TITLE

IS THERE AN ASSOCIATION BETWEEN BACTERIAL VAGINOSIS INFECTION AND HIV-1 INFECTION ACQUISITION AMONG WOMEN AGED 18-35 YEARS IN SOWETO

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DECLARATION

I, Nathaniel Weluzani Banda Chimbatata declare that this research report is my own work except to the extent indicated in the reference and acknowledgements. It is submitted for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics, to the Witwatersrand University, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

The Ethics Committee on Human Research, Witwatersrand University approved the study. The medical ethics clearance certificate number is M080984.

(Nathaniel Weluzani Banda Chimbatata)

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DEDICATION

To my wife Chikondi and beloved son Chiyambi Chimbatata for the support rendered and endurance they experienced when I was away from them for my studies.

To all relatives for not worrying about all the hours spent away from them.

To Canon Collins Trust Educational Fund for Southern Africa, for ably funding my tuition fees. Thanks for the financial support.

ABSTRACT

BACKGROUND

Studies suggest an association between Bacterial Vaginosis (BV) and HIV infection; however, its temporal effect has not been greatly investigated.

METHODS

This is a secondary data analysis of a cohort study: set out to describe the association between BV infection and HIV acquisition. There were 750 participants enrolled in the primary cohort study. The main exposure, BV, was measured from a gram stain slide prepared from a vaginal swab. The slide was read in a laboratory qualitatively and scored by Nugents scoring. A score of 7 or above was considered positive for BV. The outcome variable (HIV) was determined by dual rapid tests and confirmed in the laboratory by a third generation ELISA. Descriptive statistics was done to describe demographic characteristics and the prevalence of BV and STIs. HIV incidence rate was calculated. Kaplan Meier survival time analysis and log rank test for significance were performed. Cox regression (univariate and multivariate) was done to determine association of BV with HIV infection.

RESULTS

The baseline prevalence of BV was 52 %, 95 % CI; 45 – 59. There were 21 HIV seroconversions experienced of which 7 had BV results missing and were excluded in the analysis. The remaining 14 seroconversions were followed for a mean time of 0.40 of a year and accumulated follow up time at risk of 286 person years, this represented an HIV incidence rate of 4.9 per 100 person years of follow up, 95 % CI: 2.9 – 8.27. Kaplan Meier curves revealed a higher risk of HIV-1 acquisition among women who were BV positive than the women who were BV negative. A log rank test showed that the

probability of seroconversion was different among the women depending on BV status, chi-square value 3.8, p 0.05.

Controlling for confounding variables, seroconversion was high, but not significant, among BV positive women, adjusted hazard ratio 3.21; 95 % CI; 0.85-12.12, p value 0.08.

CONCLUSION

This study suggests that BV increases HIV seroconversion risk though statistical significance was not achieved. Vaginal cleansing education, screening and treating women with BV could maintain normal vaginal flora and reduce their susceptibility to HIV.

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God bless you all.

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LIST OF ACRONYMS

AHR	Adjusted Hazard Ratio
AOR	Adjusted Odds Ratio
ARR	Adjusted Risk Ratio
BV	Bacterial Vaginosis
CI	Confidence Interval
CT	*Chlamydia trachomatis*
ELISA	Enzyme Linked Immuno-Sorbent Assay
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
HREC	Human Research Ethics Committee
HSV2	Herpes Simplex Virus 2
NG	*Neisseria gonorrhoea*
OR	Odds Ratio
PCR	Polymerase Chain Reaction
RPR	Rapid Plasma Reagin
STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TV	*Trichomonas vaginalis*

ETHICS COMMITTEE CLEARANCE CERTIFICATE

CHAPTER 1: INTRODUCTION

1.1 BURDEN OF DISEASE HIV

Human Immunodeficiency Virus (HIV) currently affects populations worldwide. The prevalence of HIV continues to grow globally and women of reproductive age experience consistently high rates of HIV infection. About 38 million people are infected worldwide, of which 29 million are in Sub-Saharan Africa [1]. It is estimated that 8 % of all adults in Sub-Saharan Africa are HIV infected and almost 60 % of these HIV infections occur among women [2]. South Africa has one of the highest HIV infection rates in Sub-Saharan Africa and the prevalence of HIV infection has been increasing significantly with young people, particularly young women, are at the greatest risk [3]. It is estimated that 1 in 9 people are infected in South Africa with a total of 5.3 million people infected. The prevalence of HIV in women attending public antenatal care reached 27.9 % in 2003 and an estimated 11.4 % of the total population is infected [4]. Determining the factors that increase HIV infection risk in women is significant in developing HIV infection prevention strategies.

1.2 BURDEN OF DISEASE BACTERIAL VAGINOSIS

Bacterial Vaginosis (BV) is defined as a clinical syndrome characterised by a change in the normal vaginal flora where, the normal lactobacilli dominated flora is replaced by other anaerobic organisms resulting in an increase in vaginal pH [5] and a decrease in the number of hydrogen peroxide producing lactobacilli. It has complex aetiology with the

commonly associated organisms being *Gardnerella vaginalis, Mycoplasma hominis* and *Mobiluncus* species. This complex aetiology limits its proper diagnosis and treatment. It is a common vaginal infection and the leading cause of abnormal vaginal discharge [6]. Women with BV present with increased vaginal discharge and on a swab taken from the vaginal wall a low pH and clue cells are found [6]. It is a treatable condition, however treatment is difficult as it is sometimes asymptomatic and because of its multicausal aetiology and recurrences of infection is common after treatment [7]. BV occurs in 20-50 % of women in the general population worldwide and in Sub-Saharan Africa prevalence rates of 20-50 % have been reported [7].

The prevalence is even higher in women attending sexually transmitted infection (STI) clinics (40-50 %), suggesting that BV is associated with sexual activity [8]. Although there appears to be a clear association with sexual activity there is still debate about whether BV is a true STI.

BV is diagnosed based on either clinical or laboratory based examination of a gram stain by Nugent score criteria. The clinical diagnosis of BV is based on an increased vaginal discharge, increased vaginal pH >4.5, presence of clue cells on microscopy and a positive whiff test. A swab of the discharge is tested with litmus paper for pH and then prepared in a wet mount (addition of normal saline to the sample of discharge on a slide).Characteristic clue cells are seen on the wet mount examined under a microscope. Clue cells are vaginal epithelial cells coated with bacteria. They are called clue cells because they provide a clue for the presence of BV. This is followed by a whiff test which is done by adding several drops of a base (potassium hydroxide) to a sample of the vaginal discharge which then releases a characteristic amine odour or fishy smell.

In clinical practice to make the diagnosis of BV, the following four Amsel criteria need to be met:

- An increased thin yellow homogeneous vaginal discharge,
- A characteristic fishy odor on wet mount by the whiff test,
- Loss of acidity (alkaline on litmus paper test pH >4.5),
- Presence of clue cells on wet mount.

However, the clinical diagnosis may be influenced by many factors and to some extent depends largely on the expertise of the care provider [8].

The Nugent score diagnosis of BV is a widely used laboratory based method, it consists of a scoring scale system which ranges from 0-10 for assessing the vaginal flora by gram stain. A score of 0-3 is considered negative, 4 to 6 is regarded as intermediate and 7 or greater is classified positive for BV [9]. The use of gram stain with Nugent scoring is considered as the gold standard for diagnosing BV [10].

BV has been linked with adverse public health consequences. It has been associated with serious obstetric complications such as spontaneous abortions, preterm labor and post caesarean section infections [11]. In a study to assess the role of BV on pregnancy complications in Durban, South Africa, it was found out that BV was found in 52 % of the women studied, of those who were BV positive, 46 % had poor pregnancy outcomes as measured by complications such as pregnancy loss and neonatal morbidity. There was significant difference in poor pregnancy outcome among women with BV (63 %) compared to women with other infections (42 %), p value 0.005 [5].

BV has as well been linked with the acquisition of HIV infection in women [6] and has been suggested as a mediator of the association between intra vaginal practices and HIV infection.

Vaginal cleansing and use of intra vaginal herbs are also associated with BV infection [12, 13]. Other risk factors for BV include behaviours such as greater number of sexual partners, frequent vaginal intercourse, new sexual partner and less frequent use of condoms [14, 15]. These risk factors suggest that there is a link between BV and sexual activity and fuel the debate on whether BV is an STI or not.

Besides the tremendous implications BV has on public health, there has been little attention to investigate the problem [11]. Treatment of vaginal discharge by syndromic management covers BV, however there are many organisms associated with BV and it is sometimes asymptomatic, therefore identifying proper treatment is difficult and the cure rates for BV are low ranging between 70-80 % and the recurrency rates of infection after treatment are high [7].

1.3 STATEMENT OF PROBLEM

Diseases of the reproductive tract have been reported to be associated with the sexual transmission of HIV infection in both males and females [16]. The focus of this association has been on sexually transmitted infections which are known biological risk factors for acquisition and transmission of HIV infection sexually. Although BV is not a classical STI as it is unlikely to be transmitted between partners, it is associated with sexual activity and the risk factors for STIs such as increased number of sexual partners.

BV is associated with changes in the vaginal flora, which weaken the natural defence mechanism present in the vagina, which may predispose women to acquisition of other STIs. It is therefore important to describe the risks associated with BV. The prevalences of BV are relatively higher than those of STIs [8]. Despite this, few prospective studies have been done on BV as a potential female reproductive tract infection associated with the risk of HIV infection acquisition [8]. The reported magnitude of association between BV and HIV infection is variable and depends on the definition of the exposure. It is therefore important to critically investigate further the association of BV and acquisition of HIV infection. This analysis may add further knowledge surrounding the temporal association of BV and HIV infection acquisition.

1.4 LITERATURE REVIEW

Evidence describing the association between BV and HIV infection

Several cross sectional studies have shown that BV is associated with an increased risk of HIV prevalent infection; however few prospective studies have been conducted [8].
A cross sectional study was conducted in Chiang Mai, Thailand, among 144 female commercial sex workers to investigate the relationship between BV and HIV-1 seropositivity in a population at high risk of sexual acquisition of HIV [17]. BV was detected in 33 % of the participants and HIV was detected in 43 % of the participants. BV was found to be significantly associated with prevalent HIV infection using the clinical criteria of diagnosis, odds ratio (OR) 2.7; 95 % Confidence Interval (CI), 1.3-5.0. The association between BV and HIV prevalence was not significant using gram stain alone

for the diagnosis of BV. The association was also found between abnormal vaginal flora and HIV prevalent infection, OR, 2.1; 95 % CI; 1.0-4.8. Multiple logistic regression was applied to adjust for age, number of sexual encounters per week, current condom use and currently having a sexually transmitted disease (STD). After adjusting, both BV and a history of an STD were independently and significantly associated with HIV seropositivity, adjusted odds ratio (AOR) for BV infection, 4.0; 95 % CI, 1.7-9.4 and AOR for history of an STD, 6.9; 95 % CI; 2.1-22.9.

In another cross sectional study [18], involving 4718 women aged 15-59 years in rural Rakai District, Uganda, an HIV prevalence of 14.2 % was reported among women with normal vaginal flora and 26.7 % among those with severe BV. The study found an association between BV and prevalent HIV-1 infection among younger women, but not among women older than 40 years. The adjusted odds ratio for HIV-1 infection associated with any abnormal flora was 1.52; 95 % CI; 1.22-1.90 and 2.08; 95 % CI; 1.48-2.94, for severe BV.

Further to this, another cross sectional study conducted in North Carolina, USA, to investigate the association of BV with HIV infection in pregnant women, 724 women attending prenatal care were recruited at 24 to 29 weeks of gestation [19]. The study reported that HIV prevalence depended on BV status. The HIV prevalence was 0.8 %, 1.2 % and 3.3 % among women with normal vaginal flora, intermediate vaginal flora and BV respectively. A multiple logistic regression model adjusting for douching gave an odds ratio for HIV infection associated with abnormal vaginal flora of 3.9; 95 % CI; 1.03-14.9 and for BV the odds ratio was 3.7; 95 % CI; 1.1-13.2.

Besides this, a cross sectional study to determine the relationship between HIV-1 infection and BV involving 598 women was conducted in South Africa. BV prevalence was 70 % and HIV prevalence was 62 % among this group, p; 0.001. It was found out that the odds of being HIV-1 infected was 1.5; 95 % CI; 1.3 – 1.8; p; 0.001 with a BV Nugent score of ≥7. BV remained independently significantly associated with HIV-1 infection after adjusting for other factors, AOR; 2.3; 95 % CI; 1.6 – 3.3 [20].

In addition, a nested case control study was conducted in South Africa among 5110 women enrolled in a cervical cancer trial to investigate the association of BV infection and susceptibility to HIV infection among South African women. The BV prevalence was 46 % in this study, there were 86 HIV seroconversions identified in the trial and this represented an overall incidence rate of 2.1 per 100 person years. It was found in this study that diagnosis of BV on the basis of Nugent score criteria was significantly associated with increased risk of HIV seroconversion. In a multivariate model adjusting for demographic factors, STIs and other sexual behaviours the AOR was 2.01; 95 % CI; 1.12 – 3.62 [21].

In Malawi a prospective study was conducted to determine the association of BV and other disturbances of the vaginal flora with HIV seroconversion among pregnant and postnatal women [22]. The diagnosis of BV was done based on clinical criteria only. The association of BV and other risk factors with HIV seroconversion were examined using contingency tables and multiple logistic regression analyses on antenatal data, Kaplan Meier proportional hazards analyses were used on postnatal data. The study involved 1196 HIV seronegative women who were followed antenatally for a median of 3.4

months and 27 women seroconverted by the time of delivery. Postnatally, 97 seroconversions were observed among 1169 seronegative women who were followed for a median of 2.5 years. The study reported that BV was significantly associated with antenatal HIV seroconversion, AOR 3.7, p, 0.04 and postnatal HIV seroconversion, ARR 2.3, p, 0.03.

Another prospective study was done in Mombasa, Kenya, to examine the relationship between BV and HIV infection acquisition among 657 HIV negative women commercial sex workers [23]. There were 68 seroconversions within 621 person years of follow up, with an overall incidence rate of 11 per 100 person years of follow up. BV was found to be significantly associated with HIV incident infections. In a multivariate Cox regression model after adjusting for other factors, a hazard ratio of 1.9; 95 % CI, 1.1-3.2 was reported for HIV-1 infection acquisition among women with BV.

Similar findings were reported recently in a prospective study of HIV-1 incidence among women of reproductive age in Malawi. In this study, 787 HIV negative women were followed up for a period of 12 months. The women had subsequent HIV testing and 31 were found HIV positive during the follow up period representing an overall incidence rate of 4.51; 95 % CI; 2.96 – 6.06 per 100 person years of follow up. A Cox proportional hazard model was used to assess the factors associated with HIV acquisition. BV was found to be significantly associated with incident HIV-1 infection, AHR 2.52; 95 % CI; 1.07 – 5.94; p, 0.03 [24].

Likewise positive findings were reported in a multi centre prospective study done in Zimbabwe and Uganda. The study was conducted to evaluate the relationship between

BV and the risk of HIV infection acquisition. This involved 4531 HIV negative women aged 18 -35 years. Participants were followed for 15 to 24 months. The exposure variable BV was determined by gram stain and Nugent scoring. Women had subsequent HIV testing and there were 213 incident HIV infections. This represented an incidence rate of 4.12 and 1.53 per 100 woman years of follow up in Zimbabwe and Uganda respectively. Cox proportional hazard analysis was used to investigate factors associated with HIV infection acquisition. The analysis was performed for the two countries combined. BV was found statistically significantly associated with HIV infection acquisition, AHR 1.67, 95 % CI; 1.24-2.26[25].

Positive findings were also reported in a meta-analysis of published studies to assess the published literature on the extent to which BV may increase the risk of HIV acquisition. In this meta-analysis medline and other electronic data bases were systematically searched for eligible publications. In the studies reviewed the diagnosis of BV was based on clinical criteria only, Nugents score criteria only or both clinical and Nugents score criteria. The association of BV and incident HIV infection was analysed and evaluated. BV was found to be associated with an increased risk of HIV acquisition in HIV incidence studies, RR 1.6, 95 % CI; 1.2-2.1[26].

Studies described above that have demonstrated an association between BV and HIV have been summarised in the table below:

Table 1.1: Summary of the studies on association between BV and HIV infection

Study Area	Number and type of Participants	Study Design	Methods of BV Diagnosis	BV Prevalence	HIV Incidence or Prevalence	Adjusted measure of association (95 % CI)
Chiang Mai [17]	(144)Commercial sex workers	Cross sectional	Clinical / Gram stain	33 %	43 %	**OR4.0 (1.7 -9.4)**
Uganda [18]	(4718) General population	Cross sectional	Gram stain	55 %	26.7 %	**OR 2.1(1.4-2.9)**
NorthCarolina [19]	(724) Pregnant women	Cross sectional	Gram stain	20.9 %	03.3 %	**OR3.7(1.1-13.2)**
South Africa [20]	(598) General population	Cross sectional	Gram stain	70 %	62 %	**OR 2.3(1.6- 3.2)**
South Africa [21]	(5110) General population	Nested case control	Gram stain	46%	2.1 per 100 person yrs	**OR2.01(1.1–3.6)**
Malawi [22]	(1196) Pregnant women	Prospective cohort study	Clinical	59 %	7.9 per 100 person years	**OR 3.7 p; 0.04**
Kenya [23]	(657) Commercial sex workers	Prospective cohort study	Gram stain	36 %	11 per 100 person years	**HR1.9(1.1– 3.2)**
Malawi[24]	(787) General population	Prospective cohort study	Gram stain	68 %	4.51 per 100 person years	**HR2.52(1.1-5.9)**
Zimbabwe/Uganda[25]	(4531)General population	Prospective cohort study	Gram stain	50 %	4.12 per 100 person years.	**HR1.67(1.2-2.2)**
Meta-analysis[26]	23 studies	Variable	Variable	33 %		**RR 1.6 (1.2-2.1)**

The strengths of association between BV and HIV infection is variable, table 1.1. It ranges from 1.6 to 4.0 fold in the adjusted measure of association. The variation could be due to the different groups of women studied, numbers of women included in the study and different diagnostic methods used in defining the exposure and the outcome. These factors could result in the variation in the effect size of the association.

Biological Plausibility for the association between BV and HIV acquisition

The vaginal flora plays an important role in protecting women from sexually transmitted infections including HIV infection [8]. Lactobacilli are the major inhabitants of the vagina; these bacteria are responsible for the production of microbial toxins including hydrogen peroxide. Hydrogen peroxide has been shown to inhibit the growth of micro organisms including *Gardnerella vaginalis, Neisseria gonorrhoea* (NG) and HIV [8]. Lactobacilli also maintain the acidity of the vagina through the production of lactic acid. Vaginal pH which is normally less than 4.5 is inhabitable to many organisms including HIV [8]. This protective nature of the vaginal flora changes in women with BV.

The organisms that are associated with BV such as *Gardnerella vaginalis* are said to increase the incidence of sexual transmission of HIV by many mechanisms [27]. The mechanisms include vaginal microflora changes that take place during BV infection which lead to an increased vaginal pH and a decrease in the number of hydrogen peroxide producing lactobacilli. This abnormal vaginal flora causes an inflammatory response which leads to the recruitment of immune response cells that become the target cells for the HIV virus in the vagina. The inflammatory process also causes epithelial trauma, this allows the virus to pass the epithelial barrier more easily. These changes are believed to provide necessary conditions for HIV survival and replication [27].

Summary

Cross sectional data suggest an association between BV and prevalent HIV infection, although associations are varied in strength and do not provide temporal associations. This is supported by prospective studies that suggest an association between BV and

incident HIV infection, table 1.1. Many of the studies report some association between BV and HIV acquisition, but the exposure variable is not always ideally measured using the standard Nugent score criteria. The clinical diagnosis of BV can be influenced by many factors and depends on the expertise of the care provider [8, 16]. The evidence for the association between BV and HIV does seem to be conflicting if different criteria (Clinical or Nugents scoring) are used to define the exposure. The misclassification of the exposure affects the measure of effect in these studies making them hard to compare [17].

1.5 STUDY OBJECTIVES

Primary Objective

To determine the association of BV with HIV infection acquisition among women aged 18-35 years in Soweto.

Secondary Objectives

i. To describe the social and demographic characteristics of the study participants.
ii. To describe the prevalence of BV and STIs in the study sample.
iii. To estimate HIV incidence in the study sample.

1.5.1 Null Hypothesis

BV infection is not associated with HIV infection acquisition.

1.6 JUSTIFICATION FOR THE STUDY

The evidence to date is mixed in strength of study design (many cross sectional studies) and in measurement of the exposure variable, mostly the diagnosis of BV has been based on clinical criteria only [17, 18, 19]. Few prospective studies in different populations

have been conducted, this analysis of a prospective cohort study may add evidence by the strength of design and by defining the exposure variable by the laboratory based Nugent score criteria. This analysis may also offer an opportunity to demonstrate the temporal relationship between BV and HIV infection. This study population is possibly more generalisable as it is a general population of women from a general population of South African women which may provide local researchers and policy makers with relevant local data.

CHAPTER 2: METHODS

2.1 DESCRIPTION OF PRIMARY DATA

A cohort study was conducted in Soweto between Oct 2002 and January 2005. This cohort study aimed to estimate HIV incidence, condom usage and retention in the cohort in preparation for an HIV prevention trial. Women aged 18 to 35 years were invited to participate and those who volunteered were screened for HIV and pregnancy. Additional inclusion criteria included willingness to stay in Soweto for next year and willingness to give consent. The exclusion criteria included women out side the age range of 18 – 35, HIV positive, pregnancy positive and intending to move out of Soweto within next one year. There were 1089 potential participants screened and 750 were enrolled. Participants were followed up for a maximum period of one year if they completed the study, however participants included in this analysis were followed for a mean period of 0.40 of a year. At entry, the participants were further screened for biological factors that included sexually transmitted diseases, *Neisseria gonorrhoea* (NG), *Chlamydia trachomatis* (CT), *Trichomonas vaginalis* (TV), syphilis, Herpes Simplex Virus 2 (HSV 2) and BV.

2.2 STUDY DESIGN

This is a secondary data analysis of a prospective cohort study which was designed to estimate HIV incidence and condom use.

2.3 STUDY POPULATION

The study population comprised HIV negative sexually active women aged 18-35 years in Soweto.

2.4 STUDY SAMPLE

The study sample was a fixed one and consisted of all the sexually active HIV negative women who were enrolled in the primary cohort study. However, 683 participants were included in this analysis and had accumulated 286 woman years of follow up; this was due to the missing of BV results. These participants were followed for a mean period of 0.40 of a year. There were 14 incident HIV infections that had BV results available and were included in this analysis.

2.5 STUDY PROCEDURES

2.5.1 Clinical Procedures

Women were seen at the study clinic every three months. At this time HIV pre and post test counselling was done as were HIV rapid tests in parallel by trained nurses in the clinic. Structured interviews were done to collect data on potential confounders. The interviews gathered information on demographic factors that included age, level of education and social economic markers. Behavioural factor data included contraceptive use, condom use at last sexual act, number of sexual partners in the past three months, type of sexual partners and vaginal practices such as cleansing and product insertion. Pelvic examinations were done at baseline, six and 12 months visits where swabs were collected from the cervix for NG, CT, from the vaginal wall for BV and TV. Blood for HSV 2 and syphilis was also collected. Both syndromically diagnosed and laboratory diagnosed sexually transmitted infections were treated.

2.5.2 Laboratory Procedures

BV was determined by laboratory based Nugent Score criteria, a gram stain scoring system of vaginal smears. It consists of a scoring system and ranges from 0-10. In this analysis a score of 7 or above was considered positive for BV. HIV infection was diagnosed by commercially available validated rapid HIV tests done in parallel; all positive and discordant tests were confirmed by a third generation Enzyme linked immuno-sorbent assay (ELISA). A 10 % sample of all tests was quality controlled by ELISA. NG and CT were detected using Roche Amplicor® polymerase chain reaction (PCR) and TV sample were cultured in Diamond's media and assessed qualitatively in the laboratory. Syphilis testing was done by rapid plasma reagin (RPR) and reported by titre and testing for HSV 2 was done by Focus Herpe Select® HSV 2 ELISA. Summary of study visit and data collection procedures are presented below in table 2.1:

Table 2.1: Study visit procedures

Procedure	**Visit**				
	Time 0	3 months	6 months	9 months	12 months
Demographic characteristics	Yes				
Sexual/Behavioural characteristics	Yes	Yes	Yes	Yes	Yes
BV test	Yes		Yes		Yes
HIV test	Yes	Yes	Yes	Yes	Yes
CT,NG,HSV2,RPR	Yes		Yes		Yes

2.6 MEASUREMENT AND DATA SOURCES FOR VARIABLES

2.6.1 Outcome Variable

The outcome variable of interest was HIV infection. This was measured at three monthly intervals by commercially available rapid test. Testing was done in parallel by trained nurses in the study clinic. The tests used included Determine®, Unigold™ and Serocon. Tests were changed due to logistic problems with supply. Each time a new test was introduced a confirmation of test performance was done by a third generation ELISA. Throughout the study all dual positive and any discordant results were confirmed by a third generation ELISA in the laboratory. In addition quality control ELISA tests were done on the first 100 rapid tests and on a 10 % continuous quality control confirmation of rapid results.

2.6.2 Primary Exposure Variable

The primary exposure variable, BV, was also determined at baseline and at the six and twelve month follow up visits, gram stain was taken from the vaginal wall by a swab, read in the laboratory qualitatively and scored by Nugent's scoring. For this analysis the exposure was considered as a categorical variable of the presence of BV by Nugent score of 7, greater than 7 or not.

The potential confounding variables such as condom use at last sexual act, number of sexual partners last three months, behavioural and demographic factors were determined by using structured interviews. Inconsistent condom use has been associated with the risk of BV as well as the risk of HIV infection. Increased number of sexual partners is also associated with a high risk of BV at the same time a high risk of HIV infection. These

factors were therefore taken as potential confounders and have been reported in other studies as confounders [14, 15]. STIs were also considered as potential confounders as they are known biological risk factors of HIV infection transmission [16], they were also considered for potential effect modification on the association of BV and HIV acquisition as they are also independently associated with the risk of HIV infection.

Vaginal cleansing alters the vaginal normal flora and is associated with an increased risk of BV [12, 13]. Vaginal cleansing, STIs and BV could concurrently influence the risk of HIV infection. Multivariate analysis was done to control for possible confounding effect of these factors. An assessment was also carried out to check for possible effect modification of STIs and vaginal cleansing on the association of BV and HIV infection acquisition.

2.7 DATA PROCESSING METHODS AND DATA ANALYSIS PLAN

2.7.1 Data Cleaning and Dataset Set up

Data cleaning and all the data analysis was done using STATA® version 10. The primary data sets were stored in a data base in three separate files. The required variables were extracted from each of the three files. All the participants screened without an enrolment number were dropped to remain with the enrolled participants only. Standard data cleaning procedures to identify values on variables was carried out by conducting checks on all the extracted variables to determine the extent of missing values and all the variable values entered as 9, 98 and 99 for example were set as missing. Then the data files were merged using participants' unique identity numbers to have one data set file for this analysis. Participants with missing BV results were excluded from the analysis.

2.7.2 Data Coding

To facilitate analysis some of the variables in the original data set were recoded. The variables for example, type of house, were recoded to include formal and informal type of houses only. The education status of the participants was recoded to have those with no schooling at all, those with less than 12 years of schooling and those with 12 years or more of schooling. Marital status was also recoded to include two groups, those living (cohabiting) with their sexual partners and those not living (not cohabiting) with their sexual partners.

2.7.3 Descriptive Statistics

The analysis was done using STATA® version 10 and all the variable values and measures of effect have been reported with 95 % confidence intervals and p values where appropriate. The descriptive frequency of social demographic and behavioural characteristics of study participants were calculated using contingency tables. The baseline prevalence of BV and STIs in the sample was also calculated using contingency tables. The chi square test of significance was conducted on categorical data and a Student's T test on continuous variables to compare the baseline characteristics of women who were positive for BV and those who were negative.

2.7.4 Analytical Statistics

To investigate the association of BV and HIV infection acquisition the following analysis was done:

HIV incidence was calculated; the incidence was estimated as the number of seroconversions and is expressed per 100 person years of follow up. Duration of follow up time at risk was defined as the mid point time from enrolment to time of seroconversion for those who seroconverted and from time of enrolment to last time of follow up or last HIV negative result for those who remained negative.

The risk factors for seroconversion analysed in the models include age, income, education status, marital status, number of sexual partners last 3 months, condom use at last sexual act, vaginal cleansing, contraception use, BV, Nugent score, NG, CT, HSV 2, TV and syphilis. Kaplan Meier survival time analysis was performed comparing time to event of exposed to unexposed and log rank test for significance in the time to event were calculated. A univariate Cox regression analysis to determine whether exposure to BV is associated with HIV infection was done.

The variables were then included in a multivariate Cox regression analysis with a set p value of ≤ 0.1 as a point of entry to include potential confounders. Product terms of vaginal cleansing and BV, STI and BV were run respectively to check for possible effect modification of these factors on the association between BV and HIV infection.

2.7.5 Ethical Considerations

The primary prospective cohort study has ethical clearance from the Human Research Ethics Committee (HREC); reference number M02-01-09. The participants were consented and signed an informed consent prior to study participation. Participant names were not used on all data that was collected on report forms and laboratory result forms.

The study participants were told the potential risks and benefits of the study prior to their participation in the study. They were free to or not to participate and had the freedom to withdraw at any time. Treatment for any STIs was offered freely to the participants in the due course of the study. The study protocol for secondary data analysis was assessed by the University of the Witwatersrand Human Research Ethics committee for ethical review and clearance. It was approved and its ethics clearance number is M080984. Data analysis was done only after the approval of the protocol by the ethics committee.

CHAPTER 3: RESULTS

This chapter presents the study findings. The results are based on the objectives of the study and cover the social demographic characteristics of the participants, the prevalence of BV and STIs, the estimated HIV incidence in the study sample and the evaluation of the association of BV and risk of HIV infection acquisition.

3.1 SOCIAL DEMOGRAPHIC AND BEHAVIOURAL CHARACTERISTICS OF THE PARTICIPANTS

There were 1089 potential participants screened and 750 were enrolled in a primary cohort study following HIV negative results. The participants were all from Soweto Township and had comparable baseline characteristics. The median age of the participants was 23, (inter quartile range of 20 – 28 years). The majority of the participants had 12 years or more of schooling (48 %), followed by those who had less than 12 years of schooling (43 %) and then those who did not go to school (9 %). They were mainly living in formal type of houses (88 %) and more than half (66 %) of the participants had a source of income. A large proportion of the participants (79 %) reported ever having used a condom, and only 7 % of the participants reported to have been practicing vaginal cleansing. The majority of the participants (82 %) were not living with a sexual partner and only 8 % of the women reported having more than one sexual partner. The participants made use of contraception with 57 % using hormonal contraception (injectable and oral contraception) and 43 % were on non hormonal contraception (condoms and natural methods). The primary cohort study participant flow from screening visit up to last follow up visit is outlined below:

Table 3.1: Primary study participant flow chart

Visit Type	(n) Participants	(n) Participants attained end point	(n) Participants end visit
Screening	1089	264-pregnant 39 –HIV positive 36-not willing	750
Visit 0 (Baseline)	750		750
Follow up Visit 1	750	13-pregnant 5-HIV positive 99-lost to follow up	633
Follow up Visit 2	633	21-pregnant 7-HIV positive 15-lost to follow up	590
Follow up Visit 3	590	34-pregnant 5-HIV positive 11-lost to follow up	540
Follow up Visit 4	540	41-pregnant 4-HIV positive 2-lost to follow up	538

There were 21 seroconversions experienced in the entire study. In total there were 236 participants excluded during the course of the study. These included 109 participants who were found pregnant during the study and 127 participants who were lost to follow up. This analysis involved 14 HIV seroconversions among 683 participants who had BV

results and had accumulated 286 person years of follow up depending on their specific time of exposure.

3.2 DESCRIPTION OF THE STUDY PARTICIPANTS AND BASELINE BV PREVALENCE

The table below describes the baseline characteristics and baseline BV prevalences of the study participants within the specific groups.

Table 3.2: Description of Study Participants and BV Baseline Prevalence

Characteristic	n (%)	BV at Baseline n (%)	No BV at Baseline n (%)	p Value
Education(n=750)				
No Schooling	68 (9)	32 (8)	32 (9)	
<12 yrs School	320(43)	171 (45)	139 (40)	0.42
>=12 yrs School	362(48)	177 (47)	175 (51)	
House(n=750)				
Formal	662(88)	334 (88)	310 (90)	
Informal	88(12)	46 (12)	36 (10)	0.47
Income(n=748)				
Yes	491(66)	248 (65)	230 (67)	0.65
No	257(34)	132 (35)	114 (33)	
Condom Use last sex act(n=750)				
Yes	594(79)	307 (81)	268 (77)	0.26
No	158(21)	73 (19)	78 (23)	
Ever vaginal cleansed (n=750)	51(07)	29 (08)	22 (06)	0.5
Yes	699(93)	351 (92)	324 (94)	
No				
Number of Sexual Partners last 3mths (n=691)				
One	638(92)	323 (91)	297(94)	0.19
More than one	53(08)	32 (09)	20(06)	
MaritalStatus(n=750)				
Cohabiting	138(18)	71 (19)	61(18)	0.71
Not Cohabiting	612(82)	309 (81)	285(82)	
Contraception(n=750)				
Hormonal	425(57)	218 (57)	194 (56)	0.72
Non Hormonal	325(43)	162 (43)	152 (44)	

The women who tested positive for BV at baseline were not statistically different from the women who tested negative for BV within the specific participants' characteristics. The overall baseline prevalence of BV in the study was 52 %, 95 % CI; 45 – 59. There does not seem to be any differences between those participants who had their BV results and those who had BV results missing, suggesting that BV results were missing at random. Most common reason for missing BV results was that samples were not taken at the time of the clinical visit because the participant was menstruating.

3.3 BASELINE STI PREVALENCE OF THE STUDY PARTICIPANTS

Herpes Simplex Virus 2 had the highest prevalence in the study sample (54 %). This was followed by CT, TV, syphilis and NG with the prevalence of 12 %, 4 %, 2 % and 2 % respectively, as shown in the table below:

Table 3.3: Description of Baseline STI Prevalence

Sexually Transmitted Infections	**n (%)**
N.gonorrhoea (n =685)	15 (2)
C.trachomatis (n = 688)	83(12)
T. vaginalis (n = 737)	31 (4)
Herpes Simplex Virus 2(n = 733)	393(54)
Syphilis (n = 747)	18 (2)

3.4 HIV INCIDENCE AND SURVIVAL TIME OF THE WOMEN

There were 683 participants included in the final analysis on HIV incidence and the association between BV and HIV infection acquisition. Participants were followed for a possible maximum period of one year and were followed for a mean period of time of 0.40 of a year. This accumulated 286 person years of follow up time at risk for the participants who had BV results. There were 21 HIV seroconversions experienced throughout the follow up period of which 7 had BV results missing and were excluded from this analysis. The remaining 14 incident infections represents a crude HIV incidence rate of 4.9 per 100 person years of follow up, 95 % CI: 2.9 – 8.27. There were 4 HIV seroconversions among the women who were BV negative within a total follow up period of 161 person years. This represented an HIV incidence rate of 2.49 per 100 person years of follow up, 95 % CI: 0.93 – 6.63. Likewise, among the women who were BV positive 10 HIV seroconversions were experienced within 125 person years of follow up. The HIV incidence rate among this group was 8.0 per 100 person years of follow up, 95 % CI: 4.3 – 14.87.

Survival Time analysis

The Kaplan Meier survival curves for HIV seroconversion comparing women who had BV to those who did not was performed and is shown below:

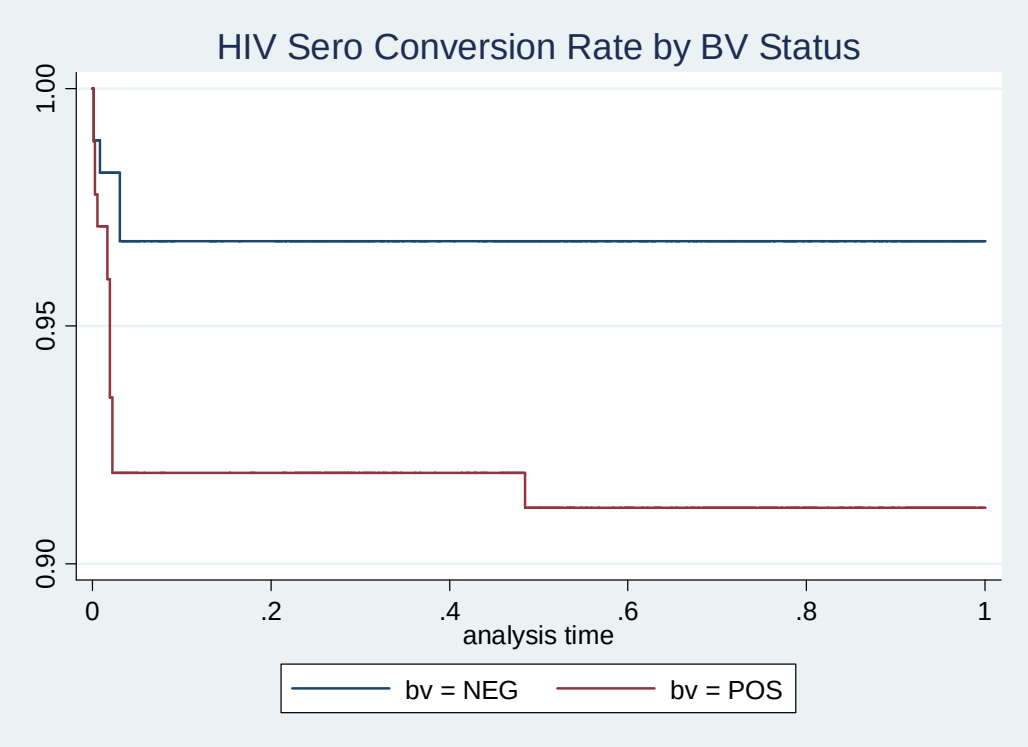

Fig 1: HIV sero conversion Rate by BV status.

A higher risk of HIV-1 acquisition was present among women who tested positive for BV than the women who tested negative for BV at every point through out the follow up period (Fig 1). A Log rank test was performed with a chi-square value of 3.8 and p value 0.05, meaning that the probability of seroconversion was different among the women depending on BV status.

3.5 ASSOCIATION OF BV WITH HIV SEROCONVERSION

The following factors were investigated for possible association with the risk of HIV seroconversion, BV, education level, age, income, contraception, marital status, number of sexual partners last three months, vaginal cleansing, BV Nugent score, NG, CT, and HSV 2, however, most of these did not meet the criteria for retention into the multivariate

model. Vaginal cleansing is also associated with BV and may concurrently increase the risk of HIV-1 seroconversion. To assess this synergistic effect, an interaction term of BV and vaginal cleansing was run and it revealed no effect modification on the risk of HIV-1 seroconversion between BV and vaginal cleansing. An interaction term was also run for STIs and BV and there was no effect modification observed.

The table below presents findings for univariate and multivariate Cox regression analysis to determine the association of BV with the risk of HIV-1 acquisition. The other covariates were also assessed. In the univariate out put, only sexually transmitted infections (NG and CT) had an association with the risk of HIV seroconversion which was statistically significant. The rest of the reported associations did not attain the statistical significance.

Table 3.4: Univariate and Multivariate Cox Analysis

	Cox Univariate Analysis		Cox Multivariate Analysis	
Variable	**HR (95 % CI)**	**P value**	**HR (95 % CI)**	**P value**
BV				
No	1			
Yes	2.97(0.93-9.52)	0.07	3.21(0.85-12.12)	0.08
Nugent Score	1.20(0.98-1.49)	0.08		
Age	1.02(0.94-1.11)	0.56		
Sex Partners Last 3months	1.94 (0.44-8.44)	0.37		
Education				
No schooling	1			
< 12 yrs Sch	2.28(0.29-17.7)	0.42		
≥ 12 yrs sch	1.58(0.2-12.52)	0.66		
Income				
No	1			
Yes	0.48(0.20-1.12)	0.09		
Ever vaginal cleansed				
No	1			
Yes	1.95(0.26-14.6)	0.51		
Marital Status				
Cohabiting	1			
Not Cohabiting	1.33(0.39-4.52)	0.64		
Contraception				
Non hormonal	1			
Hormonal	1.57(0.63-3.88)	0.33		
Gonorrhoea				
No	1			
Yes	7.4(1.61-33.67)	0.01	4.9(1.06-23.4)	0.04
Chlamydia				
No	1			
Yes	3.45(1.04-11.5)	0.04		
HSV2				
No	1			
Yes	2.22(0.70-7.09)	0.17		

3.5.1 Univariate Results

BV infection was associated with higher risk of HIV-1 infection acquisition though statistical significance was not achieved, women who were BV positive had an increased

risk of acquiring HIV-1 infection, HR, 2.97; 95 % CI, 0.93-9.52, p, 0.07 where as the risk of HIV-1 seroconversion for BV Nugent score was 1.2; 95 % CI: 0.98-1.49, p; 0.08, table 3.4. This means that for every one unit increase in Nugent score, the risk of HIV seroconversion increases by 20 %.

The results are suggestive of a decreasing trend in the risk of HIV seroconversion as the education level of the participants increased though statistical significance was not achieved, with the women who had 12 years or more of education being less likely to seroconvert than women with less than 12 years of education, HR, 1.58; 95 % CI; 0.2-12.52, p, 0.66, and 2.28; 95 % CI; 0.29-17.7, p, 0.42 respectively. Similarly participants age had no statistical significance on the risk of HIV seroconversion despite its slight positive trend towards an association, HR, 1.02; 95 % CI; 0.94 - 1.11, p, 0.56. This means that with an increase of the womens'age by one, there is an increase in the risk of HIV seroconversion by 2 %. Likewise women who had a source of income were 52 % less likely to seroconvert than the women who had no source of income, though statistical significance was not achieved either, HR, 0.48; 95 % CI; 0.20-1.12, p, 0.09.

The results show that vaginal cleansing may be associated with an increased risk of HIV-1 acquisition, hazard ratio, 1.95; 95 % CI; 0.26 – 14.6, p, 0.51. The women who practised vaginal cleansing were more likely to seroconvert than those who did not. Results also show that participants number of sexual partners could be associated with increased risk of HIV-1 seroconversion, HR; 1.94; 95 % CI; 0.44 – 8.44, P, 0.37 and that not living with a sexual partner may be associated with an increased risk of HIV-1 seroconversion, HR, 1.33; 95 % CI, 0.39-4.52; p, 0.64.

Though statistical significance was not attained, women who were on hormonal contraception were more likely to seroconvert than women on non hormonal contraception, HR, 1.57; 95 % CI; 0.63 – 3.88, p; 0.33.

The women who had NG infection had a statistically significantly higher risk of HIV-1 seroconversion, HR, 7.4; 95 % CI, 1.61-33.67, p, 0.01. Similarly, CT infection was also associated with a statistically significantly increased risk of acquiring HIV infection, HR, 3.45; 95 % CI; 1.04 – 11.5, p, 0.04. The women who were positive for HSV2 were 2.22 times, 95 % CI, 0.70-7.09, p, 0.17, more likely to seroconvert than the women who had no HSV 2; however statistical significance was not achieved in this case.

3.5.2 Multivariate Results

The variables gained entry into the multivariate model if they had a p of ≤ 0.1 in the univariate model, these included BV, BV Nugent score, income, NG and CT. Variables were retained in the multivariate model if they maintained a p of ≤ 0.1. The final model contained the variables BV and gonorrhoea. Therefore, after adjusting for other factors, BV was associated with an increased risk of HIV seroconversion, HR, 3.21; 95 % CI; 0.85 – 12.12, p, 0.08.

CHAPTER 4: DISCUSSION, RECOMMENDATIONS & CONCLUSION

This study produced no significant findings; however the findings suggest that BV is associated with an increased risk of HIV infection acquisition in women.

The study revealed a high baseline BV prevalence among women aged 18 – 35 years in Soweto. The BV baseline prevalence was 52 %; 95 % CI; 45-59 a finding which is similar to that of other studies in South Africa and many settings [5, 7, 8, 21]. The women who were BV positive were not statistically different from the women who were BV negative in any baseline demographic characteristics. This may be due to the fact that the women volunteered to participate in the primary study as such this made them to be similar in many aspects. The homogeneity could also be a result of the study population which only comprised women from a local South African setting in Soweto.

The baseline STI prevalences in this study are similar to those reported in other studies [21, 22]. The results also shows a high HIV incidence rate of 4.9 per 100 person years of follow up, 95 % CI; 2.9 – 8.27. This is relatively higher if compared to HIV incidence estimates reported in other areas in South Africa [28]. The majority of women in this study were not living with their sexual partners and had an increased risk of HIV seroconversion, HR, 1.33, 95 % CI; 0.39 – 4.52, p, 0.64. In a study to measure HIV incidence in a rural area of South Africa in KwaZulu Natal, an HIV incidence rate of 3.8 per 100 person years of follow up, 95 % CI; 3.2 – 4.6 [28] was reported. Similarly, the HIV risk of seroconversion in the KwaZulu Natal study was twice as high among women

who were unmarried but had a sexual partner than those who were married. Not living with a sexual partner, may have increased the risk of HIV seroconversion among women in this analysis as they may have been more likely to engage in risky sexual behaviours and have frequent sexual partner changes as may their partners, these factors may have increased the risk of BV infection among the women as well. High rates of infection in this case may therefore be attributed to high rates of partner change among the women.

A similar HIV incidence rate of 4.51 per 100 person years of follow up, 95 % CI; 2.96 – 6.06 was reported in Malawi among women of reproductive age [24]. It was reported in the study that BV was a strong predictor of HIV infection acquisition. Likewise the results of this analysis, show the same pattern that women who were BV positive were more likely to seroconvert than the women who were BV negative, HR, 2.97, 95 % CI; 0.93-9.52, p, 0.07. BV may affect HIV-1 acquisition in many ways; these include vaginal pH changes, changes on the integrity of the vaginal epithelium and changes in the genital microflora that occur with BV [25]. These changes may increase the women's risk of HIV infection acquisition.

After adjusting for demographic factors, other STIs and sexual behaviours, it was observed that BV was associated with the risk of HIV seroconversion, adjusted hazard ratio 3.21, 95 % CI; 0.85-12.12, p value 0.08. This suggest that BV is associated with a higher risk of HIV-1 infection acquisition with the BV positive women being 3 times more likely to seroconvert than the women who were BV negative within the follow up period. However, the confidence interval is wide (0.85 – 12.12), meaning that the population value may be anywhere in between 0.85 and 12.12. In addition the confidence

interval includes one and the p value is greater than 0.05, implying that the association is not statistically significant. The reasons for this wide confidence interval include sample size which was small and few incident infections that were included in the analysis due to the exclusion of 7 incident infections.

These results of BV being associated with an increased risk of HIV infection acquisition are also reflected in the findings of other studies conducted in different settings on the temporal effect of the association of BV and HIV infection.

A prospective study conducted in Malawi reported a significant association of BV with the risk of HIV seroconversion, an adjusted risk ratio for BV of 2.3, p; 0.03 was reported [22]. A similar pattern was also found in another prospective study done in Mombasa, Kenya [23], BV was found to be significantly associated with HIV incident infections with an adjusted hazard ratio of 1.9, 95 % CI; 1.1-3.2. Likewise findings obtained recently in a prospective study of HIV-1 incidence among women of reproductive age in Malawi, an association between BV and HIV infection acquisition was observed, adjusted hazard ratio, 2.52, 95 % CI; 1.07 – 5.94; p; 0.03 [24]. Positive findings were also obtained in a multi centre prospective study conducted in Zimbabwe and Uganda, BV was found statistically significantly associated with HIV infection acquisition, AHR 1.67, 95 % CI; 1.24-2.26[25]. Similarly, positive findings were reported in a meta-analysis to assess the extent to which BV may increase the risk of HIV acquisition. BV was statistically significantly associated with HIV infection acquisition, ARR 1.6, 95 % CI; 1.2-2.1[26].

Statistical significance was not achieved in this analysis possibly due to a small number of the BV positive women who seroconverted within a total person time of follow up of 125 person years whereas the women who were BV negative who seroconverted were followed up for a total person time of 161 person years. Lack of statistical significance could also be explained as a result of the potential bias introduced by the exclusion of the 7 HIV seroconversions in this analysis which had no BV results. If this study is compared to the Malawi study [24] which had a similar sample size, but had far more HIV seroconversions analysed than those analysed in this analysis thereby achieving statistical significance. The effect size of the association of BV with the risk of HIV seroconversion is also relatively higher in this analysis than that reported in many studies [22, 23, 24, 25].

This could result from a wide range of factors; firstly the prevalence of BV in the study participants though comparable to other settings was relatively higher. In a high BV prevalence setting it may be difficult to detect differences in seroconversion rates. Besides this, most of the participants too were not living with a sexual partner; this could be associated with frequent change of new sexual partners and hence increased risk of BV and STIs among the women and hence increased risk of HIV infection. These factors may have inflated the effect size of BV with the risk of HIV seroconversion.

The risk described in this study supports the biological plausibility of the factors described in the relationship of BV and HIV-1 acquisition. Many factors may lead to HIV-1 infection acquisition in women with BV. The normal vaginal flora which plays a protective role in women's susceptibility to sexually transmitted infections is disturbed; there is depletion of lactobacilli which may limit production of hydrogen peroxide and

other antibacterial activities which are protective against potentially pathogenic organisms such as those that cause sexually transmitted infections and possibly HIV. The low vaginal pH has been suggested to inhibit CD4 lymphocyte activation and reduce HIV target cells in the vagina [22]. In this case lack of lactic acid produced by lactobacilli could lead to an elevated pH which could be conducive to the growth and survival of the virus. BV also increases HIV-1 acquisition by changing cervicovaginal epithelial integrity and permeability and it is also suggested that it causes an inflammatory reaction in the female genital tract [25]. With these mechanisms present in BV positive women their risk of HIV infection may be increased.

4.1 STRENGTHS OF THE STUDY

This study is a cohort study which allows the exposure (BV) to have been measured prior to the outcome (HIV) and its temporal sequence adds to the growing knowledge of BV being associated with the risk of HIV infection acquisition among women. The primary exposure in the study was defined by laboratory Nugent score criteria. This criterion is classified as the gold standard for the diagnosis of BV [10]. This reduced measurement error and misclassification of the exposure.

The definition of the outcome too was confirmed by ELISA which has a high specificity [30]. This made it possible to precisely classify participants who truly had the HIV virus as outcomes, this as well reduced misclassification of the outcome. The primary cohort study too had a good follow up and retention rate, above 80 %, and in addition, the data used in this analysis is local South African data from the general population.

4.2 LIMITATIONS OF THE STUDY

The interpretation of the findings in this study is subject to important limitations:

4.2.1 Volunteers

The participants in the primary cohort study volunteered to take part as such they may differ with those who did not take part. This may have an influence on the generalizability of these findings due to selection bias this may have introduced.

4.2.2 Information Bias

Although interviewers were trained to conduct structured interviews, interviews can introduce information bias, and there is a possibility that participants may have hidden some pertinent information for this study.

4.2.3 Secondary Data Analysis

This study is a secondary data analysis of a primary cohort study that was designed for the evaluation of another research question and not the association between BV and the risk of HIV infection acquisition, therefore BV was not specifically taken as an exposure of interest and some information in this case was not available which was pertinent for the evaluation of this research question. For example, BV results for seven participants who seroconverted were missing and these were not included in the analysis. This may have biased the study findings.

4.2.4 Few number of end Points analysed

The number of HIV seroconversions analysed was low due to the exclusion of those that had no BV results; this made it difficult to find the statistical significance of the association of BV and HIV infection acquisition and made the confidence intervals wider.

4.2.5 Misclassification of end Points

In addition the confirmation of HIV status at baseline did not include PCR testing for the presence of virus prior to an antibody response, this may have misclassified already HIV positive women at baseline as HIV negative, thereby inflating seroconversion rate in the study.

4.2.6 Loss to Follow up

Though the retention rate was high, above 80% in the primary study, the women who were lost to follow up may differ considerably to the other participants in the study, thereby affecting the generalizability of the findings of the study. In addition some outcomes may have been missed among women who did not complete the follow up.

4.2.7 Un measured confounding and Limitation of Cox regression model

Further to this, unmeasured confounding may have overestimated the association between BV and HIV seroconversion. Although a range of confounding variables were adjusted for, including sexual behaviours and different STIs, the possibility of residual confounding cannot be ruled out that may have contributed to the observed adjusted hazard ratio. In addition to this, Cox regression model could not be performed on the variable ever used condom because there was one participant who seroconverted who was not using condoms as such the data could not converge. Similarly, regression could not be done on syphilis and TV because there was one participant who seroconverted who had syphilis and there was no participant who seroconverted who tested positive for TV.

4.3 RECOMMENDATIONS

Based on the limitations of the study, the following recommendations are proposed. Another study designed specifically to answer the question of whether BV is associated with HIV-1 acquisition be conducted, with an adequate sample size and powered to detect a difference in the number of seroconversions between the two groups. The study should take BV as the primary exposure of interest and gather all the information regarding BV results on all the participants. A study describing the association between the treatment of BV and HIV-1 infection acquisition would also be of interest.

4.4 CONCLUSION

In summary BV is an ongoing burden among women, evident with its high prevalence in this cohort. This study contributes to the knowledge that BV is associated with HIV infection acquisition, the women who were BV positive were more likely to seroconvert than the women who were BV negative, although not significant. The vaginal discharge caused by BV may interrupt the lives of many women so improved care should be provided to screen and treat BV in the primary health care setting. Women should also be exposed to education on vaginal cleansing as this predisposes them to BV.

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