively associated with HIV testing if the awareness affects risk perception and associated behaviours. Firstly, because individuals who have a good understanding of HIV prevention and transmission methods could be less likely to adopt risky behaviour and thus may perceive themselves to have less need for HIV testing as they think they are less at risk.[27] Secondly, people who overestimate their likelihood of getting infected through their poor health knowledge could have higher odds of HIV testing thinking they are more at risk. This notion resonates with Musheke et al[28] who assert that the effect of HIV knowledge on HIV testing may be heterogeneous in the population. For instance education may determine access and exposure to HIV information and will affect the way this information is used to modify attitudes towards HIV testing.

Also, many studies have linked HIV infection with risky sexual behaviours, including multiple sexual partners, limited ability to negotiate safer sex, which may lead to lower rates of condom use and disclosure of HIV status.[12,19,29] All of these may lead to an increased risk perception of HIV infection, leading to lower or higher uptake of HIV testing. In line with the effects of risk perception on healthcare utilisation, studies have shown that perceived susceptibility as a result of risky sexual behaviour exacerbates risk perception of HIV infection, in turn leading to underutilisation of health care services.[22] On the other hand, existing literature also suggests that perception of being at risk of a disease is a prerequisite for behaviour change,[30] and that perceived high risk of infection causes people to take precautionary measure including HIV testing and knowing the results in order to reduce the risk of getting the virus.[12,16] According to health behavioural models[31] perceived susceptibility to a particular health problem (e.g., am I at risk of HIV?), perceived seriousness of the condition (e.g., how serious is HIV/AIDS?, how hard would my life be if I get it?), and cues to action (e.g., witnessing the death or illness of a close friend

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or a family member due to HIV) may move an individual to take necessary action or change their behaviour.[31] Therefore, knowing someone who died because of HIV is also a significant cue to action to an individual to test for HIV.[32]

At community level, the likelihood of HIV testing may increase for individuals who reside in communities with higher HIV prevalence, high poverty, high risk perception of HIV infection, or within easy access of HIV intervention programmes, compared to individuals living in other communities.[7,18,22,30] While HIV/AIDS stigma may exist within self as a result of imagined fear due to perceived risk of HIV infection based on self-assessment,[33] stigma can also be observed and exerted from the outside world (health care providers, family, community, and friends). Either form of stigma may lead to more people underutilising health care services.[26]

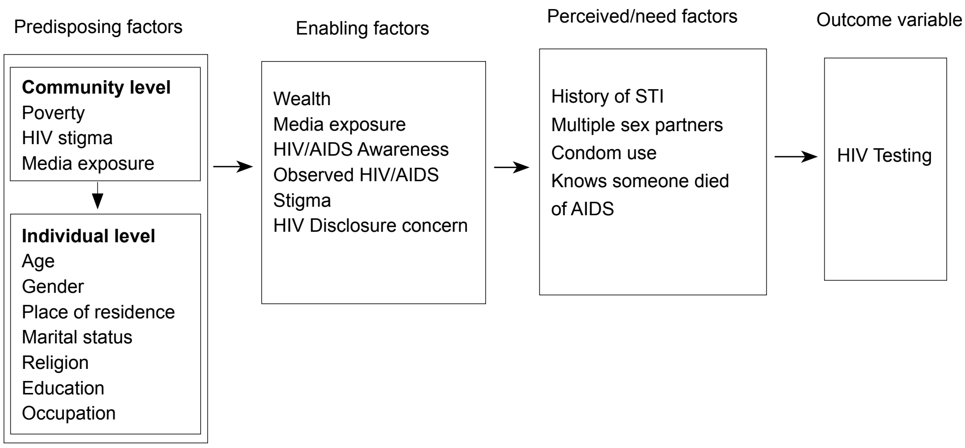
Since risky sexual behaviour may be linked to poverty in the community, people living in poor communities may perceive themselves to be at a high risk of HIV infection, and this may reduce the likelihood of uptake of HIV testing services in such communities.[34] Furthermore, availability and quality of health care services, for example availability of family planning and HIV testing services in the community, may motivate individuals to seek help.[8]

Research on the determinants of HIV testing has received considerable research attention. However, different studies have come up with different positions and it is challenging to reconcile existing findings and use them to inform our knowledge about HIV testing in Zimbabwe. Most available literature has focused on the association between HIV testing and individual-level factors.[8,16,26,35] However, implications of community influence on HIV testing has received limited attention. Also, most studies have tended to analyse the association of predisposing factors such as age, gender, marital status, etc., without taking into account the enabling factors such as resources and enabling environment that facilitates individual's behaviour. Working from the premises that community inequalities in access to health care services is as significant as individual level factors, it is essential and worthwhile to investigate and understand the effects of both individual and community-level determinants of HIV testing in the context of Zimbabwe.

CONCEPTUAL FRAMEWORK

The trajectory to HIV testing first needs to be explained before undertaking the analysis. In this regard, Andersen[36] provided a useful analytical framework for examining determinants of HIV testing. This theoretical framework is grounded in the notion that utilisation of health care services is dependent upon contextual situations, which in turn influences individual circumstances. The framework is conceptualised based on the predisposition, enablement and need for health care use.[36] What is vital in this approach is its emphasis on the joint effects of community and individual level factors that influence health care service utilisation. First, the level of HIV testing uptake is affected by predisposing factors which usually includes personal attributes of an individual such as demographic characteristics (i.e., age, gender, marital status, religion, ethnicity and others). Secondly, predisposing factors are assumed to operate through enabling and perceived need factors and these comprise of contextual and sometimes personal circumstance, representing the ability to use health care services. In this study, enabling factors are composed of an individual's income, access to media, and awareness of HIV, observed HIV stigma, HIV disclosure and confidential concern.[12] Thirdly, enabling factors are assumed to facilitate or inhibit individuals from accessing HIV testing services. However, whether an individual has a means to access health care services, there must be a need for them taking that action.[31] The need factors pertain to the perceived and evaluated assessment of one's health status which may compel the need for seeking health care services.[18] For that reason, perceived/need factors such as whether an individual had engaged in risky behaviour

Figure 1: Conceptual Framework for the Analysis of Determinants of HIV Testing in Zimbabwe.



Source: Authors' construct.

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such as having multiple sex partners, or had history of sexually transmitted diseases (STD) and whether one knew someone who had died of HIV were also considered in the framework. The conceptual framework in Figure 1 provides a schematic display of the perceived determinants of HIV testing in Zimbabwe.

The accumulated effects of predisposing, enabling and need factors govern one's trajectory to HIV testing behaviour. Given the background of growing inequality in access to health care services in Zimbabwe, issues of community influence on HIV testing becomes at fore. A growing need exists for implication of both individual and community level effects on HIV testing. Such information will help the HIV prevention programs identify communities with high concentration of individuals who are likely to have been ever tested for HIV, as well as sub-populations that are not likely to be tested.[10] Guided by this conceptual framework, this study aims to understand individual and community-level factors associated with HIV testing. Specifically, the study aims to identify the predisposing, enabling and perceived need factors associated with HIV testing with particular focus on gender differences.

MATERIALS AND METHODS

The Data

This is a secondary data analysis study based on the 2005-2006 and 2010-2011 Zimbabwe demographic and health surveys (ZDHS), the first 2 nationally representative surveys to include HIV testing. The surveys were designed to provide national estimates of HIV in the population, including HIV testing coverage. The analyses is based on respondents aged between 15-59 years residing in 402 clusters, and the sample size was 32,384 of which 27.8% of males (n=14,587) and 43.7% of females (n=17,797) were ever tested for HIV. Overall, about 93% of those who tested for HIV received their test results. The individuals responded to a questionnaire survey asking questions related to their HIV testing history, socio-economic and demography background and health indicators such as individual's sexual behaviour, HIV related knowledge, attitude and behaviour and media exposure. The sample excluded the institutional population, which includes individuals living in hospitals, prisons and other institutions.

Outcome Variables

The key outcome variables of interest were whether the respondent was ever tested for HIV and received results. For each outcome variable, possible responses were 'yes' or 'no' if an individual had ever been tested for HIV or have received results or not.

Explanatory Variables

We identified a range of independent variables based on the conceptual framework described above, their presence in existing empirical literature and the sample distribution in the ZDHS datasets. The explanatory variables were clustered into specific categories; individual and community-level predisposing, enabling and perceived/need factors. These categories were considered to give the best representation of community and individual characteristics that are associated with HIV testing behaviour. Community-level HIV awareness, stigma and media exposure variables were imputed from relevant individual-level factors.

Individual-Level Socio-Economic and Demographic Predisposing Variables

While inclusion of wealth, occupation, education and media exposure may indicate the manifestation of economic position of an individual,[30] demographic factors such as gender, parity, religion, marital status and age are considered important because they may capture behavioural factors such as the perception of risk, acceptance of testing and sexual practice.[6] The following are factors which were included at individual level.

Gender: Gender plays a key role in health seeking behaviour and there is evidence from existing literature suggesting that women, due to reasons such as frequent access to health care services through antenatal care (ANC), are more likely to test and receive HIV test results than men.[11,12] The variable was used to compare utilisation of HIV testing services between males and females. This is a binary measure coded 1 if female and 0 if males.

Age: The ZDHS sample included females (aged 15-49) and males (aged 15-59) of reproductive age. Age was recorded into 5 categories: 15-24; 25-29; 30-34; 35-39; 40+. The variable was used to compare HIV testing behaviour between different age groups.

Marital status: This variable captures differentials in HIV testing behaviour between marital status categorises. It was recoded into 4 categories: never-married, married, widowed and divorced.

Type of place of residence: This variable captures HIV testing behaviour of individuals by urban/rural residence.

Level of education: This was one of the key variables, which captured socio-economic characteristics of individuals. The variable was recoded into 3 categories: primary, secondary and higher educational attainment.

Religion: Catholic and protestant followers tend to have higher odds of health care service utilisation.This behaviour has been interpreted as a consequence of greater social diversity within mainstream religious groups that facilitates change of behaviour towards health care services utilisation.[37] A categorical variable based on religious affiliation classified into: traditional/Catholic, Protestant, Pentecostal and Apostolic categories is included in the analysis.

Occupation: Economic dependency has been one of the major barriers to women's control over their health behaviour in developing countries. However, studies have shown that a woman's occupation is an important factor that influences her access to

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health care services. This is due to the fact that women who are working and earning money will have greater autonomy and control over financial resources, and thus they are more able to pay for health care services.[16] A categorical variable based on occupation is also included in the analysis.

Enabling Factors

Another explanatory domain in the conceptual framework relates to enabling factors. These are factors which promote or inhibit use of HIV testing services and they include enabling resources and environmental factors.

Household wealth: This variable was used as a measure of economic well-being. The index was constructed by DHS using principal component analysis to assign indicator weights based on household ownership of assets. This was a key variable of interest, and five wealth quintiles (poorest, poorer, middle, richer, and richest) were used in analysing the relationship between wealth and HIV testing behaviours.

Media exposure: This variable captures how individuals' exposure to media was associated with their HIV testing behaviour. Exposure to HIV/AIDS information through mass media may lead to high levels of awareness, which may in turn influence self-assessed risk of HIV infection and the need to test for HIV.[14] This variable was measured by 3 items relating to frequency of watching television, reading newspapers and listening to radio. An additive scale was imputed and classified into 3 level categorical variables: low, medium and high.

AIDS awareness: (1) AIDS awareness was derived from eight HIV related questions which consists of prevention and misconceptions about transmission. AIDS awareness index, was classified into 3 categories: low: if participants answered 3 or less questions correctly; medium: if answered up to 5 questions correctly; and high: if answered 6-8 questions correctly.

HIV related stigma: This referred to the respondent's attitude towards HIV developed from 4 questions of HIV related stigma, namely; 'If the respondent would buy vegetables from a vendor with HIV', 'can care for relative with HIV', 'would want an HIV-positive teacher to continue teaching', and 'would want others to know if a family member became infected with HIV'. The variable was classified into 3 categories: low: if not experienced any of the stigma indicators, medium: if experienced 1-2 of the stigma indicators and high stigma: if experienced 3-4 stigma indicators.

Observed stigma: Like in Sambisa,[26] this study uses this variable to assess individual's attitude towards people living with HIV. People act upon what they see happening around them.[23] People may have been discouraged from being tested for HIV if they observed people living with HIV being discriminated against in their communities or families. In a study by the Government of Zimbabwe,[38] a significantly number of people living with HIV reported that they had been forced to change residency or denied accommodation. We therefore, constructed observed 'stigma variable' from the following set of prompt items; whether participant knows someone suspected to have HIV/AIDS who has been denied health services, knows someone suspected to have HIV/AIDS who has been denied involvement in social events and knows someone suspected to have HIV who has been verbally abused. For the observed stigma variable, the additive scale was split into two categories: yes (1 or more) or no (0)

Disclosure and confidentiality: Not much attention has been given to HIV status disclosure and confidentiality dynamics within private and public places. People do not worry about how to disclose their HIV sero-status only, but they also worry about how their health information is shared.[39] Mashuba and Hemalata[37] pointed out that people living with HIV may have to disclose their HIV status to their family members, health care workers, employers, religious leaders, counsellors and community members. The disclosure and issues with how health information is going to be shared if results come out positive can act as a barrier to access to HIV testing services. Disclosure and confidentiality concern was a variable measured by a single item; 'would want others to know if a family member became infected with HIV and coded 'yes' if someone reported that they would want others to know if a family member became infected, and 'no' if they didn't want others to know.

HIV and risky sexual behaviour variables

Finally the perceived need factors were considered. The conceptual framework suggests that for someone to take an action on his/her health there must be a perceived risk and a need to do so.[31] Here the perceived need factors are operationalized by whether someone feels he/she is at risk HIV infection because of their history of STI, multiple sex practice or knowing someone who died of HIV. Literature suggests that people get tested for HIV following the Illness or death of people they know.[39] Each perceived need variable was coded 'yes or no'.

Condom used in the last sex encounter: This is a binary variable. Those who had not used condom takes the value of 0, and those who have used take the value of 1.

Multiple sex partners: This also is a binary variable, which takes the value of zero if an individual did not have sex or had sex with only one partner and takes the value of one if an individual had sex with more than one sex partner in the last 12 months preceding the survey.

History of Sexually Transmitted Infection (STI): This is a binary variable. Those who have never had STI take the value of 0, and those who have ever had STI takes the value of 1.

Methods of Analysis

A two-level logistic regression model for binary response was

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employed. Individuals who responded to the questionnaire were considered as level-1. Other studies which investigated multilevel determinants of HIV testing behaviour considered household[7] and couples,[12] as levels of analysis. This study did not consider household as a level of analysis because the average number of individuals in a household who have been ever tested for HIV as contained in the dataset was too small to be considered as a level of analysis. The study did not also consider couples; this was premised on the fact that never married people are less likely to be tested for HIV than married, divorced or widowed people.[14,25] Therefore, in this study of the determinants of HIV testing, level-2 of analysis was represented by a cluster, which is defined as a community.

Multilevel Logistic Models

We recognize that communities may share similar socio-economic and demographic characteristics, resources and experiences[7] and it is therefore, reasonable to assume that residents of one community maybe more similar to each other with respect of their HIV testing behaviour. With such background knowledge, it can be argued that variations in HIV testing in Zimbabwe may not have been as a result of individual characteristics alone, but of the effects of community effects in which they live as whole.[10,30] For that reason, this study used 2 level logistic regression models to investigate the community and individual level factors that influence HIV testing behaviour. The basic form of 2 level random intercepts logistic regression model used can be expressed as:

$$Logit(\pi_{ij}) = Log\,[\pi_{ij}/(1-\pi_{ij})] = \beta_0 + \beta X'_{ij} + u_{0j}$$

$$\text{Var } u_{(0j)} = \sigma^2_{u0}$$

Where(π_{ij}) is the probability of having ever been tested for HIV for an individual i, in the jth community. β_0 is the regression intercept, X'_{ij} is the vector of covariates defined at individual or community level; β is the associated vector of usual regression parameter estimates, and is shared by all communities, while the random effect u_{0j} is specific to community$_j$.

Before including any explanatory variables in the models, it was crucial to know how much between communities variations were there in the propensity to test for HIV. To assess this, we look at the estimated value of σ^2_{u0}, which is the variance of the u_{0j} terms. We use a threshold model approach which measures proportions at group level:

$$\rho = \sigma_U^2/(\sigma_U^2 + \sigma_\varepsilon^2)$$

Where σ_U^2 is the total variance at community level, and σ_ε^2 is the total variance at individual level. For the multilevel logistic regression model, the level-1 residuals are assumed to have a standard logistic distribution with mean zero and variance $\sigma_\varepsilon^2 = \pi^2/3$ where π is the constant 3.14159. Thus, $\rho = \sigma_U^2/\sigma_U^2 + 3.29$.[40]

Modelling Approaches

Since our analysis is based on hierarchical data given the ZDHS multi-stage sampling design, it is necessary to use techniques that consider the possible dependence of individuals clustered in the community.[40] Conventional regression analysis techniques assume that individual observations are independent from one another. If this assumption is violated, estimates of the regression coefficients can be biased and standard errors may be underestimated. Multilevel regression techniques make it possible to take into account the possible dependence of the outcome variable between people in the same community.[40] Four models were fitted in the analysis. Model 0 was an empty model with year of survey as the only co-variate. This was fitted to decompose the total variance between individual and community level. Background demographic and socio-economic factors were then added in Model 1. The next model (Model 2) was composed of background demographic/socio-economic factors and HIV and risky sexual behaviour; enabling and need factors. The final model (Model 3) took account of community factors.

Measures of fixed effects, that is the effects of individual-level and community-level determinants of HIV testing were reported in terms of odds ratios, whilst the measures of variation (random effects) were expressed in terms of intra-class correlation coefficient (ICC). The estimation procedure was based on quasi-likelihood methods, starting with the default procedure 1st order marginalized quasi-likelihood methods (MQL) and extending to 2nd order penalized quasi likelihood (PQL). The 2nd order estimation was preferred because it is an improved approximation procedure.[41]

RESULTS

Descriptive and Bivariate Analysis

Cross-tabulation analysis, including chi-square tests were undertaken to assess the distribution of individuals who were ever tested for HIV by predisposing, enabling and need factors.Relevant means or proportions were imputed for community-level variables. The proportions of individuals who were ever tested for HIV and received their results are presented in Table 1, based on 2005-2006 and 2010-2011 ZDHS.

Percentage Distribution of Individuals Ever Tested for HIV and those Who Received the Results

Table 1 presents the proportion of individuals who ever tested for HIV; and the proportion that received results among those who were ever tested for HIV in the 2005-2006 and 2010-2011 surveys. The coverage of HIV testing was significantly higher for females (26%) *versus* males: (18 %), ($p<0.005$) in the 2005-2006 survey. By 2010-2011 the coverage of HIV testing increased for both males and females to 62 % for females: *versus* males: 38% ($p<0.005$). Among those who ever tested for HIV, 89% of males and 85% of females received results in the 2005/2006 survey; these percentages increased to 93% for males

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Table 1: Trends in HIV Testing Coverage and Receipt of Results.

Survey year	Proportion who have ever been tested for HIV			
	Males		Females	
	Weighted %	Unweighted cases	Weighted %	Unweighted cases
2005-6	17.7	7107	25.7	8849
2010-11	37.6	7480	61.5	8948
All	27.9	14587	43.7	17797
Survey year	**Proportion who received results among those who have ever been tested for HIV**			
	Males		Females	
	Weighted %	Unweighted cases	Weighted %	Unweighted cases
2005-6	88.6	1258	85.1	2274
2010-11	92.8	2812	96.4	5503
All	91.5	4070	93.1	7777

and 96% for females in the 2010-2011 survey.

Distribution of HIV Testing by Background Characteristics

A bivariate analysis was used to assess the association between each independent variable set out in the framework and the study outcomes. The Chi-square tests showed association between key variables and HIV testing, but not with receipts of HIV test results. The sample size of those who did not receive results after HIV test was too low to allow for a meaningful multivariate multilevel logistic regression analysis. Therefore, the determinants of receipts of HIV test results were excluded from further analysis. Given that over 90% of those who tested for HIV collected their results,[2] policies needs to focus mainly at increasing uptake of HIV testing in Zimbabwe.

The results in Table 2 indicated that there was an association between HIV testing and the following factors: age, place of residence, marital status, educational attainment, occupation, household wealth, exposure to media, and HIV awareness. The highest proportion (58%) was observed among females in the age group (25-29); compared to males (38%) in same age group. For both males and females, HIV testing coverage was lowest among individuals below the age of 20 years, followed by those aged 40 years and above. Because of disparities in socio-economic development between urban and rural areas, place of residence was also a key factor in determining access to HIV testing services. A higher proportion of people who ever tested for HIV were from urban than rural areas for both males and females. With respect to marital status, for females, the highest coverage of HIV testing was observed among those who were married (52%), whilst for men it was among those who were widowed (41%). As we expected, HIV testing coverage increased by educational attainment. For example only 19% of males and 36% of females with primary education ever tested for HIV, compared to 53% and 65% males and females with higher educational attainment, respectively.

Distribution of HIV Testing by Enabling and Need Factors

Table 3 shows differentials in HIV testing based on enabling and need factors, including household wealth, media exposure, HIV/AIDS awareness, observed stigma, and HIV test results disclosure concern. The proportion of individuals who were ever tested for HIV was higher among those from wealthier households. For example, 21% of males from poorest households *versus* 39% from richest household reported ever being tested for HIV. Whilst, HIV testing was higher among individuals who reported having higher media exposure or HIV awareness, it was lower among those who reported having observed HIV stigma and those with HIV disclosure concerns.

Individuals who engage in multiple sexual partnerships, have history of (STI), and do use condoms or those who knew someone who had died of HIV may consider themselves to be at a high risk of HIV infection. Therefore, they may perceive themselves as having a need to test for HIV.[12] In our sample, a higher proportion of individuals (35% of males and 57% of females) who mentioned that they have a history of STI had been tested for HIV than those who did not have a history of STI (28% of males and 43% of females). Furthermore, a higher proportion who reported having used condoms in the last sexual intercourse had been tested for HIV compared with individuals who did not use condoms (e.g. 62% *versus* 42% for females). For both males and females, those who reported having multiple sexual partners and those who knew someone who died of HIV were less likely to report having been tested for HIV than their counterparts without multiple partners or who did not know anyone who had died of HIV/AIDS, contrary to expectation.

The results from bivariate analysis suggested a significant association between variables considered in the framework. However, given that bivariate associations can be influenced by confounding factors, a multivariate analysis that simultaneously takes into account these effects was used to accurately establish the independent predictors of HIV testing behaviour.[42]

Multivariate Multilevel Analyses

Multivariate analyses here start with combining the data for males and females across the 2 surveys to assess gender differ-

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Table 2: Distribution of HIV Testing by Background Socio-Economic and Demographic Characteristics.

Background characteristics	Proportion ever been tested for HIV by background characteristics						All Cases (unweighted)
	Males			Females			
	Weighted %	N	*p*-value	Weighted %	N	*p*-value	
Age groups*			<0.05			<0.05	
15-24	16.7	6533		35.4	7717		14250
25-29	37.7	2218		57.9	3089		5307
30-34	36.3	1838		51.6	2470		4308
35-39	37.1	1462		48.0	1859		5321
40+	34.6	2536		36.5	2660		5196
Residence*			<.005			<.005	
Urban	34.6	4992		47.9	6571		11563
Rural	24.4	9595		41.3	11224		20819
Marital status*			<0.05			<0.05	
Never married	17.7	6724		22.9	4695		11419
Married	36.7	7154		51.9	10529		17683
Widowed	41.1	180		46.5	1231		1411
Divorced	34.2	529		49.1	1340		1869
Education level *			<0.05			<0.05	
Primary	18.8	4176		35.5	6065		10241
Secondary	29.6	9523		46.9	11083		20606
Higher	52.8	888		64.5	647		1535
Religion*			<0.05			<0.05	
Roman catholic	26.9	6837		42.2	4754		11591
Protestant	29.4	2202		41.3	3734		5936
Pentecostal	33.6	1944		47.2	3343		5287
Apostolic sect	25.8	3604		44.4	5964		9568
Occupation*			<0.05			<0.05	
Unemployed	18.5	4634		40.7	10278		14912
Professional	52.1	1056		72.6	1796		2852
Manual work	46.0	2121		70.8	729		2850
Agriculture	36.4	1842		58.6	1218		3060

*All variables statistically sig at 5% level <0.05

ences. Although, the association patterns observed in bivariate analysis were relatively consistent with gender, it is reasonable to recognise that the determinants of HIV testing among males and females are likely to be different.[9] For this reason, multivariate analysis is presented separately for males and females to explore the differences in predictors of HIV testing by gender. Results for the combined sample are given in Annexure, while tables 4 and 5 present estimates for females and males respectively. There is evidence of a significant gender disparity in HIV testing, with females being about twice as likely to be tested (average OR=1.8) as compared to males of similar characteristics (See Annexure).

The Determinants of HIV testing among females

Table 4 presents the odds ratios (ORs) associated with HIV testing for females. With all variables excluded, the results show females were 5.4 times more likely to have been HIV tested in 2010/2011 than in 2005/2006 survey. When all predisposing variables were included in Model 1, the odds of HIV testing substantially increased to 6.9. Females from rural areas were 36% less likely to test for HIV than those from urban areas. As females grow older, they have a tendency to avoid HIV testing. For example, females aged between 30-34 years and those who were 40 years and above were 31% and 65 % less likely than young individuals aged 15-24 years to test for HIV, respectively. Married females were 2.1 times while those who were widowed and divorced were 2.4 and 1.8 times more likely to test for HIV compared with those who never married. Also females who were professionals and those with manual jobs had 1.4 times higher odds of HIV testing than those who were unemployed. Again, the likelihood of HIV uptake increased significantly with birth order. For instance, birth order 2 was associated with 5.4 times higher odds of HIV testing than birth order 0. Furthermore, in-

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Table 3: Distribution of HIV Testing by Enabling and Need Factors.

Variable	Proportion ever been tested for HIV by enabling and need factors						
	Males			Females			
	Weighted %	N	*p*-value	Weighted %	N	*p*-value	All cases
Wealth *			<0.05			<0.05	
Poorest	20.7	2513		38.1	3227		5740
Poorer	21.3	2638		39.6	3143		5781
Middle	24.3	2712		42.9	3165		5877
Richer	30.1	3475		48.6	3925		7400
Richest	39.1	3249		46.9	4335		7584
Media exposure*			<0.05			<0.05	
Low	24.3	9446		44.1	12396		22742
Medium	37.8	3245		48.2	2662		5907
High	28.7	1877		34.4	1804		3681
HIV awareness*			<0.05			<0.05	
Low	19.2	661		23.8	1117		1778
Medium	33.8	7694		51.6	10174		17868
High	21.6	6232		34.7	6504		12736
Observed stigma*			<0.05			<0.05	
No	16.5	5148		47.3	14782		19930
Yes	22.4	9439		30.6	3013		12452
Disclosure concern*			<0.05			<0.05	
No	28.7	8226		44.3	9982		18208
Yes	26.9	6361		42.9	7813		14174
Need/risk perception factors							
History of STI*			<0.05			<0.05	
No	27.7	14204		43.3	17248		31452
Yes	35.2	383		57.2	547		930
Condom use*			<0.05			<0.05	
No	36.0	12057		42.1	16374		28431
Yes	37.1	2530		62.4	1421		3951
Multiple sexual partners*			<0.05			<0.05	
No	31.2	7501		47.4	11698		19199
Yes	24.5	6576		36.7	5931		12507
Know someone died of HIV*			<0.05			<0.05	
No	29.9	11089		47.3	14313		25402
Yes	21.7	3490		30.7	3261		6751

*Statistical significance at 5% level $p<0.05$.

dividuals with secondary or higher educational attainment were found to be 1.8 and 3.6 times more likely to have been tested for HIV compared with those with primary or no educational attainment. Compared with empty model 0, the inclusion of individual level variables in Model 1 increased the intra-community correlation to 6.8%, with approximately 21.4% of proportional change in variance unexplained.

Model 2 controlled for the enabling, perceived and need factors. The results showed that, although slightly reduced, the background characteristic factors remained significantly associated with HIV testing when enabling, perceived and need factors were controlled for. However, controlling for model 2 factors substantially explained the effect of place of residence such that place of residence was no longer significantly associated with HIV testing for females. Enabling factors such as wealth, media exposure and HIV awareness were found to be associated with HIV testing for females. Being wealthier was associated with increased odds of HIV testing. For instance, females in the richest quintile households were 1.3 times more likely to test for HIV

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Table 4: Average Odds of HIV Testing from Multilevel Logistic Regression Models (95% Confidence Intervals are given in Square Brackets)-Females.

Parameters	Model 1	Model 2	Model 3
Survey (2005-6)			
2010-11	5.39 [4.83-6.02]*	6.89 [6.08-7.79]*	6.91 [6.11-7.82]*
Residence (urban)			
Rural	0.74 [0.67-0.83]*	0.96 [0.82-1.11]	1.08 [0.91-1.29]
Age groups (15-24)			
25-29	1.02 [0.91-1.14]	0.93 [0.83-1.05]	0.93 [0.3-1.05]
30-34	0.69 [0.61-0.79]*	0.62 [0.54-0.71]*	0.62 [0.54-0.71]*
35-39	0.51 [0.44-0.59]*	0.46 [0.39-0.53]*	0.46 [0.39-0.53]*
40+	0.35 [0.30-0.40]*	0.31 [0.27-0.36]*	0.31 [0.26-0.36]*
Marital status (never married)			
Married	2.12 [1.85-2.43]*	2.23 [1.94-2.57]*	2.23 [1.94-2.57]*
Widowed	2.43 [2.00-2.95]*	2.40 [1.97-2.92]*	2.39 [1.97-2.91]*
Divorced	1.76 [1.47-2.11]*	1.71 [1.45-2.10]*	1.74 [1.45-2.09]*
Occupation(unemployed)			
Professional	1.41 [1.24-1.63]*	1.34 [1.17-1.54]*	1.34 [1.17-1.54]*
Manual work	1.41 [1.17-1.70]*	1.36 [1.12-1.64]*	1.34 [1.11-1.63]*
Agriculture	1.03 [0.88-1.19]	0.98 [0.84-1.15]	0.98 [0.84-1.14]
Religion (Catholic)			
Protestant	1.10 [0.99-1.22]	1.05 [0.94-1.17]	1.04 [0.94-1.17]
Pentecostal	1.08 [0.97-1.21]	1.05 [0.94-1.17]	1.04 [0.93-1.17]
Apostolic	0.93 [0.85-1.02]	0.96 [0.87-1.06]	0.96 [0.87-1.05]
Education (primary)			
Secondary	1.78 [1.63-1.94]*	1.56 [1.43-1.71]*	1.55 [1.42-1.70]*
Higher	3.60 [2.91-4.44]*	2.79 [2.24-3.47]*	2.78 [2.24-3.46]*
Enabling and perceived/need factors			
Wealth (poorest)			
Poorer		1.05 [0.93-1.19]	1.03 [0.91-1.17]
Middle		1.20 [1.06-1.37]*	1.14 [0.99-1.31]
Richer		1.24 [1.07-1.45]*	1.15 [0.97-1.36]
Richest		1.27 [1.05-1.53]*	1.16 [0.95-1.42]
Media exposure (low)			
Medium		1.21 [1.09-1.35]*	1.21 [1.09-1.34]*
High		1.67 [1.42-1.97]*	1.67 [1.42-1.97]*
HIV/AIDS Awareness(low)			
Medium		1.59 [1.31-1.93]*	1.59 1.31-1.93]*
High		2.04 [1.72-2.43]*	2.04 [1.71-2.43]*
Observed AIDS stigma(no)			
Yes		1.07 [0.90-1.27]	1.06 [0.90-1.26]
HIV disclosure concern(no)			
Yes		0.82 [0.74-0.90]*	0.81 [0.74-0.89]*
Knows someone died with Aids(no)			
Yes		1.23 [1.08-1.41]*	1.23 [1.08-1.41]*
History of STI (no)			
Yes		1.55 [1.27-1.89]*	1.55 [1.27-1.90]*
Community factors			
Poverty – gm			0.73 [0.56-0.94]*
Random Variance (SE)	0.239 (0.025)*	0.22 (0.024)*	0.218 (0.024)*
(VPC)=ICC(%)	6.8	6.3	6.2

VPC=Variance Partition Coefficient, ICC=intra-cluster correlation, *Statistical significance at 5% level *p*<0.05.

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than those from the poorest households. Similarly, those with higher levels of media exposure and HIV awareness had higher odds of HIV testing compared with their counterparts with lower levels of media exposure or HIV AIDS awareness. The results relating to perceived and need factors suggest that having disclosure concerns was associated with reduced odds of being tested for HIV by 18% compared with females with no disclosure concerns. On the other hand, having a history of STIs and knowing someone who died of HIV was associated with increased odds of HIV testing. The results provide no evidence of a relationship between HIV testing and observed stigma among females.

In the final model (Model 3), poverty in the community was the only community-level variable found to be significantly associated with HIV testing for females. Living in a community with a high proportion of individuals living in poverty was associated with reduced odds of HIV testing.The estimates for household wealth diminished after we controlled for community poverty such that household wealth was no longer significantly associated with HIV testing.

The results of the variance components model (i.e the empty model) and other models in Table 4 suggest that most of the variation in HIV testing for females was at the individual level. However, there were also some variation at the community level as indicated by the significant random variance in reporting of ever being tested for HIV across communities. As shown by the variance partition coefficient (VPC), the ICC was estimated at about 6-7%, even after controlling for individual and community level factors in Table 4. Thus, about 6% of the total unexplained variation in HIV testing could be attributed to unobserved community-level effects with the remaining 94% of unexplained variation attributable to individual–level factors.

The Determinants of HIV testing among males

The results for males (Table 5) slightly differed from those for females. As with females, the odds of HIV testing for males were higher in 2010/2011 than in 2005/2006, but the odds of HIV testing for males were lower by 3.03 points compared with the odds for females between the same periods (OR=5.60 females *versus* OR=2.57 males). This suggests that the increase in uptake of HIV testing was greater for women than men. In comparison with female's estimates, some results for males also differ when we controlled for background characteristics. It is noticeable that males residing in rural areas were about 1.2 times more likely than those from urban areas to have ever been tested for HIV. This finding differs with that of female which suggested that rural residence was associated with lower odds of HIV testing than urban residence. Results also revealed that being older is associated with lower odds of HIV testing for females, but not for males. Being married was associated with reduced odds of HIV testing by 32% for males, while being widowed was associated with increased odds by 47% compared to being never married. Unlike females, being a manual worker or working in agricultural sector was associated with increased odds of HIV testing for males.

Also differing from females, the odds of being tested for HIV was 21% higher for individuals belonging to Pentecostal church and 16% lower for those belonging to Apostolic faith, compared with those from Roman catholic church for males, but not for females. Education was found to be positively associated with HIV testing, with those with higher educational attainment exhibiting increased odds of HIV testing for both males and females.

Model 2 controlled for enabling, perceived and need factors. Comparing female and male, both wealth and media exposure were found to be associated with HIV testing. Like for females, being wealthier and having exposure to media were associated with increased odds of HIV testing for males. Similarly, males exposed to media messages were more likely than their counterparts with less media exposure to have tested for HIV. Among perceived and need factors that were associated with HIV testing were condom use, knowing someone who died of HIV and history of STI. The results suggest that those who did use condoms in their last sex encounter were 1.6 times more likely to have been tested for HIV than those who did not use condoms. Meanwhile, those who reported knowing someone who died of AIDS and those with a history of STI were 1.3 and 1.7 times more likely to test for HIV than their counterparts who did not know of anyone who had died of AIDS or had no history of STI. Circumcised males were about 1.3 times more likely to have been tested than those not circumcised. In the final model 3, the results showed that living in a community with a high proportion of individuals with stigma was associated with reduced odds of HIV testing. Other community level factors such as poverty and media exposure were not associated with HIV testing for males. Controlling for community-level stigma in Model 3 considerably reduced the estimates for place of residence such that rural residence was no longer significant for males.

The results for different models in Table 5 showed that most of the variation in HIV testing for males was at the individual level, but there were also some variation at community level as indicated by significant community-level random variance. The VPC (i.e., the ICC was estimated at about 2.5%, which was the proportion of total unexplained variability in HIV testing that could be attributed to unobserved community level effects. This implies that most (i.e., about 97.5%) of the unexplained variation in HIV testing was attributable to unobserved individual-level factors.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Key Findings

The main objectives of this study were to identity individual and community-level factors associated with HIV testing in Zimbabwe, and establish gender disparities. Overall the results show

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Table 5: Average Odds of HIV Testing from Multilevel Logistic Regression Models (95% Confidence Intervals are given in Square Brackets)-Males.

Parameters	**Model 1**	**Model 2**	**Model 3**
Survey (2005-6)			
2010-11	2.57 [2.28-2.91]*	2.92 [2.53-3.37]*	3.04 [2.63-3.53]*
Residence (urban)			
Rural	1.16 [1.01-1.33]*	1.17 [1.02-1.34]*	1.13 [0.99-1.30]
Age groups (15-24)			
25-29	0.30 [0.24-0.38]*	0.37 [0.29-0.48]*	0.37 [0.29-0.48]*
30-34	1.04 [0.85-1.27]	1.04 [0.85-1.27]	1.04 [0.85-1.27]
35-39	0.93 [0.76-1.14]	0.93 [0.76-1.14]	0.93 [0.76-1.14]
40+	0.94 [0.76-1.15]	0.94 [0.76-1.16]	0.94 [0.76-1.15]
Marital status (never married)			
Married	0.68 [0.59-0.77]*	0.68 [0.59-0.77]*	0.57 [0.49-0.68]*
Widowed	1.47 [1.06-2.03]*	1.47 [1.06-2.03]*	1.23 [0.87-1.74]
Divorced	1.00 [0.82-1.22]	1.00 [0.82-1.22]	0.77 [0.62-0.96]*
Occupation(unemployed)			
Professional	1.18 [1.00-1.39]	1.17 [0.98-1.38]	1.16 [0.98-1.38]
Manual work	1.37 [1.20-1.58]*	1.35 [1.17-1.55]*	1.35 [1.18-1.55]*
Agriculture	1.20 [1.03-1.39]*	1.18 [1.02-1.37]*	1.20 [1.03-1.40]*
Religion (Catholic)			
Protestant	1.21 [1.05-1.40]*	1.25 [1.09-1.45]*	1.26 [1.09-1.45]*
Pentecostal	0.96 [0.84-1.09]	0.99 [0.87-1.12]	0.99 [0.87-1.13]
Apostolic	0.84 [0.74-0.95]*	0.85 [0.75-0.96]*	0.85 [0.75-0.96]*
Education (primary)			
Secondary	1.89 [1.29-2.76]*	1.82 [1.24-2.66]*	1.88 [1.28-2.75]*
Higher	3.03 [2.01-4.57]*	2.90 [1.92-4.37]*	3.00 [1.98-4.54]*
Enabling and perceived/need factors			
Wealth (poorest)			
Poorer		1.01 [0.88-1.18]	1.02 [0.88-1.19]
Middle		1.16 [1.01-1.35]*	1.18 [1.02-1.36]*
Richer		1.25 [1.06-1.47]*	1.26 [1.07-1.49]*
Richest		1.62 [1.34-1.96]*	1.65 [1.36-2.00]*
Media exposure (low)			
Medium		1.12 [1.01-1.25]*	1.12 [1.01-1.25]*
High		1.53 [1.35-1.73]*	1.54 [1.36-1.74]*
Condom used (no)			
Yes		1.63 [1.43-1.86]*	1.63 [1043-1.86]*
Knows someone died of Aids(no)			
Yes		1.26 [1.11-1.43]*	1.26 [1.11-1.44]*
History of STI (no)			
Yes		1.46 [1.15-1.85]*	1.45 [1.14-1.84]*
Community factors			
Stigma –gm			0.55 [0.34-0.87]*
Random effects			
Variance (SE)	0.082 (0.019)*	0.084 (0.019)*	0.083 (0.019)*
(VPC)=ICC(%)	2.4	2.5	2.5

VPC=Variance Partition Coefficient; ICC=intra-cluster correlation; *Statistical significance at 5% level $p<0.05$.

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that, HIV testing is improving as indicated by higher odds in 2010/2011 than in 2005/2006 surveys. This finding applies to both males and females, but the main difference lies in the degree females were more influenced to test for HIV in the 2010/2011 survey. This may be that most of females have been responding to calls for HIV testing during maternal health care services than males. Although, males have not been a focal target for HIV testing during antenatal care, recent initiatives have encouraged men to accompany their partners to health facility for HIV testing during pregnancy.

The two objectives were addressed by applying multilevel logistic regression models to separate samples by gender to identify and compare individual and community-level determinants of HIV testing among women and men. Measures of individual-level predisposing factors (i.e gender, age, educational attainment, marital status, household wealth, media exposure) showed significant associations with HIV testing. As expected, higher odds of having been tested for HIV were observed among females than males. Several studies conducted outside Zimbabwe, reported similar results to those found here with reference to differences in HIV testing between sexes. For example, a study conducted by Weiser et al[14] in Botswana and Mitchel et al[7] in ten Southern African countries are comparable to the results found here, showing a significantly higher uptake of HIV testing for females than males. This may be explained as follows: First, it is possible that women with little control over their sexual activities of their partners and more vulnerable to infection by their partners, may have perceived themselves to be at a high risk of HIV infection and consequently this may have acted as a cue for HIV testing.[31] The second explanation could be the fact that females are more likely to be exposed than man to health care services through maternal health care.[16,30,43] As such, the disparity may have been as a result of success of the increased testing uptake during antenatal care.

The person's age was found to be a significant predictor of health care service utilisation.The effect of age on HIV testing behaviour observed was consistent with other studies; although the study by Weiser[14] suggested that coverage of HIV testing begins to increase after 40 years. This study found lower odds of HIV testing among individuals over 30 years for females and those between the ages of 25-29 for males. This could be that older adults may have already experienced feeling of isolation due to illness or loss of someone they know who died of HIV.[35] Having an HIV diagnosis may increase that sense of isolation. Older people may also face unique issues such as being widowed or divorced and are dating again, but they may have less knowledge of HIV and are less likely to protect themselves than younger people. Although studies have shown that older people visit health care services more frequently than younger people, they are less likely than younger people to discuss their sexual health with the doctors, who in turn may be less likely to ask older people about these issues. Older women who are less worried about getting pregnant may have less of a perceived need for HIV testing since they will not pass HIV to unborn babies.[44] We did not find evidence of lower uptake of HIV testing among males over 30 years of age. Further, studies are needed to examine why being older for males was not associated with HIV testing.

We found that being married, divorced and widowed were associated with higher odds of HIV testing than being never married for females, but not for males. The finding that these females are more likely to test for HIV than those who never married has also been noted in previous studies.[10,14,16] This pattern is likely to relate to the fact that never married females perceive themselves to be at lower risk of HIV; thus see no need to test for HIV.[23,45] It may also be that those married or divorced may have had an opportunity for HIV testing during marriage and as partner involvement during ANC visit.[46] This study did not come to the same conclusion with other studies in regards to HIV testing for males based on marital status.[8,16] The study found that married and divorced males were less likely to have been tested compared to those who were never married. This could indicate that married and divorced males may be relying on proxy testing if their partners were ever tested for HIV during antenatal care.[47]

Several studies have found significantly higher odds of HIV testing among individuals with higher educational attainment than those with primary education.[8,14,16] The results presented here further confirm that the odds of having been tested for HIV were significantly higher among individuals with secondary or higher educational attainment. This finding applies to both males and females, but the main difference lies in the degree to which males were influenced by education. Disparities in HIV testing by education may be caused by differences in awareness of the importance of testing, access to testing centres and riskier sexual behaviour.[21] People with higher education may have better access to health care services generally, and particularly more uptake of antenatal care among females with higher education.[48]

Wealth may make it easy to afford payment of transport to go to testing centres consultation fees since health care services are paid for in Zimbabwe.[49] The results observed here show increased odds of having been tested for HIV among the wealthier than the poorer for males, and no association between wealth and HIV testing for females after controlling for community wealth/poverty. This finding is consistent with previous studies from similar settings in Sub-Saharan African countries[12,16] which found level of HIV testing increasing with wealth for males. Several hypotheses may explain this outcome. Firstly, HIV prevalence has been found to be positively correlated with socio-economic status in many sub-Saharan African countries.[50] Thus, individuals (particularly males) who are wealthy may perceive themselves more at risk, and therefore have a higher recourse to HIV testing compared to the poor.[16] Another explanation could be that HIV testing promotion and programmes are failing to reach the most deprived populations.[23] Thirdly, the adverse living conditions associated with low socio-economic status may itself constitute a barrier to access to HIV testing,[5]

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and that the wealthier may have the opportunity to access health service easily and to choose a health service which they are comfortable with. The fact that household wealth is more important for males, while community wealth is more important for females may suggest that most of males are economically empowered and have more resources enabling them to access healthcare services than females, while females rely on public health care which is cheaper or free in Zimbabwe.

Exposure to messages about HIV testing and counselling or campaigns about condom use or abstinence can be an important factor in motivating individuals to adopt HIV testing.[14] Several studies have found positive associations between HIV testing and higher media exposure.[7,10,11] This study found similar results and suggests that being exposed to media (i.e listening to radio daily, watching and reading newspapers frequently) increased the odds of having been tested for HIV for both males and females. This might reflect the fact that individuals who are exposed to mass media may have the opportunity to learn the benefits of HIV testing, where and how HIV counselling and testing is given, and this subsequently improve their awareness towards HIV related knowledge and stigma.

Females who were exposed to HIV stigma and HIV awareness were also more likely to have been tested than those who were not. The possible reason could be those who had better knowledge or comprehensive knowledge about HIV/AIDS are more likely to know how HIV/AIDS is transmitted, the prevention mechanism and the benefit of HIV testing.[51] The positive association between exposures to stigma could be as a result of success of HIV awareness programs aimed at reducing stigma in the communities. There is no evidence suggesting an association between HIV testing and HIV stigma and awareness for males.

The association between most of the sexual behaviour factors and HIV testing conform to what might be expected. For both males and females, the odds of having been tested for HIV were higher among individuals, who used condom during their last sexual contact, and those who had multiple sex partners. Several studies have found that people are more likely to be tested if they have risky sexual behaviours, such as having multiple sex partners and inconsistent condom use.[20,48,50] In regards to HIV knowledge and awareness, the highest estimates for HIV testing coverage are observed among individuals with medium to higher HIV/AIDS awareness, echoing the results found in the previous studies.[7,13]

At community level, community poverty, HIV-related stigma and media exposure were found to be associated with HIV testing. Community level risky sexual behaviour and HIV awareness were not associated with HIV testing, consistent with findings from previous studies.[12,18,25] In the general population, it was living in the communities with higher proportions of individuals with HIV-related stigma and media exposure that were associated with HIV testing. Living in a community with higher levels of media exposure was positively associated with HIV testing. Meanwhile, the results suggest that living in communities with proportion of individuals with higher HIV-related stigma was negatively associated with HIV testing in the general population. Similarly, the results showed that males living in communities with higher levels of stigmatisation were less likely to have reported ever testing for HIV. An explanation of this behaviour could be that men may be reluctant to know their HIV status if they feel that doing so they become defined in the community as ill or weak, or feel that they can be denied opportunities such as employment.[34] Living in a community with high levels of poverty was associated with reduced HIV testing for females, but not for males. Overall, there is no evidence of an association between HIV testing and other community level factors (i.e community education and risky sexual behaviour).

Finally, the analysis focused on ascertaining the intra-community correlation to obtain a clear picture of between-community variation in HIV testing. The study found significant community variations in HIV testing, partly attributed to the individual level factors. As shown in tables 4 and 5, the random part showed that about 6-7% of the total variation in HIV testing among females (and 2-3% for males) in Zimbabwe was attributed to differences across the communities. The results were fairly consistent with the literature where the proportion of total variation in HIV testing attributed to community level factors ranged from 4% to 15%.[8,12] As in other countries, the community level variation obtained supports the theory that some differences in health outcomes are attributed to the community characteristics in which the individuals live.[8,30] The study sheds light beyond the contribution of individual characteristics to the determinants of HIV testing in Zimbabwe.

STUDY LIMITATIONS

The data used in this study came from two nationally representative surveys with household and individual response rates of 98% and 97% respectively.[2] The missing data was lower than 5%, thus not likely to have affected the estimates in the study. However, the data had some weakness which might have affected the results. Firstly, the study did not control for the availability of HIV health care services at community level. The ZDHS does not provide data to distinguish whether the number of people having an HIV test is limited by the availability of testing services or whether the testing services were underutilised and why. There is a possibility that inclusion of factors regarding health care services would have helped to explain some of the variance in the model. While knowing one's own HIV status is a proxy for person's having received counselling, the indicator does not provide the quality of the counselling services. In countries like Zimbabwe, where scaling up of HIV testing services is happening; population-based surveys conducted every few years will not capture annual progress. The information in the survey was self-reported, so to some extent under-reporting of socially unacceptable behaviours and attitudes (such as stigma) and over reporting of socially desirable behaviours were likely. In Zimbabwe, HIV testing has been heavily promoted as a responsible thing to do, so it is

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possible that some people may say they have been tested when in fact they were not. Another limitation was that the HIV testing and risk measures did not include assessments of time since the behaviours occurred, not allowing this study to examine whether people who were recently tested or who recently engaged in risk sexual behaviour differed from practising these behaviours less or more recently. Although, variables for females domain such as 'problems of getting money, permission and transport to go to a health care centre are related to HIV testing behaviour, it would be a mistake to use them to make a general claim about their relative importance using variables available in ZDHS because most of these questions were not completed by the respondents. Otherwise the representation of the whole county is a major strength of this study as it allows generalization of findings for the country as a whole, for both genders.

CONCLUSION AND WAY FORWARD

Overall the multilevel results showed little variations in HIV testing across communities in Zimbabwe. The variations were mainly explained by individual level variables such as background characteristics rather than enabling, perceived need factors that were included in the models for both sexes. At community level, variables found to be contributing to the variations in HIV testing differed by gender. It was found that community poverty was negatively associated with HIV testing for females, while community stigma was important for males. Controlling for community level factors did not have much effect on community variation in HIV testing. Therefore, policies should focus on predisposing and enabling factors in order to improve HIV testing in Zimbabwe. For males' household wealth and HIV factors such as risky sexual behaviour are critical for improvements in HIV testing. Given that household wealth was a significant predictor of HIV testing for males, the introduction of financial incentives may stimulate males to access HIV testing services by providing compensation for transport cost and opportunity cost of time associated with accessing HIV testing services. Monetary incentives could reduce barriers and stigma as it may provide a broader reason for going to testing centres. More importantly perhaps for both sexes, awareness creation on HIV counselling and testing service utilization should focus on avoiding stigmatizing and discriminatory behaviours, so that clients can develop positive attitudes towards people living with HIV/AIDS. Introducing couple-oriented HIV counselling and testing services where men accompany their spouse to HIV screening during pregnancy may help increase the HIV testing uptake for males.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Annexure

Annexure: Average Odds of HIV Testing from Multilevel Logistic Regression Models (95% Confidence Intervals are given in Square Brackets) Males and Females.

Parameters	Model 1	Model 2	Model 3
Survey (2005-6)			
2010-11	4.16 [3.80-4.55]*	5.30 [4.67-6.02]*	5.60 [4.90-6.37]*
Gender (males)			
Females	1.98 [1.85-2.13]*	1.84 [1.63-2.07]*	1.83 [1.63-2.07]*
Residence (urban)			
Rural	0.79 [0.72-0.86]*	1.12 [1.00-1.26]*	1.18 [1.04-1.34]*
Age groups (15-24)			
25-29	1.30 [1.17-1.43]*	1.20 [1.08-1.33]*	1.20 [1.08-1.33]*
30-34	0.90 [0.80-1.01]	0.83 [0.74-0.94]*	0.83 [0.74-0.94]*
35-39	0.75 [0.66-0.86]*	0.69 [0.61-0.79]*	0.69 [0.61-0.79]*
40+	0.60 [0.53-0.69]*	0.55 [0.49-0.63]*	0.55 [0.49-0.63]*
Marital status (never married)			
Married	2.01 [1.78-2.26]*	2.30 [2.03-2.60]*	2.30 [2.03-2.59]*
Widowed	2.39 [1.98-2.88]*	2.50 [2.07-3.03]*	2.51 [2.07-3.04]*
Divorced	1.83 [1.56-2.16]*	1.80 [1.52-2.13]*	1.80 [1.52-2.13]*
Occupation(unemployed)			
Professional	1.31 [1.18-1.46]*	1.21 [1.08-1.35]*	1.21 [1.09-1.35]*
Manual work	1.28 [1.15-1.42]*	1.20 [1.08-1.34]*	1.21 [1.08-1.34]*
Agriculture	1.03 [0.93-1.14]	1.00 [0.91-1.11]	1.01 [0.91-1.11]
Religion (Catholic)			
Protestant	1.08 [0.98-1.18]	1.04 [0.95-1.15]	1.05 [0.96-1.15]
Pentecostal	1.18 [1.08-1.29]*	1.17 [1.06-1.28]*	1.17 [1.06-1.29]*
Apostolic	0.98 [0.90-1.05]	1.01 [0.93-1.09]	1.01 [0.93-1.09]
Secondary	1.67 [1.55-1.80]*	1.49 [1.38-1.61]*	1.50 [1.39-1.62]*
Higher	3.62 [3.04-4.31]*	2.82 [2.36-3.38]*	2.84 [2.37-3.40]*
Enabling and perceived/need factors			
Wealth (poorest)			
Poorer		1.00 [0.90-1.11]	1.01 [0.90-1.12]
Middle		1.12 [1.01-1.25]*	1.13 [1.01-1.26]*
Richer		1.25 [1.10-1.42]*	1.24 [1.09-1.41]*
Richest		1.40 [1.20-1.62]*	1.38 [1.18-1.60]*
Media exposure (low)			
Medium		1.22 [1.12-1.34]*	1.21 [1.10-1.32]*
High		1.62 [1.36-1.92]*	1.52 [1.28-1.82]*
HIV/AIDS Awareness(low)			
Medium		1.75 [1.51-20.2]*	1.76 [1.52-2.03]*
High		1.85 [1.58-2.16]*	1.86 [1.59-2.17]*
Observed AIDS stigma(no)			
Yes		0.81 [0.73-0.91]*	0.81 [0.73-0.91]*
HIV disclosure concern(no)			
Yes		0.83 [0.76-0.91]*	0.83 [0.76-0.91]*
Knows someone died with Aids(no)			
Yes		1.30 [1.14-1.48]*	1.29 [1.13-1.48]*
History of STI (no)			
Yes		1.66 [1.38-2.00]*	1.66 [1.38-1.99]*
Community factors			
HIV/AIDS stigma-gm			0.62 [0.42-0.92]*
Media exposure-gm			1.47 [1.14-1.89]*
Random Variance (SE)	0.129 (0.016)*	0.112 (0.015)*	0.11 (0.015)*
(VPC)=ICC(%)	3.8	3.3	3.3

VPC=Variance Partition Coefficient, ICC=intra-cluster correlation, *Statistical significance at 5% level *p*<0.05.

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*Corresponding author
David Mc Conalogue, MPH
Consultant in Public Health
Gloucestershire County Council
Shire Hall, Westgate Street
Gloucester, GL1 2TG, UK
Tel. 01452 324249
E-mail: dmcconalogue@nhs.net

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Implementation of a Non-Communicable Disease (NCD) Screening Programme in a Rural African HIV Clinic

David Mc Conalogue, MPH[1*]; Bongekile Nxumalo, BSc[2]; Fred Busawala, BSc[2]; Ashley Sharp, MPH[1]; John Walley, PhD[3]

[1]*Health Education North West, Mersey Deanery, Liverpool L3 4BL, UK*
[2]*Good Shepherd Hospital, Siteki, Swaziland*
[3]*Nuffield Centre for International Health and Development, Leeds Institute of Health Sciences, University of Leeds, Leeds, UK*

ABSTRACT

Objectives: The objective of this study is to investigate the feasibility and outcomes of an integrated screening programme for risk factors associated with diabetes and hypertension in a busy HIV treatment clinic in rural Swaziland.
Methods: The screening programme identifies patients with risk factors for hypertension and diabetes mellitus (DM). Patients with one or more risk factor also had their blood glucose (BG) tested (random or fasting). High readings for BP or BG were referred for follow-up diagnostic tests at their local community health facility.
Results: Four hundred (6.9%) of 5,821 patients screened positive for at least one risk factor, of which most common was high body mass index (BMI) (5.5%), followed by high BP (3.2%), and relative with diabetes (0.7%). Three point six percent of patients with a risk factor had high BG, and a further 10% had a reading indicative of pre-diabetes. There were problems with patient's attendance and information flow to/from the community facilities. Only 3.7% of patients with high BP, and 23% of patients with high BG, were known to have had full follow-up diagnostic tests. Only one patient was confirmed to have DM, and six patients were confirmed to be hypertensive.
Conclusions: This programme suggests it is feasible to integrate non-communicable diseases (NCD) screening programmes in low-resource sub-Saharan African HIV treatment services. However the known yield was low, and there were challenges to ensure follow-up diagnosis in the health centres. There is a need to do the confirmatory second test prior to a referral to community facilities for follow-up care. The screening of HIV clinic patients in this population may not be cost-effective, and a higher priority may be the general/outpatient population.

KEY WORDS: HIV; Diabetes; Hypertension; Non-communicable disease (NCD); Screening; Africa.

ABBREVIATIONS: DM: Diabetes Mellitus; BMI: Body Mass Index; BG: Blood Glucose; NCD: Non-communicable Diseases.

Background

Non-communicable diseases (NCD) account for more than 60% of global deaths, 80% of which occur in low and middle income countries.[1] It is projected that by 2030 NCD will account for more than 50% of the mortality in low income countries, surpassing communicable diseases.[1] The emergence of non-communicable diseases in low income countries is partially the result of improved outcomes from high burden communicable diseases, such as HIV.[2,3] Large injections of international funding, and improvements in the diagnosis and management of HIV, and other high burden diseases is resulting in much longer life expectancy for those affected.[4] However, as people are now living longer, they are also going on to develop NCD co-morbidities,[5] which

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is resulting in a double burden of disease for developing countries.[6,7]

The evidence base shows that HIV treatment is associated with the development of diabetes, hypertension, and metabolic syndrome, normally related to type and length of treatment.[8-19] In the case of diabetes, this may be associated with the development of metabolic syndrome, which in turn is associated with poorer glucose control.[20,21] There is some evidence that the HIV virus itself is associated with lower prevalence rates for diabetes and hypertension.[10,11,15,22] Similarly, treatment naïve HIV+ patients may actually have a lower prevalence of hypertension.[10,11,15]

International aid programmes are becoming more successful at reaching HIV+ patients in low resource settings with effective treatment,[23] which will extend their life, and also lead to a large proportion of some populations on life-long HIV treatment. The increase in life expectancy, and the interaction between HIV treatment and the development of diabetes and hypertension, will contribute to projected large increases in NCD in the coming years.[3]

Donor funded health system development programmes have tended to focus on vertical programmes,[24] dealing with the more prevalent or highest incidence infectious diseases; principally HIV, malaria, and TB. However, there is a growing emphasis on integrated care, which recognizes that patients with HIV are also at a higher risk of being affected by NCD.[25,26] One of the key strategies to avoid the impact of NCD is investment in the technology, processes, and structures to be able to effectively identify, diagnose, and treat people at the earliest possible stage. There is currently poor investment for this in sub-Saharan African countries.[27-30] Cost-effective methods need to be tested and learned from to push this agenda forward, integrating NCD screening, diagnosis, and treatment into the pre-existing vertical structures, where appropriate. Our study is an early attempt to share learning regarding this.

This study describes the development and outcomes of a screening programme for diabetes, hypertension and cardiovascular risk in a rural hospital HIV unit in Swaziland. The challenge is to implement a practical screening protocol that is affordable and can deal with the heavy patient flows of a busy HIV unit. The aim of the study is to investigate the feasibility and outcomes of an integrated screening programme for risk factors associated with diabetes and hypertension in a busy HIV clinic in rural Swaziland with limited additional resources.

METHODOLOGY

Study Design and Setting

This study was carried out in the Lubombo region of Swaziland, in Southern Africa. Swaziland has a population of 1.1 million people, and has the highest estimated prevalence of HIV infection in the world.[31]

The study took place in a busy hospital-based HIV treatment clinic in a rural province. The clinic has nearly 6,000 registered patients, who are seen in the clinic at least once every three months. This programme aimed to screen all patients during the three month period for risk factors associated with diabetes and hypertension. The challenge for the programme was to screen the clinic population with limited extra resource, and without substantially disrupting patient clinic flows.

Primary care services are delivered to this rural population through a network of government delivered health centres, which provide a basic package of health services. Health centres are staffed by nurses and healthcare assistants. Follow-up diagnostics were referred to the patient's local health centre for completion.

In this study, high BP was defined as BP≥140/90 mmHg (systolic or diastolic). High BG was defined as either: random blood glucose (RBG) (have eaten within 8 hours before test) ≥11 mmol/L or fasting blood glucose (FBG) (have not eaten for eight hours before the test) ≥7 mmol/L, tested using portable blood glucose monitors.

There were two stages to the screening process, described below.

Stage 1: All patients attending the HIV treatment clinic have measurements taken by a nurse or healthcare assistant for:

- BP (using an automated BP machine);
- Weight and height, to calculate body mass index (BMI);
- Questioned whether they have a first degree relative who is diabetic.

A positive screen was defined as any one of the following:

- BP ≥140/90 mmHg (systolic or diastolic);
- BMI ≥25 kg/m^2;
- Have a first degree relative who is diabetic.

Stage 2: Patients who were positive went on to stage 2 screening and had the following additional information recorded:

- Manual check of BP (to confirm automated reading); and
- BG check (random or fasting) using a glucometer.

Patients fulfilling the following criteria were judged to have screened positive for diabetes mellitus and/or hypertension, and were sent for repeat testing at their health centre facility to confirm diagnosis:

- RBG ≥11 mmol/L or FBG ≥7 mmol/L;
- Manual BP ≥40/90 mmHg (systolic and/or diastolic).

Health centres were asked to take one further BG read-

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ing, preferably during the same week. If this test was RBG ≥11 mmol/L or FBG ≥7 mmol/L, the patient was confirmed as diabetic. Hypertension was confirmed in patients with two further BP readings, preferably during the same week. If the patient had two consecutive BP readings ≥140/90 mmHg (systolic or diastolic) they were confirmed as hypertensive. Patients with positive diagnostics should be referred back to the hospital-based HIV treatment clinic for management and follow-up.

Study Population

All HIV positive patients attending the Good Shepherd Hospital Antiretroviral Clinic who were 16 years or older, and did not have a previous diagnosis of diabetes or hypertension, during the period December 2014 to end of February 2015 were included in the study. All patients were asked to verbally consent to screening.

Data Collection

Data was collected for all patients who had a risk factor at stage one of the screening process. A standardised data collection tool was used, which captured patient data on: unique patient identifier; date of birth; gender; weight; height; body mass index; BP; and BG reading (specified as random or fasting). The data collection tool also captured information on risk factors: BMI≥25; family member with diabetes; an BP≥140/90. Additionally, the form captured results from follow-up diagnostic tests carried out in health centres.

Patients who screened positive for high BG and/or high BP at the 2nd stage of screening had a standardised referral proforma completed in the hospital-based HIV treatment clinic. This form captured data on follow-up tests performed at health centres.

Data was collated into an excel spreadsheet. Where information was missing from the data collection tool, the patient's case notes were consulted.

Data Analysis

Data was analysed using Microsoft Excel™. Descriptive statistics were calculated for all patients who screened positive for at least one risk factor at the first screening stage: mean age; mean weight; mean height; proportion of patients with risk factors (BMI≥25; family member with diabetes; BP≥140/90; Symptoms of diabetes; symptoms of hypertension; high BG); proportion of patients with follow-up diagnosis tests; and proportion of patients with diagnosis of hypertension or diabetes.

Ethics

This study was approved under a larger study for NCD decentralisation of care, by the Swaziland Ethics Committee, and also by University of Leeds Ethics Committee.

RESULTS

First Stage of Screening

From 1st December 2014 to 28th February 2015, 5,821 patients attended the hospital-based HIV treatment clinic. All patients had first stage screening, of which 400 (6.9%) screened positive at stage one, and had at least one risk factor (BP ≥140/90 mmHg (systolic and/or diastolic), BMI ≥25 kg/m^2, or a first degree relative who is diabetic).

Table 1: Summary Data from First and Second Stage of Screening.

Mean age (years)		44.9
Weight (Kg)		80.3
Height (cm)		162
Body mass index	Mean	30.3
	Median	30
	IQR	27-33
1st BP test	BP<140/90	51% (205)
	BP≥140/90	47% (189)
	Not recorded	2% (6)
Family member with DM		41 (10.25%)
1st BG Result	Negative	78% (312)
	Pre-DM	10% (41)
	Positive	3% (13)
	Not recorded	9% (34)

IQR: interquartile range; BP: Blood Pressure; DM: Diabetes Mellitus.

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Table 1 describes the outcomes for participants who screened positive for a risk factor at first stage of screening. BMI was the most common risk factor identified; 5.5% of those who were screened had a BMI indicative of being overweight or obese (BMI≥25 kg/m^2), which is substantially lower than the estimated prevalence of 19.7% for the national population.[32] The second most common risk factor was high BP; 3.2% of the total screened had a high BP reading (systolic or diastolic ≥140/90 mmHg). This is substantially lower than estimated prevalence of 33.2% for the national population.[32]

Only 1.2% of patients from the total patient population had a known first degree relative with diabetes.

Second Stage of Screening and Diagnosis

The second stage of screening captured further information regarding a patient's BG (random or fasting), and manually confirmed blood pressure readings. Screening and diagnosis outcomes are summarised in Figures 1 and 2 of those patients screening positive for a risk factor in the first stage of screening, 92% had BG measured using a glucometer. Eight five percent of those who were tested had a negative result. Eleven point two percent of those tested had BG levels which were suggestive of being pre-diabetic. Three point six percent of those tested had a BG level considered high. As a proportion of the total patient population (5,821), only 0.22% were identified as having a high BG level (RBG≥11 mmol/L or FBG≥7 mmol/L), and 0.7% had a reading indicative of pre-diabetic RBG ≥7.8 mmol/L).

All patients who were identified as having a high BP or BG reading were referred to their local health centre for follow-up diagnostic tests (one further BG and/or two further BP readings). Thirteen patients with a measure for high BG levels were referred to their local health centre for a follow-up diagnostic test (one further BG measurement). Only three of the 13 patients referred for a further diagnostic test at their clinic returned a result. Of the three patients, one patient was confirmed as diabetic, another patient returned a result indicative of pre-diabetes, and the remaining patient had a negative result.

One hundred eighty-nine patients had a high BP reading recorded and were sent for follow-up diagnosis testing at their local health centre. Only 31 patients (16.4%) returned at least one follow-up BP test result. The first follow-up BP test was positive for 25 of the patients who attended for a follow-up test (80.6%). However, only seven patients in total had two follow-up BP tests, which represents 3.7% of those with a high BP reading at screening. Of the seven patients who had both follow-ups, six were diagnosed with high BP.

DISCUSSION

This study shows that it is feasible, with a relatively small investment, to screen a large population of patients attending a HIV clinic in a resource-constrained part of rural sub-Saharan Africa. By developing a two stage screening process, which identified the patients at highest risk of hypertension or diabetes, a large number of patients could be reviewed at relatively little cost.

Figure 1: Screening Flow Diagram in HIV Clinic.

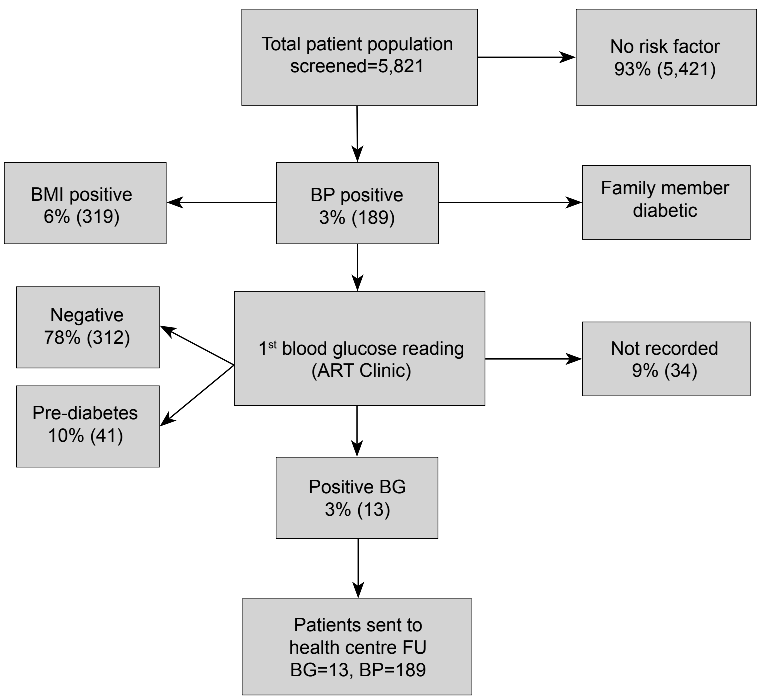

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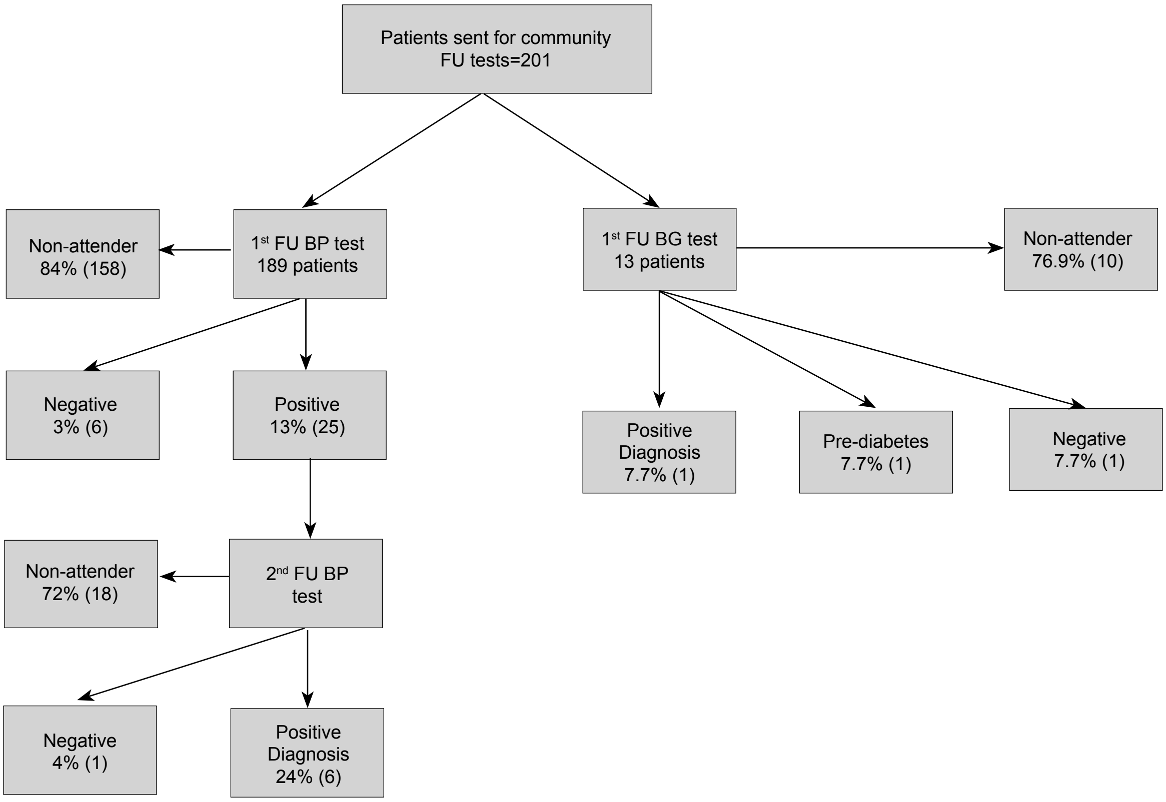
Figure 2: Health Centre Follow-up Diagnostics Flow Diagram.



This process enabled the patient clinic flows to remain largely unaffected and ensured that time and cost resources related to the administration of glucometer tests were minimised.

However, the study found relatively few people with high BG and high BP in comparison to the estimated prevalence for the country.[32] Prevalence data for Swaziland predicts high BP prevalence rates for adults as 33.2%, compared to our study rate of 3.2% (patients with at least one high BP reading). Similarly estimated prevalence of DM in Swaziland is 3.6%,[33] whereas our study identified less than 1% of the study population with high BG levels. In the case of high BG this could be a reflection of the poor sensitivity of our first stage of screening criteria; as only those with at least one risk factor went on to have a measurement taken for BG levels. It may also be a reflection of the rural location of the study population; with rural populations often experiencing substantially lower prevalence of DM than their urban counterparts.[29] However, our study suggests that prevalence estimates for high BP in Swaziland may not provide an accurate picture of this subset of the population who are receiving HIV treatment in a rural area of the country. The low rates of high BP and BG recorded could also be a reflection of the demographics of our study population, who had mean average age of less than 45-years-old. We already know that there are substantial variations in prevalence of high BP and BG within and between populations,[29,34,35] so it is reasonable to assume that there will be substantial variation across the population from which our sample is drawn.

There are also issues with the sensitivity of BG monitoring to predict diabetes,[36] although it is a cost-effective method, and appropriate for use in resource constrained environment with high patient volumes. BMI was chosen as a risk factor to predict poorer cardiovascular outcomes; however, evidence suggests that waist circumference is a stronger predictor of high blood glucose and high blood pressure.[37] In our study BMI was chosen as a pragmatic measure as clinic staff felt more confident measuring and interpreting this rather than waist circumference.

While our screening protocol was able to identify key non-communicable disease risk factors in a time and cost effective manner, it appeared to be very ineffective in delivering timely follow-up diagnostic tests. The low numbers are likely to be due to a number of factors: poor communication between health centre and hospital; poor travel infrastructure to health centres; cost of using public transport (where available) to reach health centres; and poor awareness of the potential seriousness of diabetes and hypertension as health issues.

It is possible that poor communication of the results from the health centre to the hospital-based HIV treatment service, and/or low capacity and capability in diabetes and hypertension diagnosis and management at health centres, were key factors in follow-up rates. Recently, implemented training of health centre staff in diabetes and hypertension diagnosis and treatment, increased availability of diabetic and hypertension medication at health centres, and improved support in diabetes

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and hypertension diagnosis and management, should improve this situation substantially. As mentioned, HIV unit clients found to have a high BP or glucose were expected to be sent to the community clinic for confirmatory repeat of these tests, and then be sent/come back for further case management. However, distances and travel infrastructure to health centres may still be substantial barriers for patients, who may have low-income and have relatively poor understanding regarding the significance of their risk factors to health outcomes.[38] It should also be noted that the hospital base and health centres are located in a rural region of Swaziland, with limited travel infrastructure. Moreover, the cost of using public transport, where available, can represent a substantial barrier for patients who have limited financial resource to call upon. In retrospect it would have been more effective if follow-up tests had also been performed at the hospital-based HIV treatment service as part of the patient's normal treatment package.

Adherence to HIV treatment in Swaziland is higher than the average for sub-Saharan Africa, with retention at 6 months reaching 92% and 65% at 60 months.[39] Decentralised HIV treatment in health centres has been established for several years in Swaziland. This patient group are used to obtaining regular healthcare input through health centres. In theory, targeting this group, who also have higher risk of developing non-communicable disease, for identification and management of diabetes and hypertension could be a cost-effective development. However, even where risk factors were identified, the health centres and the patients attending were not possibly not sensitised sufficiently as to the health impact of these conditions. Moreover, as discussed, there are also substantial transport and finance barriers to accessing follow-up care.

The published evidence is that there is a link between HIV and the development of non-communicable disease risk factors. This is likely to increase substantially during the coming 20 years. However, prevalence data for diabetes and hypertension is highly variable across sub-Saharan African countries, and also across in-country populations. It is important that population health risk factors are understood before implementation of screening and management services for diabetes and hypertension, as this is a key factor in approach to screening and its likely cost-effectiveness. The prevalence rates of high BG and high BP in our HIV patient population appear to be much lower than estimated prevalence for the national population, which highlights important information gaps required to support screening programme implementation decisions. Our study suggests the feasibility of integration of NCD screening programmes in low-resource sub-Saharan African HIV services. However, there were inappropriate design assumptions made and important challenges to ensure timely follow-up diagnosis.

LIMITATIONS

The use of glucometers to assess risk of poor glucose control has limitations particularly in terms of sensitivity,[36] and has been criticised as a routine screening method.[40] However, this was assessed as being the only feasible option for use in a resource constrained service, with high patient numbers, and limited laboratory testing facilities.[41-43] While this study provides valuable insights into the feasibility of the development of a screening protocol in a HIV treatment setting, it cannot tell us how sensitive or specific the screening criteria is, as patients did not undergo comparative gold standard diagnosis. Nevertheless, the risk factors were chosen based on evidence of their strength of association with common NCD. For patients who were identified as having a risk factor, or who were subsequently diagnosed with hypertension or diabetes, this study cannot inform whether these patients had improved outcomes compared to patients who were not identified. It is important that future studies look at this, to assess whether investments result in improved patient outcomes.

CONCLUSION

It is feasible to integrate NCD screening programmes into HIV care, even in this high HIV prevalence low-resource sub-Saharan African setting. However the known yield was low in part due to the expectation to attend for a confirmatory second test at a community facility and then return. The BP and glucose screening of HIV clinic needs to be piloted with the screening and confirmatory test at the HIV unit. Then the cost-effectiveness screening at the HIV unit can be assessed, and in comparison with screening in, for example, the hospital general outpatients department.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Research

*Corresponding author
Kingsley Badu, BSc, MPhil, PhD
Department of Theoretical and Applied Biology
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
E-mail: kingsbadu@gmail.com

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Intestinal Parasitemia and HIV/AIDS Co-infections at Varying CD4+ T-cell Levels

Samuel CK Tay, BSc, DVM, MSc, MPH[1]; Eric Nii Okai Aryee, BSc, MSc[2]; Kingsley Badu, BSc, MPhil, PhD[3*]

[1]*Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

[2]*Madina Polyclinic- Kekele Ghana Health Service, Madina, Accra, Ghana*

[3]*Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*

ABSTRACT

Background: Intestinal parasites, especially coccidian parasites, cause gastrointestinal symptoms such as severe diarrhoea which increases morbidity and mortality rates in people living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS), particularly in Sub-Saharan Africa. We examined the prevalence of intestinal parasites in people living with AIDS at different CD4+ T-cell levels.
Method: Case-control studies were conducted over a four month period including a total of 672 participants, between the ages of 8 and 72 years. HIV screening and confirmatory tests were done. We examined stool samples by wet mount, followed by formol-ether concentration and staining with Modified Field's and Ziehl Neelsen techniques. We also carried out fluorescence-activated cell sorting (FACS) analyses to obtain their CD4+ T-cell levels.
Results: The prevalence of intestinal parasites were significantly higher (25.2%) among HIV seropositives than HIV seronegative individuals (13.3%), (p<0.001). Coccidian parasites: *Cystoisospora belli* (formerly *Isospora belli*), Cryptosporidium and the round worm *Strongyloides stercoralis* infections were found exclusively in HIV seropositives. Cryptosporidium infections were more frequently observed in the rural cohort (p=0.039). *C. belli, Cryptosporidium*, *Giardia lamblia* and *Strongyloides stercoralis* infections were significantly higher in diarrhoeic stools. Microsporidia and *Cystoisospora belli* were found mostly in individuals with CD4+ T-cell levels of ≤200 cells/μL. Participants with CD4+ T-cell count of ≤50 cells/μL were associated with diarrhoea.
Conclusion: The prevalence of opportunistic coccidian parasites remains high in HIV-infected individuals with low CD4+ T-cell counts. Routine diagnosis is recommended to ensure comprehensive care for HIV patients.

KEY WORDS: HIV/AIDS; Intestinal parasites; *Diarrhoea,* CD4+ T-cell count.

ABBREVIATIONS: HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; NSAID: Non-Steroidal Anti-Inflammatory Drugs; ART: Antiretroviral Therapy; CI: Confidence Interval.

INTRODUCTION

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) and intestinal parasites co-infections are linked in a vicious cycle which, results in a major public health burden for developing countries.[1] Currently, about 23.5 million people, an estimated 69% of all people living with HIV and AIDS, live in sub-Saharan Africa.[2,3] Moreover, 90-92% of all pregnant women and children living with HIV are reportedly living on the continent.[3,4]

In Ghana, the HIV prevalence is relatively low, but the rising trend in the last three years is a matter of concern.[5] According to HIV sentinel survey report for 2016, the national

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prevalence is estimated to be 2.4% representing "a second consecutive upsurge from the 2014 prevalence of 1.6 % and 1.8 % in 2015".[5] The survey also found that HIV prevalence is higher in urban areas (2.5%) than rural (1.9%). New infections remained unchanged at 1.1%. The prevalence of the disease was highest among the 45-49 age groups at 5.6%, followed by 35-39 year group at 3.5% whilst 15-19 being the lowest at 0.6%.[5] The survey also found that the proportion of HIV subtype 1 is 98.5% compared to 1.5% for dual HIV type 1 and 2 infections but no subtype 2 sole infections.[5]

Although, antiretro viral therapy is available in Ghana, the coverage hardly reaches all the patients who need them. According to the Centers for Disease Control and Prevention (CDC), 66,366 adults were receiving Antiretroviral Therapy (ART) at the coverage of 62% by 2012 (http://www.cdc.gov/globalaids/global-hiv-aids-at-cdc/countries/ghana/default.html). In the 2015 UN Sustainable Development Goals, the world committed itself to stopping HIV/AIDS by 2030, it is therefore imperative that infections that aggravate disease progression, such intestinal parasites are closely investigated.

HIV/AIDS infection is associated with high prevalence of gastrointestinal infections including parasite-associated diarrhoea due to apparent dysfunctional immunity.[6-8] The progressive decline of the mucosal immunologic defense mechanisms in HIV/AIDS patients predisposes them to early, intermediary, or late gastrointestinal infections.[9] Progressive HIV infection have been characterized by an increase in Th2-like responses, which may either be a consequence or a cause of the immune deterioration.[10-15] This has been shown to be responsible for increasing host susceptibility to a myriad of intestinal opportunistic agents, such as *Cryptosporidium parvum*, *Cystoisospora belli* and *Microsporidia* species.[16] Diarrhoea affects up to 90% of HIV patients, increasing in frequency and magnitude as the disease progresses.[17,18] Helminths are known to cause T-cell dysfunction, thereby worsening the already compromised immune system.[19] Diarrhoea, as a result, has been recognized as one of the most frequent causes of morbidity and death in people living with HIV.[20] At the national level, data on intestinal parasites in people living with HIV/AIDS is virtually non-existent since there are no specific guidelines that require standard investigation and diagnosis of intestinal parasitic infections in HIV patients. The objective of the study was to determine the patterns of intestinal parasitic infections in people living with HIV/AIDS, and its association to diarrhoea at different $CD4^+$ T cell levels.

METHODS

Study Area

This study was based on case-control surveys conducted at two hospitals in the Ashanti region of Ghana which is known to have HIV prevalence of 2.6%.[5] The surveys were conducted at HIV/AIDS Voluntary Counseling and Testing centers (VCT) at the two hospitals. The Nyinahin Government Hospital was located in a rural area and St. Patrick's Hospital at Offinso was located in a peri-urban area. The surveys were carried out between April and July, 2011.

The study was conducted in two districts of the Ashanti region (forest zone) in the middle belt of Ghana; the Atwima Mponua District and the Offinso Municipality. The Atwima Mponua District is located in the western part of Ashanti Region, and lies between longitude 2°00'W and 2°32'W and latitude 6°32'N and 6°75'N. The district is within the wet-semi equatorial zone and has two peaks of rainfall in the year. About 92% of the people reside in the rural countryside, with only about 8% living within an'urban'settlement. The Offinso Municipality, on the other hand, is located in the north-western stretch of Ashanti. It is about 40 km away from the regional capital of Kumasi, and lies between longitude 1°65'W and 1°45'E and latitudes 6°45'N and 7°25'S. The district covers an area of 1255 km^2. The high population growth rate in these localities can be attributed to high immigration and the spillover population from the Kumasi metropolis giving rise to about 40% of the populace in urban dwellings. The district represents a peri-urban settlement. Nyinahini and Offinso are district capitals of the Atwima Mponua District and Offinso Municipality, Ashanti Region, Ghana, respectively.

Sample Size

This was determined based on assumptions of the binomial distribution to estimate the confidence interval (CI) of HIV prevalence at 95% with an estimated population size of less than 3000, described earlier.[21,22] $n=z^2(pq)/d^2$, where z is the critical value of standard normal distribution, p is the baseline parasite prevalence and d is the level of precision.[21,22] For 95% CI and precision level of 5%, the minimum number of participants required to achieve adequate statistical power was 323.

Inclusion and Exclusion Criteria

All patients attending the VCT clinic, who were at least one year old, regardless of their HIV status (seropositive or negative) and gender, and not on non-steroidal anti-inflammatory drugs (NSAID) were eligible to be included in the study. Informed consent was sought from all eligible patients, and participants were only recruited when consent was given.

Study Participants and Recruitment

The study took place in two hospitals, (The Nyinahin Government hospital and St. Patrick's Hospital) specifically at HIV VCT clinic.

Researchers visited the clinics twice per week until desired sample size was realized. Researchers approached all persons attending the clinic including family members for consent and participation. The study was duly explained to them and participation was purely voluntary. Informed consent mainly adult

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consent forms and parental/guardian/assent consent forms were administered.

A total of 341 HIV patients (seropositives) consented to the study and these constituted the case group. Another 331 HIV seronegative gave their consent to participate in the study; this group constituted the negative control group. Demographic data and antiretroviral drug usage information were obtained for each individual who consented to the study. Each participant was provided with sterile screw-capped containers to provide stool samples (collected in the morning) on 2 consecutive days during their scheduled visits. The stool samples were examined for ova, larvae and cyst of parasites regardless of the presence of diarrhoea. In addition, 3 mL of blood taken from participants were put in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tube for $CD4^+$ T-cell count.

HIV Testing

Screening: All consenting study participants regardless of the knowledge of their status were screened for the presence or otherwise of HIV-1 and HIV-2 or both, using the First Response HIV Card Test (PMC Ltd, Shree Indl Estate, India). Briefly, (according to manufacturer's instructions) 10 µl of serum was dropped into the sample well and 35 µl of assay diluent was added. The results were read and interpreted within 5-15 minutes. The presence of only one band in the control line in the result window indicates a negative result. However, two-color bands, one control and the other for HIV-1 indicated reactivity for antibodies to HIV-1. Two-color bands, one control and the other for HIV-2 indicated reactivity for antibodies to HIV-2. All three color bands indicated reactivity for antibodies to HIV-1 and HIV-2.

Confirmation: All seropositive individuals were re-tested with the Qualitative Immunoassay test for confirmation of their HIV status. The HIV-1/2 Oral Quick Rapid Test (OraSure Technologies, Inc., Bethlehem, PA 18015, USA) was used according to manufacturer's instructions. Briefly, the rapid test device was removed from its pouch and the padded-end was used to swab the upper and lower gums of participants. The padded-end of the rapid test device was inserted into the buffer, and the test result was read and interpreted after 20 minutes.

Stool Examination

A total of 1,344 stool samples were obtained from 672 participants. These were subjected to routine stool examination, which included saline and iodine mounts to screen for helminth ova and larvae, protozoan cyst, and trophozoites.

Direct wet mount of stool sample in normal saline (0.85% NaCl) was prepared immediately upon arrival in the laboratory and examined under light microscopy (x10 and x40 objectives) for the presence of vegetative forms, larvae, and ova of helminthes. Field's stain and Lugol's iodine staining was used to detect *Giardia lamblia* flagellates and cysts of protozoa, respectively. The formol-ether concentration technique was employed to concentrate stool samples for further confirmatory microscopic examination. Examination of fecal smears after special staining (Modified Zhiel Neelsen and Modified Field's staining techniques for the detection of *Cryptosporidium*, *Isospora belli*, *Cyclospora cayetanensis* and Microsporidia spores, respectively) was done according to Chessbrough.[23]

$CD4^+$ T-cell Count

The fluorescence-activated cell sorting (FACS) count (Becton Dickinson Immunocytometry system, Singapore) was used for the immunophenotyping of lymphocytes. Briefly, CD4 reagent tubes were vortexed and opened with the coring station for 50 µl of whole blood to be added. These were vortexed and incubated for one hour in the dark at room temperature. The tubes were uncapped and 50 µl of fixative added. The tubes were recapped and vortexed for 5 seconds upright before subjecting it to the FACS Count instrument for the immunophenotyping of lymphocytes.

Data Analysis

Statistical analysis was carried out using SPSS (2007) version 17.0. Data was summarized using frequency tables. The proportions of parasites were compared between the $CD4^+$ T-cell counts with chi-square test. The relationship between the $CD4^+$ T-cell count and the presence of diarrhoea were assessed using the chi-square analysis with significance level set at 0.05.

Ethical Considerations

The study was conducted with the approval of the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

RESULTS

Demographic Characteristics

A total of 672 patients gave their consent to participate in the study; these comprised of 341 HIV seropositives and 331 seronegatives. Overall, there were 81.5% females (n=548) 18.5% males (n=124). The percentage of male and female participants under each study group (seropositive or seronegative) was similar, and followed the same trend as that mentioned for the overall percentages (Figure 1).

When the study population was stratified into age groups, it was realized that majority of the HIV seropositives 65% (n=225) belonged to the most productive age bracket 25-45 years. Only 3.2% (n=11) belonged to the paediatric population, that is less or equal to 14 years (Figure 2).

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Figure 1: Total Number of Participants Stratified into Gender and HIV Status.

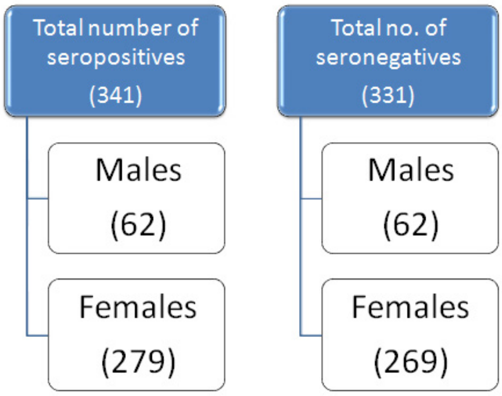

Figure 2: Age Distribution among HIV Seropositive (in Red) and HIV Seronegative in Black.

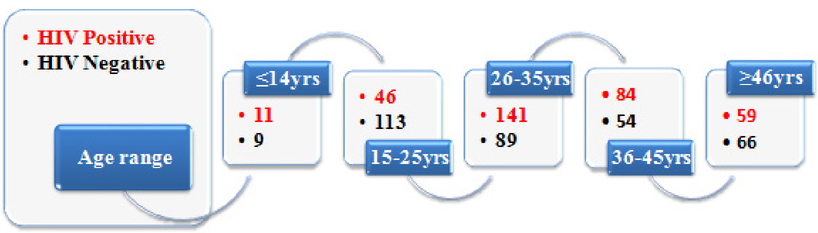

Table 1: Parasite Prevalence among HIV Seropositive and Seronegative Participant.

Parasite	HIV seropositive N=341 (%)	HIV seronegative N=331 (%)	Total (N=672)	*p*-value
Giardia lambia	39 (11.4)	39 (11.8%)	78 (11.6)	0.905
Entamoeba histolytica	4 (1.2)	0 (0)	4 (0.6)	0.124
Cystoisospora belli (formerly *Isospora belli*),	9 (2.6)	0 (0)	9 (1.3)	0.004
Cryptosporidium spp.	7 (2.1)	0 (0)	7 (1.0)	0.032
Microsporidia	1 (0.3)	0 (0)	1 (0.1)	1
Cyclospora cayetanensis	1 (0.3)	0 (0)	1 (0.1)	1
Strongyloides stercoralis	13 (3.8)	0 (0)	13 (1.9)	<0.001
Enteriobius vermicularis	1 (0.3)	1 (0.3)	2 (0.3)	1
Ascaris lumbricoides	0 (0)	1 (0.3)	1 (0.1)	0.493
Hookworm	3 (0.9)	2 (0.6)	5 (0.7)	1
G. lambia and *E. coli*	1 (0.3)	0 (0)	1 (0.1)	1
G. lambia and *E. histolytica*	4 (1.2)	1 (0.3)	5 (0.7)	0.373
C. belli and *S. stercoralis*	1 (0.3)	0 (0)	1 (0.1)	1
Microsporidia and *C. belli*	2 (0.6)	0 (0)	2 (0.3)	0.499

HIV^+=HIV Positive; HIV^-=HIV Negative; N=Number of Participants, *Coccidian parasites: *p*-value of <0.001 with HIV positive participants.

Parasite Prevalence among HIV Seropositive and Seronegative Participants

The overall prevalence of intestinal parasites among the study subjects was 19.3%. A total of 130 participants had at least one parasite. *Giardia lamblia* was the most common parasite encountered, and had similar prevalence in HIV seropositives (11.4%) and seronegatives (11.8%) (Table 1). Parasite prevalence was significantly higher in HIV seropositive participants (25.2%), than the control group (13.3%) [(χ^2=15.3, p=0.000) (Table 1)]. This confirms the susceptibility of HIV patients to opportunistic parasitic infections.

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Among the HIV seropositive cohort, 21 (6.2%) were infected with the coccidian parasites (*Cystoisospora belli*, *Cryptosporidium, Cyclospora cayetanensis*) including the fungi-like unicellular intracellular parasite Microsporidia. There were single infections as well as co infections with more than one parasite. Parasites such as *Cystoisospora belli* (2.6%), *Cryptosporidium sp* (2.1%), Microsporidia (0.3%) and *C. cayetanensis* (0.3%) as well as *S. stercoralis* were single infections exclusively found with HIV seropositives. The predominant helminths was *S. stercoralis* (3.8%) followed by Hookworm (0.9%) and *E. vermicularis* (0.3%) among seropositive participants. Again, there were co-infections with *Giardia lamblia* and *E. coli*, *Giardia lamblia* and *Entemoeba histolytica*, *C. belli* and *S. stercoralis* as well as Microsporidia and *C. belli* albeit in low prevalence. *Ascaris lumbricoides* occurred in only 1 HIV negative participant (0.3%) Table 1.

CD4⁺ T-cell Levels among Parasite and HIV Co-infected Participants

Using CD4⁺ T-cell estimate as a marker of relative risk of developing HIV related opportunistic infections,[24] we observed 29.3% of HIV seropositives were in the acute (asymptomatic) infection stage, 44.3% were in the intermediate (symptomatic) stage, and 19.7% were in the late (symptomatic) disease stage. Still 6.7% were in the most advanced HIV disease stage (Table 2). *Giardia lamblia* infections were found at all CD4⁺ T-cell levels with lower prevalence (4.3%) among the participants with the least CD4⁺ T-cell count (<50 cells/µl), but this was not statistically significant (p=0.852) (Table 2). *C. belli*, Microsporidia, and *C. cayetanensis* were also found in patients with the least CD4⁺ T-cell count. Although, *S. stercoralis* occurred exclusively among HIV seropositives (p<0.001) (Table 1), its occurrence was not significantly associated with CD4⁺ T-cell level.

CD4⁺ T-Lymphocyte Levels and Diarrhoea

Out of 341 participants belonging to the HIV seropositive group, 110 presented diarrhoeic stools; representing 32.3%. Intestinal parasites were observed in 32.7% of the diarrhoeal stools. The highest incidence of diarrhoeic stools (78.3%) was observed among participants with CD4⁺ T-cell count <50 cells/µL (Table 3). Contrary, only 2% of participants with CD4⁺ T-cell count of ≥500 cells/µL presented diarrhoeal stools.

Coccidian parasites were detected more commonly in HIV seropositives with diarrhoea than in participants with hel-

Table 2: CD4⁺ T-cell Levels among Parasite and HIV Co-Infected Participants.

	CD4⁺ T- cell count (cells/ul)				
Parasite	**>500 (N=100) (29.3%)**	**200-500 (N=151) (44.3%)**	**50-200 (N=67) (19.6%)**	**<50 (23) (6.7%)**	***p*-value**
Giardia lambia	50 (11.9%)	16 (9.9%)	11 (16.4%)	1 (4.3%)	0.852
Entamoeba histolytica	0 (0%)	3 (1.9%)	1 (1.5%)	0 (0%)	0.079
Cystoisospora *belli*	0 (0%)	0 (0%)	3 (4.5%)	6 (26.1%)	<0.001
***Cryptosporidium* spp.**	2 (0.5%)	1 (0.6%)	3 (4.5%)	1 (4.3%)	0.442
Microsporidia	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)	0.002
Cyclospora cayetanensis	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)	0.002
Hookworm	4 (1.0%)	1 (0.6%)	0 (0%)	0 (0%)	0.343
Strongyloides stercoralis	2 (0.5%)	5 (3.1%)	5 (7.5%)	1 (4.3%)	<0.001
Enteriobius vermicularis	1 (0.2%)	0 (0%)	1 (1.5%)	0 (0%)	0.423
Ascaris lumbricoides	0 (0%)	1 (0.6%)	0 (0%)	0 (0%)	0.571
G. lambia* and *E. coli	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0.503
Cystoisospora *belli* and *S. stercoralis*	0 (0%)	0 (0%)	1 (1.5%)	0 (0%)	<0.001
Microsporidia and Cystoisospora *belli*	0 (0%)	0 (0%)	0 (0%)	2 (8.7%)	<0.001
G. lambia and E. histolytica	1 (0.2%)	2 (1.2%)	2 (3.0%)	0 (0%)	0.068

HIV⁺=HIV Positive; HIV⁻=HIV Negative; N=Number of Participants; ND=Not Determined.

Table 3: Correlation between CD4⁺ T-Lymphocyte Levels and Diarrhoea.

	CD4 T⁺-cell count(cells/L)					
	>500	**200-500**	**50-200**	**<50**	**Total**	***p*-value**
Diarrhoea Stools	2 (2%)	6 (4.0%)	23 (34.3%)	18 (78.3%)	49 (14.4%)	<0.001
Non Diarrhoea Stools	98 (98%)	145 (96.0%)	44 (65.7%)	5 (21.7%)	292 (85.6%)	
Total	100 (100%)	151 (100%)	67 (100%)	23 (100%)	341 (100%)	

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Table 4: Presence of Parasites in Diarrhoeic Stools of HIV Positive and Negative Participants.

	HIV+ (N=341)			HIV- (N=331)		
Parasite	**D**	***ND***	***p*-value**	**D**	**ND**	***p*-value**
Giardia lambia	10 (40.0%)	29 (9.2%)	<0.001	18 (62.1%)	21 (7.0%)	<0.001
Entamoeba histolytica	0 (0%)	4 (1.3%)	0.736	0 (0%)	0 (0%)	nd
Cystoisospora *belli*	8 (16.3%)	1 (0.3%)	<0.001	0 (0%)	0%	nd
***Cryptosporidium* spp.**	3 (6.1%)	3 (1.0%)	0.04	0 (0%)	0 (0%)	nd
Microsporidia	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Cyclospora cayetanensis	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Hookworm	0 (0.0%)	3 (1.0%)	1	0 (0%)	2 (0.6%)	1
Strongyloides stercoralis	5 (10.2%)	8 (2.7%)	0.026	0 (0%)	0 (0%)	nd
Enteriobius vermicularis	1 (2.0%)	0 (0%)	0.14	0 (0%)	1 (0.3%)	1
Ascaris lumbricoides	0 (0%)	0 (0%)	nd	0 (0%)	1 (0.3%)	1
G. lambia* and *E. coli	2 (4.1%)	2 (0.7%)	1	0 (0%)	1 (0.3%)	1
Cystoisospora *belli* and *S.stercoralis*	1 (2.0%)	0 (0%)	0.14	0 (0%)	0 (0%)	nd
Microsporidia and Cystoisospora *belli*	2 (4.1%)	0 (0%)	0.02	0 (0%)	0 (0%)	nd
G. lambia and E. histolytica	2 (4.1%)	2 (0.7%)	0.1	0 (0%)	1 (0.3%)	1
Total diarrhoea (143) (21.3%)	36/110 (32.7%)	52/283 (18.3%)		18/33 (54.5%)	27/325 (8.3%)	

HIV+=HIV Positive; HIV-=Negative; N=Number of Participants; nd=Not Determined; D=Diarrhoea Stools; ND=Non Diarrhoeal Stools.

minths and other protozoa (Table 4). The incidence of diarrhoea among participants infected with *Giardia lamblia* only, was 40% and 62.1% in HIV seropositive and seronegatives respectively. The incidence of diarrhoea among HIV seropositives infected with *C. belli, Cryptosporidium* and *S. stercoralis* infections were 16.3%, 6.1% and 10.2%, respectively. Microsporidia, *C. cayetanensis* and *E. vermicularis* occurred with diarrhoea in at most 2% of participants.

DISCUSSION

Intestinal parasitic infections and HIV/AIDS have been the major public health problems and remain a vital cause of morbidity and mortality in developing countries. Both problems are linked in a vicious cycle.[1] The introduction of antiretroviral therapy has lessened the prevalence of gastrointestinal infections in HIV patients. This notwithstanding, several people with HIV infection still suffer from intestinal parasite infections.[1,6-8,25]

Intestinal parasitic infections found among HIV patients from low-income countries have been reported with prevalence 15% to over 80% in recent times.[1,6-8,25]

In the current study, the overall prevalence of intestinal parasite among the study population was 19.3%. However, the prevalence of intestinal parasites in the HIV seropositive group was significantly higher (25%) than that observed in the HIV seronegative group which was 13.3%. The observed prevalence in this study is similar to others carried out in Zambia which reported 25% prevalence among HIV-infected cohort.[26] Other reports from India, Ethiopia and Tanzania were comparably higher ranging from 30% and above.[27,28] However, much lower prevalence of 10.6% among HIV patients have also been reported elsewhere.[29]

It was also observed that opportunistic parasitic infections mainly the coccidian parasites occurred exclusively in HIV/AIDS patients with a corresponding depletion of CD4+ T cell count. This has been attributed to the modulation of immune response in the advance stages of the disease.[10-15] The highest prevalence of parasitosis was observed among participants in the CD4+ T-cell level ≤50 cells/μL. This category forms 56.5% of participants in the advanced stage of the disease. The most predominant parasites recovered among this group of participants belonged to the coccidian groups (47.8%), which are well known as opportunistic parasites in HIV disease. With the exception of one participant, all participants with mixed parasitic infections had CD4+ T-lymphocyte level <200 cells/μl. This observation has been echoed by other studies.[6,30]

Typically, the dynamics of HIV-1 infection is known to follow a familiar pattern where there is the acute phase in which there is massive depletion of CD4+ T cells of the gastrointestinal tract,[31] followed by the chronic phase, where there is a gradual reduction in CD4+ T cells which results in high risk of opportunistic infections, and then AIDS sets in. Recently it has been found that there is significant preferential loss of Th17 cells within the gastrointestinal tract of HIV-infected individuals[32] as a result of microbial translocation after the initial structural and immunological disruption of the gut mucosa in the acute phase.[33] *Giardia lamblia* was the most common parasite among the participants. Its occurrence, among both HIV seropositive (11.4%) and seronegative (11.8%) was similar. Previous studies have demonstrated that although infection with *Giardia lamblia*

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and HIV correlated with enteritis or enterocolitis, its incidence does not differ amongst HIV-positive and negative patient populations.[34] This underscores the non-opportunistic nature of the *G. lamblia* reviewed by Cimerman et al.[35]

The helminths observed in this study were *A. lumbricoides*, *E. vermicularis*, hookworm and *S. stercoralis*. Helminths infections generally were low among the study groups when compared to findings of similar studies elsewhere reporting prevalence of 37.04%.[36] However, *S. stercoralis* was only associated with HIV seropositive individuals and hookworm infections were higher (5%). Modjarrad et al[36], reported relatively higher prevalence of intestinal helminths (24.9%) with *A. lumbricoides* and hookworm being prevalent among HIV-1 patients in an urban African setting.[37] Apart from *S. stercoralis*, other helminths had lower prevalence in our study when compared to others carried out in similar developing countries[20]; this may be due to the widespread administration of anti-helminths and co-tri-moxazole among the study participants (56% of participants were already on ART at the time of stool collection).

Studies have shown that, reconstitution of the immune system following ART administration alone resolves *Cryptosporidium* infections without specific treatment for the parasite.[38,39] This is because ART acts against the aspartyl-protease of the parasite depriving the parasite of an essential protein.[40,41] More than 56% of participants were already on ART at the time of stool collection. It is likely that as a patient's $CD4^+$ T-cell level increases with the administration of ART, opportunistic infections are not established even if they are exposed to infection.

Diarrhoea is a life threatening complication often associated with HIV, causing severe weight loss; both conditions are independent predictors of mortality in HIV/AIDS.[42] The incidence of diarrhoea among HIV seropositives in this study was significantly high. Among the HIV cohort diarrhoea episodes increased with declining immunity, with the highest diarrhoea prevalence (78.3%) occurring at the least $CD4^+$ T-cell count <50 cells/µL. (Table 4). *Giardia lamblia*, *Cystoisospora belli*, *Cryptosporidium*, and *S. stercoralis* were associated with diarrhoeal stools of HIV seropositive patients (Table 4). Among the opportunistic coccidian parasites in HIV seropositives *Cystoisospora belli* (3.5%) was predominant followed by *Cryptosporidium* (2.1%). Microsporidia and *C. cayetanensis* had a prevalence of 0.9% and 0.3%, respectively, these occurred exclusively among HIV seropositives. All participants with *Cystoisospora belli* infections presented with diarrhoea. This strong association with diarrhoea may be associated with patients who were ART naïve and presenting themselves very late to the hospitals. They often present with wasting, general weakness and diarrhoea. *Cyclospora cayetanensis*, an emerging parasite, was found in only one participant with diarrhoea.

On the other hand, the presence of diarrhoea without parasites in stool can be quite intriguing. About 32.7% of HIV seropositives had diarrhoea even in the absence of parasites (Table 4). This can arise from bacteria etiology, lactose intolerance or insufficient sensitivity of the diagnostic procedure.[39] It has been shown however, that no etiological agent is found in 15-50% of HIV patients with chronic diarrhoea.[41] Munnink et al observed that unexplained diarrhea in HIV- infected patients were not due to novel pathogens [immunodeficiency-associated stool virus (IAS virus)][42] or previously unknown pathogens, but may be due to HIV-1 itself having a "virotoxic" effect on the enterocytes that results in intestinal mucosal abnormalities leading to diarrhoea.[40] Again some anti-retroviral agents especially the protease inhibitors have been reported to cause diarrhoea.[43] This appears to be changing the etiology of diarrhoea in some parts of the world where parasite related diarrhoea appear to be falling, while the number of unexplained and drug-induced diarrhea seems to be going up.[44] It is thus conceivable to state that the interpretation of diarrhoea associated with parasitic infections must be made cautiously.

In spite of the high prevalence (25%) of intestinal parasitosis in HIV patients, there are currently no clear guidelines that require its diagnosis. Moreover, the high burden of intestinal parasitosis results in diarrhoea and weight loss which are independent predictors of mortality in HIV patients. In order for HIV patients to obtain comprehensive healthcare, it is recommended that efforts are made towards diagnosing intestinal parasites in HIV patients especially those with $CD4^+$ T cell counts less than 50 cells/µL.[45]

COMPETING INTEREST

The authors declare no competing interest.

AUTHOR'S CONTRIBUTION

SCKT and KB conceived the study, participated in its design, supervised the field work, data analysis and drafted the manuscript; EOA conducted the field and laboratory data collection and performed the experiments. All authors read and approved the final manuscript.

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