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Correlation between nutrition status and genital shedding of HIV-1

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CORRELATION BETWEEN NUTRITION STATUS AND GENITAL SHEDDING OF HIV-1

Peter Mwiti Arimi

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirement for the Master of Science in Medicine in the field of Epidemiology and Biostatistics.

DECLARATION

I, Peter Mwiti Arimi, declare that this research report is my own work. It is being submitted for the Master of Science in Medicine in the branch of Epidemiology and Biostatistics in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university

…… [Signed]……day

of……. [Month], 2007

DEDICATION

I dedicate this report to my wife, Irene Kathambi Arimi and my children; Powell Mugambi Arimi and Dennis Mwirigi Arimi

ABSTRACT

Background:

Correlation of nutritional status with genital shedding of HIV-1 has not been described. Genital shedding patterns have not been described in Botswana previously

Methods:

I conducted a cross-sectional study to describe genital shedding patterns in Botswana and to correlate nutritional status with genital shedding of HIV-1. Between July 2005 and December 2005, samples were collected and analyzed from 50 women participating in an ongoing micronutrient supplementation clinical trial that is examining the effect of supplementation on HIV disease progression

Results:

HIV-1 RNA was isolated from both baseline and three months CVL in 24% of the study population, and these were labelled **continuous shedders (CS).** No HIV-1RNA was isolated from both baseline and three months CVL in 64% of the study population, and these were labelled non-**shedders (NS).** In 14% of women, HIV-1 RNA was either isolated from baseline CVL only (4/50) or from the three months CVL only (3/50) and these were labelled as **Intermittent Shedders (IS).**

Women who had detectable genital HIV-1 RNA at baseline had lower haemoglobin compared to those who were not shedding (Hb11.7 (95% CI 10.8 ; 12.5) vs. Hb 12.5 (95% CI 12.0 ; 13) P = 0.0877), showing a strong trend, albeit a non significant haemoglobin difference. Women who had detectable genital HIV-1-RNA at baseline had

significantly lower CD4 cell percentage compared to those not shedding (22% (95% CI 19 ; 24) vs. 30 % (95% CI 27 ; 34) $P < 0.01$) and a significantly higher log viral load (4.7 log (95% CI 4.2 ; 5.1) vs. 3.6 log; ((95% CI 3.5 ; 4.0) $P < 0.01$). Overall there was a non significant higher prevalence of genital infection in women who were shedding HIV-1 at baseline, compared to those who were not (73% vs. 46% $P = 0.123$). No HIV -1 RNA was isolated in all the19% of the women in the study who were using some form of contraception

Conclusions:

The preliminary analysis showed three patterns of HIV shedding in this study population, namely Continuous shedders, Intermittent shedders and Non-shedders. Women with detectable genital HIV-1-RNA at baseline had more advanced disease, and by extension poor nutritional status, than those not shedding, as shown by higher plasma viral load, lower CD4 count, lower haemoglobin level and higher prevalence of genital infections. This study generates hypothesis on the role haemoglobin may play in genital shedding of HIV-1 in females

Recommendation:

Due to the small sample size, these results will need to be validated by larger studies of appropriate design. Timely treatment of anaemia in HIV positive women may be important in reducing HIV transmission associated with presence of HIV-1RNA in genital secretions

ACKNOWLEDGEMENTS

I am indebted to my supervisor Dr. Saul Johnson, MD, of University of the Witwatersrand, School of Public Health for his guidance, review and approval of the protocol and write up of this report.

I am equally indebted to Dr. Adriana Campa, PhD, RD, of Florida International University and also the Dikotlana Basadi Co-Principal Investigator for availing herself as my co -supervisor

I wish to extend my sincere appreciation to Prof. Marianna K Baum of Florida International University and Prof. Richard Marlink of Harvard School of Public Health International AIDS Initiative, the Principal Investigators for allowing me to compile this report by use of secondary data from the Dikotlana Basadi pilot study.

I thank the Botswana Harvard School of Public Health AIDS Initiative Partnership (BHP) management for allowing me to use the organization's facilities – Computers, email, access to Data, and all staff who were involved in the processing, storage, shipping and analysis of samples

Sponsorship: *This material is based upon work supported by Florida International University Development Fund, which provided financial support for Dikotlana Basadi Pilot Study*

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Acronyms

AIDS	Acquired Immuno-Deficiency Syndrome
Basadi	Women
BHHRL	Botswana-Harvard HIV Reference Laboratory
BHP	Botswana-HSPH AIDS Initiative Partnership
BMI	Body Mass Index
BV	Bacterial Vaginosis
CVL	Cervico-Vaginal Lavage
Dikotlana	Micronutrient
DMC	Data Management Centre
DNA	Deoxyribonucleic acid
FIU	Florida International University
HAI	Harvard School of Public Health AIDS Initiative
HIV	Human Immunodeficiency Virus
HSPH	Harvard School of Public Health
KOH	Potassium hydroxide
PCR	Polymerase Chain Reaction
PBS	Phosphate buffer saline
PMH	Princess Marina Hospital
RNA	Ribonucleic acid
STDs	Sexually Transmitted Diseases

1.0. Introduction

1.1 Background

HIV has become the world's leading infectious cause of adult death. More than 90% of the world's current AIDS-related deaths occur in poor countries, with Sub-Saharan Africa, and in particular, Botswana among the countries hardest hit by AIDS. In 2005 there were an estimated 270,000 people living with HIV, in a country with a total population below two million, giving Botswana an adult HIV prevalence rate of 24.1%, the second highest in the world after Swaziland **[1]**

The Dikotlana Basadi pilot study, conducted by Botswana Harvard Partnership in collaboration with Florida International University, Harvard School of Public Health and Botswana Ministry of Health generated preliminary data for a larger longitudinal observation study (Dikotlana Basadi) to study the effect of micronutrient supplementation on genital shedding of HIV-1. Data from the pilot study will be presented at the 2006 AIDS Conference **[2].**

For this report, I have utilized and analyzed secondary data collected at baseline, prior to initiation of micronutrient supplementation, from 50 women participating in Dikotlana Basadi pilot study.

1.2 Problem statement:

Cervical and vaginal HIV-1 shedding is considered a measure of the capacity of an HIV-1 infected woman to transmit the virus sexually, or vertically from mother to child and has been associated with nutritional status **[4][5][6]**. Haemoglobin level, serum Albumin and Body mass index (BMI) are good measures of nutritional status [3]. Studies to correlate nutritional status with genital shedding of HIV-1 have not been done. Genital shedding patterns have not been described in Botswana previously **[4].**

1.3 Justification for the study:

A better understanding of the relationship between genital tract HIV-1 shedding and nutritional status will enable development of better strategies to help reduce sexual HIV transmission, mother to child HIV transmission and optimal care to those infected with HIV. Access to sophisticated laboratory facilities, which could support such assessments, is often limited in Sub-Saharan Africa. Identification of basic prognostic indicators of HIV transmission is essential in such resources limited settings.

1.4 Study objectives

1.4.1 Main objective:

The main objective of the study was to examine the relationship between clinical parameters of nutrition – Body Mass Index (BMI), Haemoglobin level and serum albumin level with genital shedding status. Genital shedding was defined as the presence of HIV-1 RNA in genital secretions.

1.4.2 Specific Objectives

1. To determine nutrition status, through the parameters of body mass index, haemoglobin and serum albumin in the study population
2. To determine genital shedding status in the study population
3. To describe genital shedding patterns in females
4. To determine other covariates that may have a bearing on the health status and by extension the nutritional status of the study population – CD4 count, plasma viral load, STDs, vulvovaginal candidiasis, and bacterial vaginosis.
5. To correlate nutritional status with genital shedding status

1.4.3 Hypothesis:

Poor nutritional status is correlated with genital shedding of HIV -1RNA

1.5 Literature review

Genital HIV-1 shedding is a risk factor for mother-to-child transmission of HIV-1 **[5].** It is postulated that women who shed HIV-1 in the genital tract are likely to be more infectious to their sexual partners **[6] [7].** Human immunodeficiency virus (HIV)-1 RNA burden in cervicovaginal lavage specimens is correlated to plasma HIV-1 RNA Viral load and CD4 cell count **[8]**.

Anaemia predicts a higher risk of death in low income countries, as shown in Haiti where low haemoglobin level appeared to be one of the strongest predictors of death in people starting antiretroviral therapy **[9]** and in Senegal where a study found that haemoglobin

below 10g/dl significantly predicted an increased risk of death in patients initiating antiretroviral therapy **[10]**.

Nutritional deficiencies are common in HIV-1-infected individuals **[11]** and micronutrients have been associated with disease progression in HIV infection **[12].** Serum albumin has been shown to predict outcome and predict survival **[13].** Weight loss is associated with adverse disease outcomes in HIV **[14]** and serial measurements of body mass index predicts the development of AIDS **[15] [16]**

The integrity of epithelial membranes, including mucosal immunity of the cervix and the vagina, has been associated with nutrition status **[17].** Vulvovaginal candidiasis is associated with an increased number of copies of cell-associated and cell-free HIV-1 RNA in cervicovaginal secretions **[18]** while Bacterial Vaginosis is associated with increased expression of HIV-1 RNA levels in the female genital tract **[19].** Several studies have observed increased genital HIV shedding in patients with sexually transmitted diseases (STD) **[20]**.

2.0 Materials and Methods

2.1 Study design

This is a cross-sectional study with women recruited sequentially to correlate nutritional parameters of haemoglobin, serum albumin and body mass index with genital shedding of HIV-1. CVL Specimens were collected at baseline and again at 3 months to describe

female genital tract shedding patterns in the study population. Samples from the first 50 Dikotlana Basadi women were analysed for the pilot study

2.2 Study population

2.2.1 Source of data

Between July and December 2005, 50 HIV -1 positive women were enrolled into the Dikotlana Basadi pilot study, recruited from an ongoing 5 year Dikotlana micronutrient supplementation clinical trial. The Dikotlana Basadi pilot study was carried out in order to generate some background data to be used for the design of an observational (Dikotlana Basadi) cohort to study the effect of micronutrient supplementation on genital shedding of HIV-1.

The parent protocol, the Dikotlana micronutrient supplementation study, is an ongoing double blind, randomized 2x2 factorial study where participants are randomized to receive supplements: multivitamin or selenium or combination of both or placebo. The protocol for the original study was approved by the institutional review boards of the Harvard Human Subjects Committee, Florida International University and Botswana Human Research Development Committee of Ministry of Health.

In this study I utilized baseline data from the 50 women participating in the Dikotlana Basadi pilot study. The protocol for this study was approved by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg - clearance certificate **# M050821**

2.2.2 Inclusion and exclusion criteria

Our participants consisted of healthy non symptomatic HIV-1 infected females

Inclusion Criteria - HIV-l infection confirmed by Elisa, Lack of AIDS defining conditions, CD4 > 350, BMI of equal or more than18, Age 18 or older and signature on informed consent form. **Exclusion Criteria -** Participation in another blinded clinical trial, taking antiretroviral medications and Pregnancy

2.3 Clinical assessments.

These were performed at the BHP Micronutrient Study Clinic located at the Princess Marina Hospital in Gaborone, Botswana. Baseline demographic information was obtained and blood specimens were collected for micronutrients, syphilis, haemoglobin, serum albumin, CD4 count, and viral load tests. These samples were then transported for analysis at the Botswana Harvard HIV reference laboratory, Princess Marina Hospital, Gaborone, Botswana. Body mass index was calculated by dividing the participant's weight in kilograms by the square of her height in meters, according to standard protocols **[21].**

2.4 Collection of genital specimens

Samples were collected by the following methods in the following order:

1) Vaginal swab – for Bacterial vaginosis, Trichomonas vaginalis and Vaginal candidiasis
2) Cervicovaginal lavage (CVL) – for genital HIV-1C RNA
3) Endocervical swab – For Naeseria gonorrhoea and Chlamydia trachomatis

At baseline and at 3 months, genital speculum exam was done to collect gynaecological specimens. If the participant was menstruating at the time of the visit, the visit was re-scheduled for 10-14 days later in order to avoid obtaining genital fluids contaminated with systemic blood

2.4.1 Vaginal Dacron swab

Collection of vaginal secretions was done by rolling the swab along the posterior vaginal wall, rotating it 360 degrees. Two swabs, one on the right and the other on the left posterior vaginal wall, were used. These vaginal specimens were analyzed using direct microscopy at the study clinic. 1st swab was used for wet mount analysis of Bacterial vaginosis and Trichomonas vaginalis while the second swab was used for KOH analysis of Candida albicans

2.4.2 Cervicovaginal lavage (CVL)

Following the vaginal swab, 10 mL of sterile nonbacteriostatic saline was drawn in a 10-mL syringe to which a soft plastic cannula (plastic transfer pipette) had been attached. A continuous stream of saline was directed into the cervical os. The pooled lavage was aspirated from the posterior fornix, emptied through the catheter into a 15-mL conical polypropylene tube, and transported on ice to the laboratory within 6 hours for processing and storage at -70^{0}C until assayed

2.4.3 Endocervical Dacron swab

Following the lavage, Dacron swab was inserted 1 cm into the cervical os and gently rotated 360^0 one time, to avoid trauma. Directly after sampling, the swab was placed into a sterile tube containing 1 ml of PBS. The swab was gently agitated in the PBS for 15 seconds. Excess liquid was expressed from the swab by rotating the swab 360^0 while

gently pressing it against the inside of the tube. The swab was removed and the specimen was transported to the lab within 6 hours of specimen collection and stored at -70^{0}C until assayed.

2.5 Laboratory methods

2.5.1 Procedures at the Botswana Harvard HIV Reference Laboratory

2.5.1.1 CVL specimens.

Specimen volume, colour, and presence of gross blood were noted by use of Blood indicator strip (Boehringer Mannheim Diagnostic, Chemstrip 4 Ob. Fisher Cat # BC417144 or BM cat # 417144). The specimens were then centrifuged at 800 go for 15 minutes in the polypropylene tube. Supernatant was separated, and filtered through a 0.45-µm millipore low-protein binding disk filter. The supernatant was divided into four 1-mL and four 0.25-mL aliquots. Nucleic acid isolation reagent (0.75 mL; Tri-Reagent-LS; Molecular Research Center) and acryl polymer gel (0.01 mL; Microcarrier Gel-TR; Molecular Research Center) was added to each of the 0.25-mL aliquots and homogenized. All samples were then stored at -70°C until assayed for HIV-1 RNA

2.5.1.2 Isolation of Naeseria Gonorrhoea and Chlamydia Trachomatis

Isolation was determined from endocervical Dacron swab specimen by Polymerase Chain Reaction (PCR), using the Amplicor STD Swab Specimen Collection and Transport Kit (Roche Diagnostic Systems, Inc, Branchburg, NJ). Recommended storage and dilution techniques was utilized to reduce the inhibitory effects of contaminants **[22]**

2.5.1.3 Syphilis serology

Serologic testing for syphilis was performed using the rapid plasma reagin test, or RPR (VD-25; Murex Diagnostics, Dartford, UK). Any positive result was confirmed by the *Treponema pallidum* hemagglutination assay, or TPHA (Fujirebio, Tokyo).

2.5.1.4 Micronutrient samples.

Whole blood collected in royal blue, trace mineral free tubes was centrifuged and separated into plasma and washed red blood cells. Samples were stored at -70°C until assayed

2.5.1.5 Immune measures - Lymphocyte phenotype

Blood was collected on EDTA bottles and transported to BHHRL laboratory within 6 hours for flow cytometric analysis. Quantification of absolute lymphocyte count, absolute CD4+ cell count and percentage was performed within 24 hours by automated, four-color (CD3/4/8/45) flow cytometry on a Becton Dickinson FACS Calibur. The results were expressed as number of cells/uL.

2.5.1.6 Viral load determinations

Blood specimens were collected into EDTA bottles. The plasma was separated within 6 hours of collection and frozen at -70°C until assay. Samples were processed to extract human immunodeficiency virus type 1 (HIV-1) RNA from plasma specimens by automated sample processing protocol (Roche Diagnostics, Indianapolis, Ind.) and reagents (Roche Applied Science, Indianapolis, Ind.). This test was performed according the instructions of the manufacturer. The dynamic range of the test was from 400 (2.6 $\log_{10}$) to 750,000 (5.87 $\log_{10}$) HIV-1 RNA copies/ml

2.5.2 Analysis of shipped samples

2.5.2.1 Determination of HIV-1RNA from CVL

This was performed at the Anderson Fearing HIV Research Laboratory located at the Brigham and Women's Hospital in Boston, Massachusetts. Stored CVL samples were shipped in dry ice for determination of HIV-1RNA. Extraction of CVL supernatant samples was performed as described in the NucliSens package insert (Organon Teknika). One millilitre of CVL supernatant fraction (9.4% of specimens) or 1 mL of CVL/nucleic acid isolation reagent (250 µL/750 µL; 90.6% of specimens) was lysed in 9 mL of lysis buffer. Extracted RNA was then amplified and detected using the HIV RNA quantification HIV-1 QT Amplification and Detection Reagents, according to assay instructions. All results were reported as copies of HIV-1 RNA per millilitre of CVL. The limit of detection of the HIV RNA quantification assay was set at 400 HIV-1 RNA copies/mL of CVL.

2.5.2.2 Determination of Micronutrients

This was performed at Florida International University. Stored micronutrient samples were shipped in dry ice to Florida International University for analysis. Plasma selenium and other micronutrients determination was done by use of a standardized methods **[23] [24] [25] [26]**. Micronutrient reference was obtained from the National Institute of Standards and technology (Standard Reference Material 1598), using bovine serum.

2.6 Statistical Analysis

The population demographic, nutritional and immunologic baseline characteristics were described. The prevalence of nutritional deficiencies on important parameters, prevalence

of genital infections and use of hormonal contraception in the study population were described. The results of cervical vaginal lavage (CVL) quantification of HIV-1 RNA were reported. Genital shedding was defined as the presence of HIV-1 RNA in genital secretions. HIV-1 RNA results from CVL specimens collected at baseline and at 3 months were used to describe shedding patterns in the study population. Analysis of Variance (ANOVA) technique was used to compare mean differences in CD4%, Log viral load and haemoglobin between the shedding patterns identified.

To correlate nutritional status with genital HI-1 RNA shedding, only baseline parameters were used. Because of the small sample size and to generate meaningful analysis report, the shedding patterns were combined and analyzed as two baseline categories - those who were shedding HIV-1 RNA and those who were not. Two sample t test was used to assess for mean differences in baseline Haemoglobin, CD4 count, viral load, STD, Body Mass Index and Albumin between women who were shedding HIV RNA and those who were not. The resulting results were described using exact 95% confidence interval (CI). All reported P-values are 2-sided and not adjusted for multiple testing. Logistic regression model was used to asses for important explanatory predictors of genital shedding in the study population and to control for confounders. Because of low actual individual count in STDs and genital infections, they are not included in the regression model

3.0 Results

3.1 Population characteristics

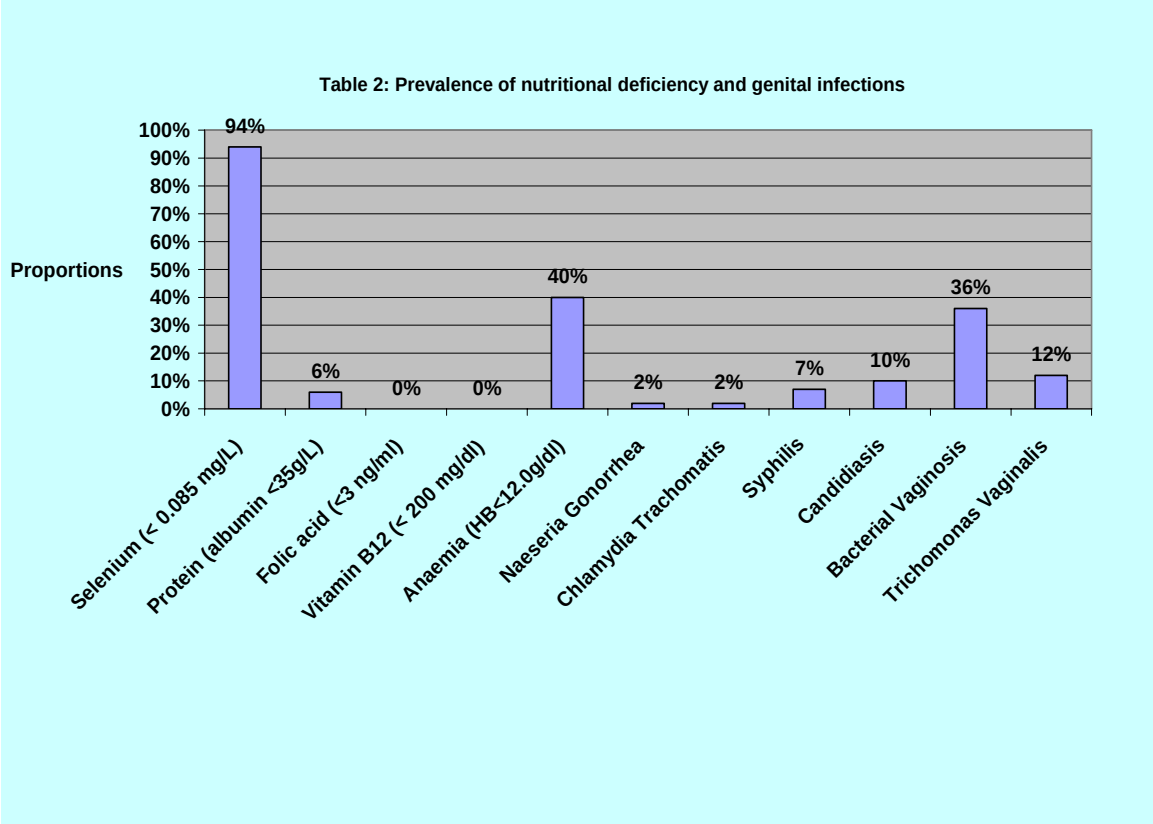
A total of 52 women were enrolled in the study. Of these, CVL results were available for 50 participants. Study participants were healthy, asymptomatic HIV-1 infected women with mean CD4 of 471 and mean viral load of 11,100 RNA copies per ml. Table 1 below shows baseline population demographic characteristics. There were no differences in mean age, education status, marital status, income levels, number of pregnancies and age at first menses between women who were shedding HIV-1 in their genital secretions compared to those who were not shedding

Table 1: Baseline demographic characteristics *

	HIV shedding		
	No (n = 35)	Yes (n = 14)	P value
Age in years (mean ± SD)	33.7 ± 7.6	32.3 ± 6.6	0.56
Education (n (%))			
Primary or less	10 (30)	6 (46)	0.64
Jr. Secondary	15 (46)	5 (39)	
Sr. Secondary or above	8 (24)	2 (15)	
Marital status			
Single	19 (58)	7 (54)	0.81
Married	4 (12)	3 (23)	
Cohabitating	9 (27)	3 (23)	
Other	1 (3)	0 (0)	
Income			
<300	12 (36)	4 (31)	0.28
300-1000	12 (36)	8 (62)	
>1000	9 (28)	1 (7)	
Number of pregnancies			
0	3 (9)	2 (14)	0.82
1-2	15 (43)	7 (50)	
3-4	15 (43)	5 (36)	
5 or more	2 (5)	0 (0)	
Age at first period	15.8 ± 1.8	16.1 ± 1.3	0.57

* The cells may not add up to the totals due to the missing values.

Table 2 below shows baseline nutrition and STI status: a 94% selenium deficiency (defined as Selenium < 0.085 mg/L), 40% anaemia prevalence (Hb < 12.0 g/dl), 6% protein deficiency (serum albumin < 35 g/l) with no deficiencies observed in folic acid or vitamin B12. Other studies have previously reported specific nutritional abnormalities in asymptomatic HIV infected individuals **[27]**. In this study, 36% of the women had bacterial vaginosis, 10% had Candida albicans, 12% had Trichomonas vaginalis, 7% had syphilis, 2% had Naeseria gonorrhoea, 2% had Chlamydia trachomatis as detailed in ***appendix 1***, and this is in agreement with a recent study of STDs in Botswana **[28]**



3.2 Shedding patterns

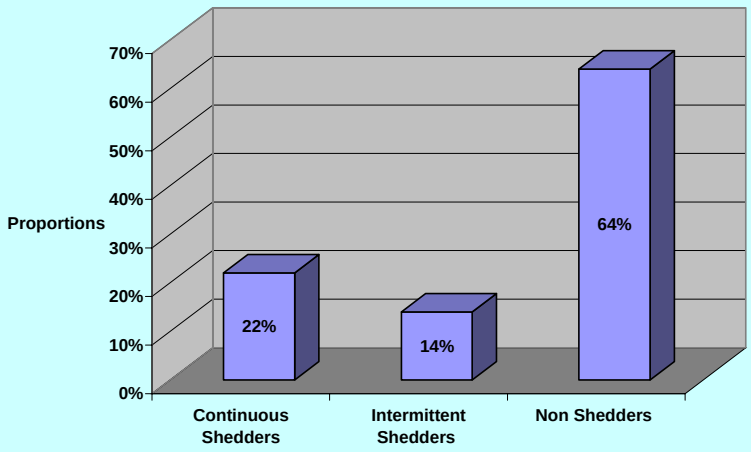
Of the 50 women whose CVL results were available, 15(30%) had detectable HIV-1 RNA virus in their genital secretions at baseline compared to14 (28%) at three months.

As shown in table 3 below, HIV-1RNA was isolated from both baseline and three months CVL samples from 24% of the study population. These were labelled as **continuous genital shedders (CS)**

No HIV-1RNA was isolated from both baseline and three months CVL samples from 64% of the study population. These were labelled as **non-shedders (NS)**

In 14% of women, HIV-1RNA was either isolated at baseline only (4/50) or at three months only (3/50) and these were labelled as **intermittent shedders (IS)**

Table 3: Genital shedding patterns in the study population



3.2.1 Comparison of Hb, CD4 and Viral load between the shedding patterns

ANOVA test was used to compare parameters between the shedding patterns. As shown in table 4 below, baseline haemoglobin was higher among the non-shedders compared to Continuous & Intermittent shedders, but the difference was not statistically significant between the three groups. Baseline viral load significantly differed between the three shedding patterns, with the highest viral load in the Continuous shedders, intermediate viral load in the Intermittent shedders and lowest viral load in the Non shedders. Baseline CD4 count significantly differed between the three shedding patterns, lowest count in the continuous shedders, intermediate count in the Intermittent shedders and highest count in the Non shedders.

Table 4: Comparison of Haemoglobin, Log Viral Load and CD4 percent between shedding patterns

Haemoglobin*	**Mean ± Sd**	**N**	**P = 0.1161**
Continuous	11.7 ± 1.8	11	
Intermittent	11.5 ± 0 .9	7	
Non - Shedders	12.6 ± 1.5	32	

*Bartlett's test for equal variances shows normal distribution (P = 0.250)

Plasma Log Viral Load*	**Mean ± Sd**	**N**	**P < 0.001**
Continuous	4.8 ± 0.6	11	
Intermittent	4.4 ± 0.9	7	
Non - Shedders	3.7 ± 0.8	32	

*Bartlett's test for equal variances shows normal distribution (P = 0.562)

CD4 percent*	**Mean ± Sd**	**N**	**P < 0.01**
Continuous	20.8 ± 3.7	11	
Intermittent	24.8 ± 5.4	7	
Non - Shedders	30.7 ± 10.5	32	

*Bartlett's test for equal variances shows non normal CD4% distribution among the three shedding patterns (P = 0.001)

3.3 Comparison of nutritional parameters by baseline shedding status

Two sample t test was used to compare mean differences between the nutritional parameters of the two baseline shedding status groups - those who were shedding HIV-1 RNA and those who were not and results are shown in table 5 below. Mean BMI, albumin, Selenium, folic acid and Vitamin B12 levels were distributed evenly among women who were shedding and those who were not shedding at baseline**.** Also mean age, income level, educational status, marital status, age at first pregnancy and number of pregnancies did not differ among women who were shedding HIV-1 and those who were not shedding. Haemoglobin level was lower among women who were shedding HIV-1 compared to those who were not shedding, but the difference showed a strong trend albeit not statistically significant**.**

Table 5: Baseline nutritional variables

	Non shedders (N=35)	**Shedders (N=15)**	
Variable	**Mean (95% CI)**	**Mean (95% CI)**	**P-value**
Plasma Selenium (mg/l)	.068 (.064 - .071)	.069 (.063 - .075)	0.8143
Plasma Vitamin B12 (mg/dl)	715.8 (637.0 - 794.7)	768.5 (634.8 - 902.1)	0.4856
Plasma Folic acid (ng/ml)	6.2 (5.4 - 7.0)	5.5 (4.8 - 6.1)	0.3005
Haemoglobin (g/dl)	12.5 (12.0 - 13.0)	11.7 (10.8 - 12.5)	0.0877
Serum Albumin (g/l)	40.6 (39.1 to 42.0)	40.0 (37.8 to 42.0)	0.6063
Body Mass Index	25.1 (23.4 - 26.8)	25.2 (22.4 - 28.0)	0.9340

3.3.1 Comparison of CD4, Hb and Viral Load by shedding status

As indicated above and also shown in table 6 below, women who had detectable genital HIV-1-RNA at baseline had lower haemoglobin compared to the those who were not shedding (Hb11.7 (95% CI 10.8 ; 12.5) vs. Hb 12.5 (95% CI 12.0 ; 13) P = 0.0877), showing a strong trend, albeit non significant haemoglobin difference. Women who had detectable genital HIV-1-RNA at baseline had significantly lower CD4 cell percentage compared to those not shedding (22% (95% CI 19 ; 24) vs. 30 % (95% CI 27 ; 34) P < 0.01) and a significantly higher log viral load (4.7 log (95% CI 4.2 ; 5.1) vs. 3.6 log (95% CI 3.5 ; 4.0) P < 0.01).

Table 6: Comparison of baseline CD4 cell count percent, Haemoglobin and log viral load by shedding status

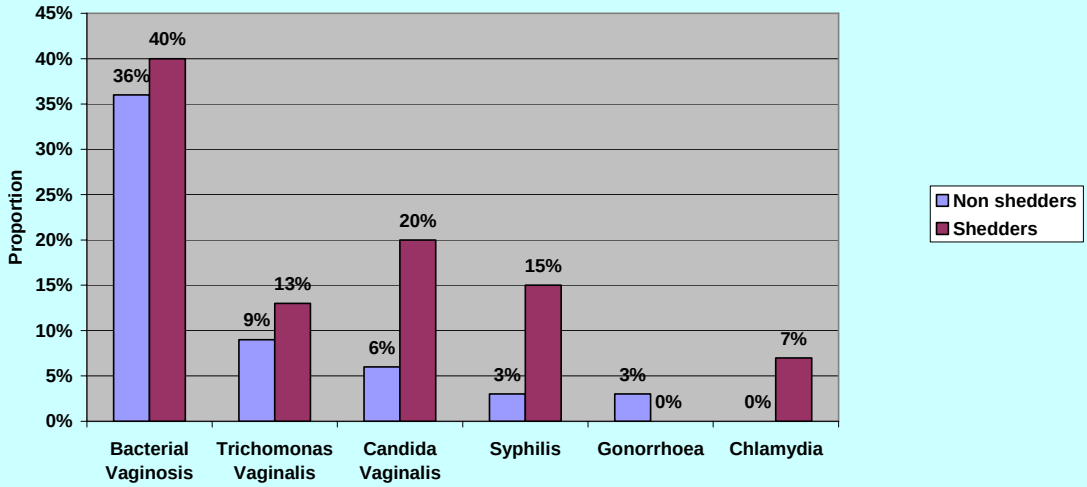
	Non shedders (N=35)	**Shedders (N=15)**	
Variable	**Mean (95% CI)**	**Mean (95% CI)**	**P-value**
Haemoglobin	**12.5 (12.0 - 13.0)**	**11.7 (10.8 - 12.5)**	**0.0877****
CD4 cell count percent	**30 (27 - 34)**	**22 (19 - 24)**	**0.0045***
Plasma (Log VL)	**3.6 (3.5 - 4.0)**	**4.7 (4.2 - 5.1)**	**0.0007***

* Shows statistical significant results
** shows a strong trend, albeit not statistically significant

3.3.2 Comparison of genital infections, hormonal contraception by shedding status

As shown in table 7 below, overall there was a higher, non significant, prevalence in baseline genital infection among women who were shedding HIV-1, compared to those who were not (73% vs. 46%; P = 0.123). No HIV -1 RNA was isolated in all the19% of the women in the study who were using some form of contraception

Table 7 : Prevalence of STDs and other Vaginal infections among shedders and non shedders



3.3.3 Explanatory predictors of genital shedding status

Logistic regression was used to estimate odds of genital shedding. Participants were 1.7 times more likely to shed HIV-1 RNA in their genital secretions if they were anaemic (HB <12.0g/dl) (OR 1.7, (95% CI 0.5 ; 5.8), P = 0.386), showing that that anaemia may be a good predictor of genital shedding, albeit not statistically significant. Participants were seven times more likely to shed HIV-1 RNA in their genital secretions if they had

CD4% of less than 27% (OR 6.9 (95% CI 1.3 ; 35.1) P = 0.02), showing that CD4% is significantly predicts genital shedding. Participants were also seven times more likely to shed HIV-1 RNA in their genital secretions if they had plasma viral load of more than 20 000 copies per ml (OR 6.75 (95% CI 1.8 ; 25.6) P < 0.01), showing that plasma viral load significantly predicts genital shedding

3.3.4 Control of confounding

Logistic regression was used to adjust for confounding. Those who had anaemia (Hb<12.0g/dl) were 2.3 times (95% CI 0.5 ; 8.7, P = 0.232) more likely to shed HIV-1 RNA in the genital secretions after adjusting for CD4%. Similarly those with CD4 count of less than 27% were 7.9 times (95% CI 1.48 ; 42.35, P = 0. 02) more likely to shed after adjusting for anaemia.

Those who had anaemia (Hb<12.0g/dl) were 1.9 times (95% CI 0.49 ; 7.4, P = 0.353) more likely to shed HIV-1 RNA in the genital secretions after adjusting for plasma viral load. Similarly those with plasma viral load of more than 20 000 were 7 times (95% CI 1.8 ; 27.2, P < 0.01) more likely to shed after adjusting for anaemia.

Those who had CD4 count less than 27% were 3.9 times (95% CI 0.67 ; 22.3, P = 0.132) more likely to shed HIV-1 RNA in the genital secretions after adjusting for plasma viral load. Similarly those with plasma viral load of more than 20 000 were 4.3 more (95% CI 1.02 ; 17.8, P=0.047) more likely to shed after adjusting for CD4 count.

Those who had anaemia were 2.1 times (95% CI 0.5 ; 8.4, P = 0.308) more likely to shed HIV-1 RNA in the genital secretions after adjusting for plasma viral load and CD4 count. Those who had CD4 count of less than 27% were 4.1 times more (95% CI 0.69 ; 24.8, P = 0.12) likely to shed HIV-1 RNA in the genital secretions after adjusting for anaemia and plasma viral load. Those who had plasma viral load of more than 20 000 were 4.1 times (95% CI 0.95 ; 18.1, P = 0.059) more likely to shed HIV-1 RNA in the genital secretions after adjusting for anaemia and CD4 count

4.0 Discussion, conclusion, recommendation and limitations

Discussion

Three shedding patterns, **continuous shedders, intermittent shedders and non-shedders** were described in our study population. The CVL RNA detection rates in our study were similar to findings from other investigators **[29] [30]**. Baseline differences in genital shedding predictors may be due to preventable or treatable conditions operating at local genital mucosa level. Understanding covariates of these shedding patterns require further research, to better understand their impact on hetero - sexual as well as mother to infant transmission of HIV during pregnancy and birth process

Data on this study showed a strong trend, albeit not significant, that low haemoglobin level may be a predictor of shedding HIV-1 RNA in genital secretions. From the data available, it was not possible to classify anaemia by aetiology. In untreated HIV, anaemia is thought to be caused by direct HIV viral infection of haematological progenitor cells.

The odds of shedding predicted by anaemia was not affected after adjusting for both CD4 count and Plasma viral load

Data on this study also showed that CD4 count and Viral load were important predictors of HIV-1 shedding in the genital secretions and this finding is also supported by findings from other investigators **[31].**

Conclusion

Women who were shedding HIV-1 RNA had higher plasma viral load, lower CD4 count, lower haemoglobin and higher prevalence of genital infections, indicating a more advanced HIV disease compared to non-shedders. Clinically persons with more advanced HIV disease is accompanied by poor nutritional status compared to those with less advanced HIV disease status. This study generates hypothesis on the role haemoglobin may play in genital shedding of HIV-1 in females.

Recommendation

Further larger prospective studies of appropriate study design are required to find out the role played by anaemia in HIV transmission.

Limitation

The main limitation of this study was the small number of participants. It was not possible to classify anaemia by aetiology from the data available. This was a cross sectional study and a prospective study is more appropriate to study the effect of nutrition

on genital shedding over time. However the findings of this study could be useful in calculating the sample size for a larger study, validating collection instruments and laboratory protocols to answer the question of whether poor nutritional status is associated with increased genital HIV-1 RNA shedding or not.

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Appendix 1: Baseline genital infections by shedding status *

Genital infection	Non shedders (N=35)	Shedders (N=15)	P - value
Bacterial Vaginosis	12/33 (36%)	6/15 (40%)	0.809
Trichomonas Vaginalis	3/33 (9%)	2/15 (13%)	0.656
Candida Vaginalis	2/33 (6%)	3/15 (20%)	0.143
Syphilis	1/30 (3%)	2/13 (15%)	0.154
Gonorrhoea N=49 • Positive • Negative • Undecided	 1/49 (3%) 33/49 (94%) 1/49 (3%)	 0/49 (0%) 14/49 (100) 0/49 (0%)	1.00
Chlamydia N=49 Positive Negative Undecided	 (0) 34 (97) 1 (3)	 1/49 (7%) 13 (93) 0 (0)	0.49
Presence of any genital infection	16/35 (46%)	11/15 (73%)	0.123

* The cells may not add up to the totals due to the missing values.