Is the use of hormonal contraception a risk factor for incident sexually transmitted infections in a cohort of women aged 18 to 35 in Soweto South Africa?

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of

Master of Science in Medicine in the field of Epidemiology and Biostatistics

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Examiners comments included March 2010

Declaration

I, Jocelyn Anstie Moyes (8412861) declare that this research report is my own. It is being submitted for the degree of Master of Science in Medicine in the field of Epidemiology and Biostatistics at the University of the Witwatersrand, Johannesburg. This report has not been submitted before for any other degree or examination at this or any other University.

March 2010

To my children, Samuel and Thomas, who both arrived during the course of this degree, thank you for the time to finish this report.

1.1 Ethics committee approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Moyes

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080842**
PROJECT	Is the use of Hormonal Contraception a Risk Factor for Incident Sexually Transmitted Infections in a Cohort Study of HIV Negative Women Aged 18 to 35 in Soweto? *a* Secondary Data Analysis
INVESTIGATORS	Dr J Moyes
DEPARTMENT	School of Public Health
DATE CONSIDERED	08.08.29
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.09.01

CHAIRPERSON
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr s Delany-Moretlwe

DECLARATION OF INVESTIGATORS)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

1.2 Abstract

Introduction

This secondary data analysis of a prospective cohort study set out to describe the association between the use of hormonal contraception and sexually transmitted infection (STI) acquisition in a cohort of 752 HIV negative women who were followed up for a year.

Methods

Outcome variables were measured by standard laboratory tests (PCR for *Chlamydia trachomatis* (CT) and *Neiserria gonorrhoea* (NG)*,* culture for *Trichomonas vaginalis* (TV) and gram stain with Nugent score for Bacterial Vaginosis (BV). Exposure variable information was collected by structured interview. Basic descriptive statistics were applied to describe the characteristics of the cohort, including a comparison of women who used contraception and those who did not. A time series analysis including incidence rates for the outcomes (CT, NG, TV and BV), Kaplan Meier curves for time to event measurement and Cox regression models (univariate and multivariate), for the estimation of risk were applied.

Results

The analysis found no significant difference between women who use hormonal contraception and those who did not with respect to baseline demographic characteristics. Incidence rates per 100 women years to follow up with 95% confidence intervals were: CT 13 (7 to 17), NG 2 (1 to 4), TV 6 (4 to10), BV 72 (63 to 83). Kaplan Meier curves showed no significant difference in time to event between

women who used contraception and those who did not. Adjusted hazard ratios for women who used contraception was 1.12 (0.69 to1.82) for CT, 0.47 (0.17 to 1.30) for NG, 1.06 (0.48 to 2.34) for TV and 0.27 (0.05 to 1.52) for BV.

Conclusion

This analysis did not reveal any significant associations between the use of hormonal contraception and the acquisition of STIs, however the trends in risks follow those reported in the literature.

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1.5 Glossary of Abbreviations

AHR	Adjusted Hazards Ratio
AOR	Adjusted Odds Ratio
BV	Bacterial Vaginosis
CI	Confidence interval
CT	*Chlamydia trachomatis*
DALY	Disability Adjusted Life Years
DMPA	Depot medroxyprogesterone acetate
HIV	Human Immunodeficiency Virus
HR	Hazards Ratio
HSV 2	Herpes Simplex Virus type 2
Net EN	Norethisterone Enanthate
NG	*Neisseria gonorrhoea*
OC	Oral contraception
OR	Odd Ratio
PAP	Papanicolaou test
PCR	Polymerase Chain Reaction
RPR	Rapid plasma reagin
SIV	Simian Immunodeficiency Virus
STI	Sexually Transmitted Infection
TV	*Trichomonas vaginalis*

2.0 BACKGROUND AND LITERATURE REVIEW

Sexually transmitted infections (STI) are still a major health concern in both the global and South African contexts. Factors contributing to this burden include individual physical factors, risk behavior factors, social and health care factors. Due to the high burden of disease and the associated morbidity, strategies to decrease the risk of acquiring STI are important. Hormonal contraception is widely used; if the use of hormonal contraception is an additional risk for acquiring sexually transmitted infection then strategies to address type of contraception use and dual use of barrier methods would be extremely important.

2.1 Disease burden of sexually transmitted infection

STIs are still a major public health concern despite effective antibiotic treatment for all the bacterial infections. Globally, on an annual basis, an estimated 150 million new cases of *Chlamydia trachomatis* (CT) and *Neiserria gonorrhoea* (NG) occur (1). The incidence of infections increases to 333 million cases when *Treponema pallidum (*Syphilis) and *Trichomonas vaginalis (*TV) are included (2). These treatable inflammatory bacterial infections are associated with severe complications including pelvis inflammatory disease, infertility and adverse pregnancy outcomes (3). An additional complication of STIs, particularly ulcerative infections, is HIV acquisition and transmission (4, 5).

This large burden of disease is the second largest cause of the loss of healthy life years in women of reproductive ages in the developing world (6).

CT and NG infections are thought to lead to the loss of as many as 7 million disability adjusted life years (DALYs) (7). The burden of disease due to STIs in South Africa is estimated to be 49 000 DALY in 2000, with disease in women contributing to 31 000 of these DALYs (8). Factors contributing to this include migrant labour, poor access and quality of health care in disadvantaged communities, the socio-economic disadvantage of women, low condom use, high level of urbanization (compared with other African countries) and a well developed transport system (8).

In South Africa STIs are highly prevalent. In a survey of women attending rural family planning clinics the prevalence of NG was 3-4% and CT 8-12% (9, 10). South African young women, in a population based survey, were particularly affected with as many 5% of women aged 15 being infected with CT , increasing to 13% of women aged 24 (11). Results from a study conducted in Kwa-Zulu Natal showed that 25% of women in the reproductive age group had at least one curable STI (CT, NG, TV or syphilis) on any given day (10). This alarming number is particularly concerning as half of these infections were asymptomatic (10).

Only three studies have looked at trends over time and in these studies the prevalence of CT and syphilis in pregnant women in Hlabisa, Kwazulu Natal, declined significantly between 1995 and 2000 (3). However a series of surveys in Carltonville, North West Province, showed an increasing prevalence of syphilis, NG and CT (in men and women, in the general population and in high risk groups) between 1998 and 2000 (3). In the same area, the detection of Herpes Simplex

Virus type 2 (HSV2) increased while the prevalence of syphilis remained unchanged in the period between 1993/4 and 1998 (12).

The South African Department of Health conducts annual surveys of HIV and syphilis prevalence in women attending antenatal clinics. These surveys report that the prevalence of syphilis has been declining since 1997 (3). The Department of Health also collects data on STI in public health clinics. These surveys have not described any significant trends in either direction (3).

When compared with other African countries the burden of STIs in South Africa is high, with the prevalence of NG, CT and TV in South Africa higher than those reported in surveys conducted in several other African countries, including Tanzania, and Uganda (3).

Although Bacterial Vaginosis (BV) has not been included in the classical definition of a STI it is the most common cause of symptomatic vaginal discharge in women of reproductive age (13). BV and the absence of lactobacilli are associated with sexual activity (14). The prevalence of BV ranges from 4% in college women in the USA, to as much as 60% in some STI clinics (15). BV is associated with pelvic inflammatory disease, endometritis, infection after gynaecological procedures, increased risk of other sexually transmitted diseases, chorioamnionitis, premature labour and premature rupture of membranes (16).

2.2 Hormonal contraception use

Hormonal contraception has provided many women with a family planning option that is safe, convenient and reliable. Global figures reflect that as many as 100 million women worldwide use hormonal contraception, which includes the oral contraceptive pill and long acting progestin injectable: depot medroxyprogesterone acetate (DMPA) (17). It is estimated that 65 million women use the oral contraceptive world wide and 40 million of these are in the developing world (18). This is a 65% increase from 14 million women in 1977 (18). In South Africa, 32% of currently sexually active women use injectable hormonal contraception and 56% of women aged 15 to 49 have used injectable hormonal contraception at some stage in their reproductive life time (19). There are two options for injectable contraception in South Africa, DMPA and Norethisterone Enanthate(NET-EN). There are very few countries that use NET-EN, and about 14% of injectable uses in South Africa use NET-EN (19).

2.3 The link between the use of hormonal contraception and the acquisition of sexually transmitted infections

The available published data includes cross sectional and prospective cohort studies. Most of the data concentrates on the association between the use of oral contraception and the acquisition of CT. Many of the studies are analyses of data collected for other purposes and so lacked the ability to adjust for confounders, the definition of exposure to hormone is not defined, or the use of barrier methods is not mentioned. Substantially more data is available describing the association between the use of hormonal contraception and the acquisition of HIV. The evidence to

support this is based in prospective cohort studies carried out in Kenya and Uganda(20, 21). In a population of commercial sex worker who used oral contraception the risk of acquiring HIV was 1.46 times that of women who did not use contraception (Hazard ratio(HR) 1.46 95% confidence interval (CI) 1.00 to 2.13). Women using DMPA were slightly more at risk of acquiring HIV than those women not using contraception (HR 1.76 95% CI 1.28 to 2.34). A more general population cohort of women in Uganda did not have the same risk with incident rate ratios of 1.12 (95%CI, 0.48 to 2.56 for oral contraception uses and 0.84 (95%CI, 0.41 to 1.72). Many of the analyses conducted on the association between the use of contraception and the acquisition of bacterial STI are based on the same cohorts.

2.3.1 *C. trachomatis* infections

A quantitative review calculated a pooled odds ratio (OR) for 29 case-control studies for the association between the use of oral contraception and the acquisition of CT infections. A pooled summary crude OR of 1.93 (95% CI 1.7 to 2.1) was reported suggesting a significant increased risk of acquiring *C. trachomatis* infection in women who used oral contraception (22). The authors also calculated odds ratios for those studies where confounding was not measured and this analysis did not alter the summary OR 1.92 (95% CI 1.73 to 2.12) significantly. There was little change to the odds ratio for studies that did adjust for confounders OR 1.98 (95% CI 1.67 to 2.34). However, when comparing oral contraception users to users of barrier methods, women using barrier methods were 66% less likely to acquire CT infections. (OR 0.34 95% CI 0.22 to 0.54) (22).

Five prospective cohort studies, summarized in table 2.1, report associations between the use of hormonal contraception and the acquisition of CT. Three of these studies are in general population women: In Belgium the study reported a crude relative risk (RR) of 8.8 (95% CI 1.3 to 59) (22); In the United States of America (USA) the study reported an adjusted hazard ratio (AHR) of 4.3 (95% CI 1.7 to 11.1) (24) and a single study, conducted in Sweden, did not follow the trend and reported a crude OR of 0.67 (95% CI 0.27 to 1.68) (34). In addition two studies drew cohorts from commercial sex workers (CSW) in Kenya and reported an AHR of 1.5 (95% CI 1.1 to 2.9) for the association between the use of oral contraception and acquisition of CT infections in HIV negative women and an AHR of 2.2 (95% CI 0.7 to 7.3) in HIV positive women (25, 45).

Similar increased risks are reported for the association between the use of DMPA and the acquisition of CT infections. Adjusted hazard ratios of 1.6 (95% CI 1.1 to 2.4), 3.1 (95% CI 1.0 to 9.4) and 4.3 (95% CI 1.7 to 11.1) are reported (24, 25). In two studies presented at an international conference in 2007, but not yet published, very similar results are reported. Morrison et al in a study conducted in three countries reports AHR for OC uses of 1.39 95% CI 1.09 to 1.77) and AHR for DMPA uses of 1.09 (95% CI 0.84-1.41(47). Pettifor et al in a smaller study reports an adjusted rate ratio of 1. 04(95% CI 0.62-1.76) (48).

2.3.2 *N. gonorrhoea* infections

There is much less published literature on the association between the use of oral contraception and the acquisition of NG infections. A review of 21 cross sectional studies reported associations in only four studies. In these studies the risk of NG infection among oral contraception users varied with adjusted odds ratios (AOR) ranging from 1.53-5.25 (95% CI 1.09 to 26.25) being reported (26). Similar finding were presented at an international conference AHR 1.39 (95% CI 1.09-1.77) (47). Interestingly in the presence of DMPA use, cross sectional studies reported a decreased (but not significant) odds of acquiring NG infection OR 0.23 (95% CI 0.01 to 2.37) when compared to IUD users, and an OR 0.23 (95% CI 0.01 to 3.65) when compared to women using not using contraception (24). Prospective studies report no difference in risk with adjusted HR 1.1 (95% CI 0.8 to 1.6) and 1.0 (95% CI 0.6 to 1.7) reported (25, 45). Similar results were presented at an international conference from two studies of women using DMPA with AHR 1.50 (95% CI 1.10-2.05) and ARR of 1.02(95% CI 0.5-2.11) (47, 48).

2.3.3 *T. vaginalis* infections

Data describing the association between the use of oral contraception and the acquisition of TV are divided on whether there is any risk association. Cross sectional data reports non significant associations including decreased and increased odds of infection (odd ratios ranging from 0.4 to 3.96 (95% CI 0.14 to 88.65). Significant results, all with a protective association, range from OR of 0.38 to 0.71 (95% CIs 0.15-0.98) (26). Prospective cohort study data follows the decreased risk trend with an AHR of 0.9 (95% CI 0.7-1.3) reported (26). Evidence linking the

use of DMPA with TV infection is based on a single cross sectional study reporting OR 1.0 (95% CI 0.19-1.3) (26) and a single prospective study reporting an AHR of 0.6 (95% CI 0.4-1.0) (25). A study presented at an international conference reported ARR 0.66 (95% CI 0.24-1.85)(48)

2.3.4 Bacterial Vaginosis infections

A prospective cohort study reports a decreased risk of BV in both women who use oral contraception and those who use injectable contraception, AORs of 0.76 (95% CI 0.63-0.9) and 0.64 (95% CI 0.53-0.76) respectively (15). In a similar study, amongst CSW, AHR of 0.8 (95% CI 0.7-1.0) and 0.7 (95% CI 0.5-0.8) for oral contraception users and injectable contraception users were reported (25).the results from a study presented at an international conference are similar in trend ARR 0.85 (95% CI 0.73-0.99) (48).

Table 1.1 Summary of published literature

Author	Objectives	Population	Study design	Summarized results	Comments
Cottingham and Hunter 1992 (22)	To assess the strength and consistency of the association between the use of OC and CT.	Many different: mostly studies conducted in Europe and North America.	Quantitative review	• Pooled estimated unadjusted OR for 29 case control studies was 1.9 (1.77-2.11) neither study quality nor prevalence affected this result. • When compared to barrier methods the OR increased to 2.91 (1.86-4.55)	• Review methodology clearly described • Studies were grouped according to whether they were prospective or case control, data were extracted and pooled estimates of unadjusted OR were made for all • Many of the studies were cross sectional and case control in design • Most of the studies were secondary analysis of data for other objectives • Many of the studies did not control for confounders • Compared single exposure (OC) to single outcome (Chlamydia)
Mohllajee et al 2006 (26)	Review of literature published	Many different populations	Systematic review	• Wide range of OR reported in the different studies, no summary odd ratios calculated	• Most recent articles included in this table under original articles
Baeton et al 2001 (25)	To examine the relationship between use of OC or DMPA and	CSW, Mombasa Kenya, HIV negative	Prospective cohort study 1202	• Women using OC were at increase risk of CT infection HR 1.8 (1.1-2.9) and decreased risk of BV	• Good study design, methods well described • Length of hormone exposure documented

	STI acquisition.		enrolled and 948 included in analysis. Median follow up was 421 days.	HR 0.7 (0.5-0.8), • Non significant increased risk of NG1.4 (0.9-2.1) and TV 0.9 (0.7-1.3) • Women using DMPA were at significantly increased risk of CT infection HR 1.6 (1.1-2.4) and decrease risk of BV HR 0.7 (0.5-0.8, TV HR 0.6 (0.4-1.0) and non significant AHR 1.1(0.8-1.6) for NG infections • Consistent condom use was associated with a significant decrease risk of GC, CT and BV.	• Exposure defined as either OC or DMPA • Outcomes measure by standard laboratory methods • Confounders included in analysis include age, education level, years in CSW, parity, number of sexual partners, number of sexual contacts and condom useage.
Morrison et al 2004 (24)	To measure the effect of oral contraceptives (OC) and DMPA on the acquisition of cervical infections	Women attending reproductive health centres in Baltimore, USA	Prospective cohort 1003 women enrolled, 819 included in the analysis. Follow up analysis as per segment exposed to hormone.	• OC not associated with infection AHR 1.5 (0.6-3.5) • DMPA was associated with infection HR 3.6 (1.6-8.5). • Cervical ectopy was not a predictor of infection	• Grouped cervical infections together as single outcome. • Hormonal exposure well documented and commenced at start of study • Laboratory methods to define end points • Confounders included in analysis age, ethnicity, socioeconomic, number of partners and condom use.

Avontis et al 1990 22)	To estimate the incidence of genital infection in women using hormonal contraception or IUD	General population women, in Belgium	Prospective cohort , 128 women on OC and 108 with IUD. 24 months of follow up.	• Crude RR 8.8 (1.3-59)	• OC compared to IUD • No confounders in model • Single outcome, CT
Lavery et al (45)	To evaluate the relationship between the use of hormonal contraception and the acquisition of cervical STI among HIV infected women	HIV positive CSW in Kenya	Prospective cohort, 242 women contributing 799 person years of follow up	• OC AHR CT 2.2 (0.7-7.3), NG 0.6 (0.3-1.3) • DMPA, AHR CT 3.1(1.0-9.4). NG 1.0 (0.6-1.7)	• Design similar to other Kenya study (23). Women who seroconverted in cohort follow up for this study. • Single exposure, well defined • Separate pathogenic outcomes defined • Good reporting on collection of data on confounders • Adjusted for confounders in analysis
Rahm et al, 1991 (34)	To describe the incidence of CT infections in sexually active teenage girls	Teenage girls in Sweden	Prospective cohort. 301 girls in 1 year follow up	• RR 0.67 (0.27-1.68) • OC only	• Statistical methods not well described, seems to be a crude RR. • Does describe ectopy and changes in this over time

Pettifor et al 2007(48)	To describe the association between the use of injectable contraception and the risk of GC, CT, BV and TV infections	Family planning clinic, South Africa	Prospective cohort study. 551 women 1 year of follow up	• Rate Ratio (RR) • ARR CT 1.04(0.62-1.76) • ARR GC 1.02 (0.50-2.11) • ARR TV 0.66(0.24-1.85) • ARR BV 0.85(0.73-0.99)	• Not yet published in a peer review journal • Injectable contraception only • Small sample size • Exposure not defined
Morrison et al (2007)(47)	To describe the association between the use of OC and DMPA and the acquisition of CT and GC	Women attending family planning clinics in Uganda, Zimbabwe and Thailand	Prospective cohort study. 6109 women, 2 year follow up	• OC AHR CT 1.39(1.09-1.77) GC 1.7 (1.25– 1.31) • DMPA AHR CT 1.09(0.84 -1.41) GC 1.50(1.10-2.05)	• Not yet published in peer review journal • Large cohort of general population women, long follow.

2.4 Proposed biological changes that may increase the risk of infection

There are several documented changes that occur in the genital tract associated with the use of hormonal contraception that change the normal defense mechanisms of the vaginal and cervix leading to an increase in susceptibility to infection. Firstly the use of DMPA leads to a hypo estrogenic state which is characterized by thinning of the vaginal epithelium which has been demonstrated to increase transmission of simian immunodeficiency virus (SIV) in macaques (27). Secondly the use of DMPA has been associated with a reduction in lactobacilli resulting in an increase in vaginal pH (28-30). A low vaginal pH is thought to contribute to vaginal natural defense mechanisms against bacterial and viral infective agents. Thirdly in a state similar to pregnancy, the oestrogen and progesterone hormonal influence of contraception may suppress the local immune system in the vagina. The importance of these local immune changes remains unclear (32, 33.) A forth possible mechanism of action is cervical ectopy, which is the extension of the columnar endocervical lining onto the outer surface of the cervix. This exposes a single layer of cells and blood vessels to trauma during intercourse and infection. Cervical ectopy is common in adolescents (33-36), pregnant women (35) and in women who are using oral contraception. (34, 36, 37) Cervical ectopy has been associated with increased risk of incident CT infections, HR 2.3 (95% CI 0.9-6.0) (34, 23) and with prevalent CT infections (34,37,38,39). This is caused by the infective agent's preference for infecting columnar epithelium such as is found in the cells exposed by cervical ectopy. Interestingly the same associations are not shown for gonorrhea infection (23, 30). In addition to the changes in vaginal environment caused by hormonal changes

animal studies suggest that progesterone and oestrogen may enhance the growth and persistence of the bacteria associated with genital infection. (25, 31, 32). There is no evidence that looks specifically at the use of NET-EN or that compares the changes in NET-EN uses to DMPA uses.

2.5 Summary

As the use of hormonal contraception is widespread and forms the backbone of family planning services in South Africa and STIs are highly prevalent in South Africa, it remains important to consolidate the evidence of the risk of STI in the presence of hormonal contraception. Additional concerns about the potential increased burden of STI globally, at present, may introduce the need to reassess messaging to women using hormonal contraception. The existing data suggests that there is an increased risk associated with the use of hormonal contraception and the acquisition of STI, particularly CT infections. Although there is not as much data for the risk of NG and TV the trends are similar in the available data. The protective effect of hormonal contraception use on the acquisition of BV is encouraging, given the increased risk of HIV acquisition associated with BV and the impact of BV on pregnancy outcomes. Most data have been collected in Europe and America, with the exception of the Kenyan cohorts. There is currently no published data that describes this association in the South African setting.

2.6 Justification for this secondary analysis

Sexually transmitted infections are still prevalent in South Africa and hormonal contraception use is very common (9, 10, 19). The link between the use of hormonal contraception and the increased risk of acquisition of STI follows a trend of slightly increased risk but no evidence, at this stage, is strong enough to assign causality.

This secondary analysis of the risk of acquiring STIs in women who use hormonal contraception arises from the availability of appropriate data following the conduct of a prospective cohort study. This cohort of South African women in an urban setting, a setting at high risk of STI, will provide locally appropriate data and will add to the evidence collected globally to justify a randomized control trial to document the association between the use of hormonal contraception and the acquisition of sexually transmitted infections.

2.7 Background to the parent study

During preparation for a phase three trial of a candidate vaginal microbicide a cohort of HIV negative women aged 18 to 35 were enrolled into a prospective cohort study. The objectives of the study were to estimate HIV incidence, condom usage and cohort retention. Additional inclusion criteria included: in good health, expect to be sexually active during the 12-month study period, use the Soweto primary health care services regularly, have an identifiable address (or mechanism to make contact) during the study, use a recognised method of contraception, were willing and competent to give informed consent, were willing to undergo clinical evaluations,

including pelvic examination and specimen collection according to the protocol, and were willing to receive regular HIV/STI risk reduction counselling. Enrolled participants were screened for CT and NG endo-cervical swab and BV and TV by vaginal swab. Participants attended the study clinic 12 weekly for a period of one year and were tested for STIs at week 24 and 52.

2.8 Research Question

Is the use of hormonal contraception associated with incident STI infections in a cohort of HIV negative women in Soweto South Africa?

2.9 Objective

To describe the association between the use of hormonal contraception and incident sexually transmitted infections.

3.0 METHODS

This secondary data analysis examines the relationship between the use of hormonal contraception and the acquisition of sexually transmitted infections in a prospective cohort study conducted in Soweto, South Africa. The cohort of volunteers was made up of HIV negative women aged 18 to 35 attending primary health care clinics in the Soweto area. Enrolment into the study included written informed consent. All 752 women enrolled into the cohort study were included in this analysis.

3.1 Clinical procedures

Women were examined in the clinic at enrolment and then at six and twelve months of follow up. All STIs were treated syndromically at the clinic visit and followed up with treatment for laboratory diagnosed infections. Genital examination included the collection of samples from the vaginal wall for BV and TV. Endocervical specimens were collected for CT and NG by a Dacron tipped swab. Data on exposure variables were collected by structured interview conducted by a trained nurse. Data were collected, by structured interview, for possible confounder variables; age, level of education, type of sexual partner, number of sexual partners and condom use. All interview forms were reviewed for quality control in the clinic and again at the data centre. Data were double entered into an Epi-Info™ database.

3.2 Lost to follow up

Women were discontinued from the study on the diagnosis of HIV or pregnancy. In addition women who did not complete follow up were censored at the last follow up visit they attended. Women contributed to the study follow up time up to the last clinical visit that they were seen at.

3.3 Measurement of outcome variables

The following laboratory based tests were performed to define a sexually transmitted infection: Samples for NG and CT were tested qualitatively by polymerase chain reaction (PCR) (Amplicor ® Roche Diagnostics Systems, Inc. Branchburg, N.J). Swabs taken from the vaginal wall were examined by microscopy for the presence of BV and scored by Nugents criteria; a positive BV result was a Nugents score of greater than seven. Specimens for TV were cultured in Diamonds media and examined qualitatively in the laboratory. For the purpose of this analysis each baseline STI was considered treated and cured, so that infections identified at the six month or twelve month follow up visits were classified as incident infections. For the time series analysis each incident infection provided a failure event, this 'failed' women at the point of diagnosis of infection. This analysis then excludes a repeated measure or clustering of a second infection in an individual.

3.4 Measurement of exposure variable

The primary exposure variable considered in this analysis is the use of hormonal contraception: specifically the injectable contraception (either DMPA or NET- EN) and the oral contraceptive pill. A combined hormonal contraception exposure variable was used as the numbers in the separate groups were small. Any form of hormonal Participants were asked about their present method use at each of the clinical visits; however no retrospective history on length of exposure to hormones was recorded nor was any biological confirmation made of hormonal exposure. The exposure to hormonal contraception was consider as a baseline exposure and switching of methods was not accounted for. The categories of response are defined as: injectable contraception either DMPA or NET- EN, oral contraception, condoms, sterilized, traditional, intrauterine device or natural methods. Contraception use was regrouped to hormonal and non hormonal. Hormonal methods included the injectable contraception (DPMA and NET-EN) and oral contraception and non hormonal methods included condoms, sterilized, traditional, IUD and natural methods.

3.5 Measurement of possible confounding variables or effect modifying variables

Identification of possible confounders variables was based on evidence from previous studies (23,24). These include demographic factors such as age and variables that define increased risk of STI infection including marital status, number of sexual partner and condom use.

Recoding of variables included type of sexual partner which was regrouped to include cohabiting partner, not cohabiting and casual partners. This defines sexual risk behaviour where women who do not cohabit with their partners are more at risk of STI including HIV. Age was regrouped into age groups to define at risk age groups, namely those under 24 (divided into two groups). Education level was defined as primary, secondary and tertiary levels of education.

Vaginal cleansing practices or douching was considered an effect modifier and data on these practices were collected by structured interview. Women were asked if they had ever practiced douching or not. Douching interferes with the normal vaginal flora leading to a decrease in the normal vaginal defenses, adding to the risk of acquiring an infection (13).

3.6 Data analysis

3.6.1 Data cleaning and preparation of the dataset

Data were double data entered into an Epi-Info™ database. The data set was cleaned and the code book was verified by comparing the structured interview guides with the dataset. Some recoding had been done on the data base and was removed prior to this analysis. The dataset consisted of two separate datasets, one for screening, which included the baseline demographic information of all participants and a second dataset which contained follow up information. The datasets were merged in STATA® version 8 by participant identification number. The merge also included a process of excluding variables that were not relevant to

this analysis. The final dataset then contained baseline and follow up data on 752 women. Variables were then renamed to more simple names to allow for easier analysis. Some STI tests were repeated at non clinical visit as laboratory results were not available or specimens were inadequate or the visit coincided with menses. A process of assigning the laboratory results to a primary clinical visit, either baseline, six months or one year was done. This was achieved by tabulating the STI results and then listing the unique identifications for STI results assigned to a non clinical visit and matching these with missing results for the preceding clinical visit. Any additional missing data on either outcome or exposure variable are quantified in the result section.

3.6.2 Descriptive statistics

Descriptive statistics were used to describe the important frequencies and proportions of risk factors (type of hormonal contraception use, age, number of sexual partners, type of sexual partners, sexual hygiene practices and socioeconomic factors) in the cohort for women using hormonal contraception and those not using contraception. Comparison by chi square test was done on categorical data and by Student t test for continuous data. All frequencies and proportions are reported with 95% confidence intervals and p values where appropriate. Baseline prevalence of STIs of was calculated and reported as proportions.

3.6.3 Power calculation

The cohort sample size is 752 women followed up for one year. Based on this sample size a power calculation has been done. The calculation calculated the power to detect a difference between using injectable contraception and non injectable contraception on the incidence of sexually transmitted infection. This was based on the published work which detected a 3.6 times increased risk of acquiring a CT infection (22). The power to detect a threefold (18% incidence) increase in incidence was 99%, the power to detect a smaller difference in incidence decreased to 30%.

Table 3. 1 Power size calculation

Sample size	**Incidence**	**% difference detected**	**Power**
In each group		***Alpha at 0.05 (2 sided)***	
376	6%	67%	30%
376	12%	50%	79%
376	18%	33%	99%

3.6.4 Survival time analysis

For the survival time analysis in the 'long' format of the data the data was declared survival time data (stset). Each incident infection provided a failure event and time to event was calculated on the date of the visit at which the women 'failed'. The survival time to each infection was plotted on Kaplan Meier curves and a log rank

test was used to compare the hormonal and non hormonal contraception users for each of the infections. The incidence rate for each infection was calculated and reported as an incidence rate per person time to follow up.

3.6.5 Adjusting for confounders and effect modifiers

A univariate analysis calculated a hazard rate for STIs in the presence of hormonal contraception or not for each of the STI. These were calculated in a survival time Cox regression model. Cox regression models for unadjusted hazard ratios are reported with confidence intervals (95%) and p value. Univariate analysis for each potential confounder was done and hazard ratios with p values of less than 0.10 for the separate confounders were then included into the multivariate analysis. Confounders were selected as those variables that would potential affect the relationship between exposure to hormonal contraception and the acquisition of a STI. For example age, marital status, number of sexual partner, condom use and education as these variables may affect both the choice of contraception and the risk of acquiring an STI. Each confounder was separately entered into a Cox model with the exposure to estimate a hazard ratio. If the p value for this model was less than 0.1 the confounder was included in a final Cox model. A Cox regression model was used as the model allows for the longitudinal time varying nature of a cohort design but with a single failure per participant.

Only douching was considered an effect modifier and so this interaction was assessed in a Cox regression model, initially as a univariate comparison and then if

the association fitted the criteria of p <0.1, the variable was added to a multivariate model with the appropriate confounding variables.

3.6.6 Statistical tests for assumptions and fit of models

The significance of each model was assessed by the log likelihood and p value. The assumption of proportional hazard, which is the only the assumption for a Cox regression model, was tested and the graphs to compare the proportional hazards over time are reported. This was tested for by plotting variables over time by the "stcoxkm" command and comparing the resulting lines.

3.6.7 Ethics clearance

This analysis received clearance from the University of the Witwatersrand Human Research Ethics committee on 29/08/2008 reference M080842. The parent study received approval from the HREC M02-01-09. All volunteers were offered information sheets in their language of choice (English, IsiZulu or SeSotho) and all enrolled volunteers signed informed consent prior to any study procedures taking place. Consent was revisited at each clinic contact and the option to withdraw was available at any time. Participants were offered all test results and treatment for STIs was available free of charge. Any medical condition that the study staff were not able to manage was handled with an assisted referral to a tertiary hospital close to the study site. A similar approach was used to social problems with the study site

having an extended interactive network of Government and Non Governmental Organizations (NGO) referral organizations.

4.0 RESULTS

4.1 Participants

Figure one illustrates the flow of participants through the study describing the reasons for participant withdrawal and loss to follow up. In the period between October 2002 and March of 2004 1089 women were screened to enroll 752 women into the cohort. Of those screened 264 (24%) were HIV positive 39 (4%) were pregnant and the remainder of the screening failures were due to other exclusion criteria such as being unwilling to undergo HIV testing, not living in the Soweto area and unable to provide reliable contact details. During the study 109 women became pregnant and 21 acquired HIV infection, resulting in their discontinuation from the study. Of the 752 women enrolled 544 completed the cohort study (excluding the 130 that reached the endpoints of pregnancy or HIV infection) leaving an overall retention rate for the study of 87%. All 752 women where included in this analysis. In total 567 person years to follow up were recorded.

4.2 Missing data

At each visit point there are laboratory results missing for participants; this is highest for CT and NG. At the first clinical follow up visit 57 (10%) CT results were missing and 58 (10%) NG results were missing. At the first clinical follow up visit 35 (9%) of BV and 34 (6%) of the TV results were missing. There were fewer missing results at the second clinical follow up visit, with the following test results missing;

CT 38 (7%), NG 39 (7%), BV 20 (4%) and TV 23 (4%). For this analysis women were included until a failure point, this may have been at either the first or second follow up visit. So if data was missing at the first visit but there for the second visit the second visit would then have formed the failure point. The main reason for missing data was inhibition of PCR testing for NG and CT. The reasons for this are both the methodology of sampling in the clinic and the limitations of PCR testing. The reasons for missing BV and TV results were likely to be participant who presented to the clinic during menstruation and other data were collected but the biological tests were not done. These missing results may well have affected the definition of the outcome variables by misclassification. This missing data was likely to be random.

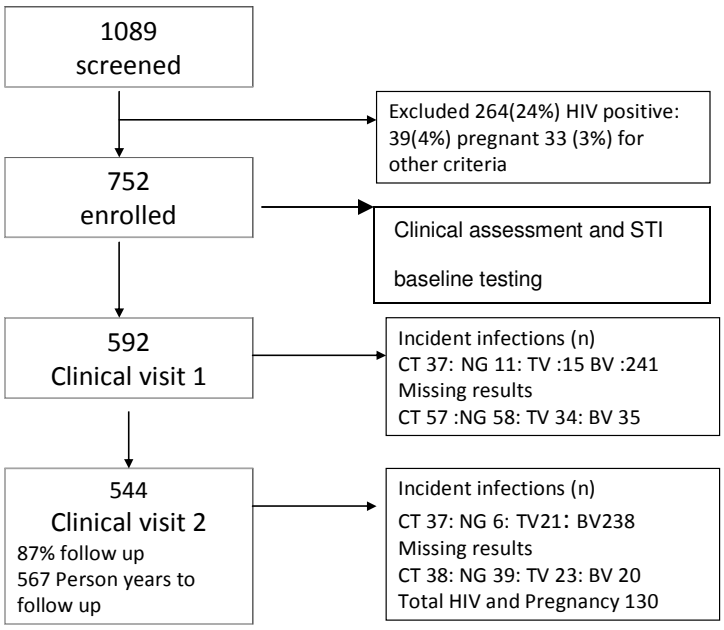


Figure 1 Study flow diagram for women screened for and enrolled into a cohort study, Soweto, 2002 to 2004.

4.3 Descriptive data

Of the 752 women enrolled, more than half the women (425/56%) used hormonal contraception as their preferred method of protection. Of these 110 (26%) used oral contraception and 315 (74%) used injectable contraception. The mean age of women participating in the study was 24.6 (SD 5) years. As illustrated in table 4.1, although the median number of years of education was high (11 years IQR 10-12, most women (81%) reported being unemployed. Despite being unemployed, a large number of women (66%) had a source of income and the vast majority lived in formal housing (91%). There were few differences in baseline characteristic between women using hormonal contraception and those not using contraception. Significant differences are seen in mean age, age group, women who had ever used a condom and type of housing. Women using hormonal contraception were significantly older (p= 0.000 for age group and p=0.003 for mean age) and more likely to live in formal housing (p=0.008) and women who did not use hormonal contraception were more likely to have used condoms (p=0.000).

Table 4.1 Baseline characteristic of women using hormonal contraception and not using hormonal contraception in Soweto, 2002 to 2004 (n=752).

	All women N (%)	*Hormonal contraception N (%)*	*Non hormonal contraception N (%)*	*P value*
Age groups				
<20	101 (13)	41 (10)	60 (18)	0.000
20 to 24	328 (44)	173 (41)	155 (47)	
25 to 29	172 (23)	126 (30)	46 (14)	
≥30	151 (20)	85 (20)	66 (20)	
Level of education				
7 years and less	81 (11)	43 (10)	38 (12)	0.173
8 to 12 years	307 (41)	186 (44)	121 (37)	
> 12 years	364 (48)	196 (46)	168 (52)	
Socioeconomic markers				
Type of housing				
Informal	86 (11)	60 (14)	26 (8)	0.008
Formal	666 (90)	365 (86)	301 (92)	
Ever used a condom				
Yes	594 (79)	306 (72)	288 (88)	0.000
No	158 (21)	119 (28)	39 (12)	
Number of sexual partners (In the last 3 months)				
no sexual partner	2	-----	2	0.048
One	638 (92)	372 (94)	266 (90)	
>1	53 (8)	24 (6)	21 (10)	
Type of partnership				
Married or co-inhabiting	138 (18)	138 (20)	53 (16)	
Regular not co-inhabiting	591 (79)	331 (78)	260 (80)	0.017
No partner	23 (3)	9 (2)	14 (4)	
Vagina cleansing n(%)				
Yes	51 (7)	33 (7.76)	18 (6)	0.222
No	701 (93)	292 (68.71)	309 (94)	

4.4 Sexual risk factors

At baseline the women in this cohort were at risk for the acquisition of STI. Condom use was low. 53% of women did not use a condom at the last sex act and 21% had never used a condom. The majority (79%) of women did not cohabit with their regular sexual partner. However the majority of women (92%) reported only having one sexual partner and vaginal cleansing practices were not prevalent, only 7% of the women reported douching. These risk behaviours are reflected in the measured STI prevalences at baseline in the cohort with BV at 53% (95% CI 49 to 57), TV 4% (95% CI 1.4 to 5.4), CT 11% (95% CI 8.7 to 13.2) and NG 2% (95%CI 1 to 3).

4.5 Incident infections

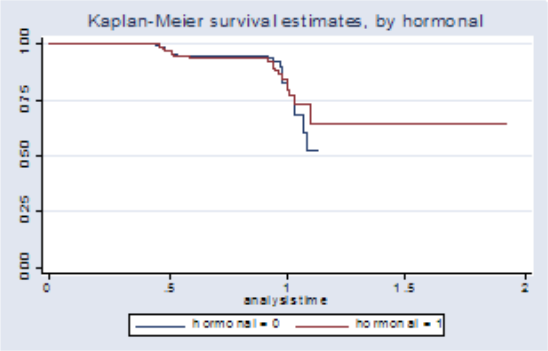
The incidence rates (Table 4.2) followed the trend of the baseline prevalences of STIs with new BV cases reporting the highest incidence rate of 72 per 100 women years of follow up, (95% CI, 63 to 83) for hormonal contraception users. Incidence rates for CT infections were 13 per 100 women years of follow up (95% CI, 9 to 17) for women using hormonal contraception The number of new TV and NG infections were lower, with incidence rates of 6 per 100 women years of follow up (95% CI, 4 to 10) and 2 per 100 women years to follow up (95% CI, 1 to 4) respectively for women using hormonal contraception.

Table 4.2 Incidence rates for STI's in women enrolled in a cohort study, Soweto 2002 to 2004.

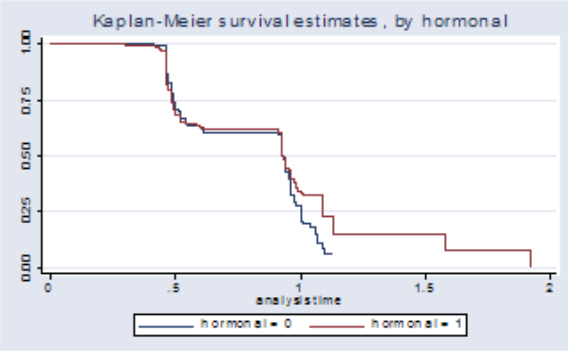
	Hormonal contraception			*Non hormonal contraception*		
	Person years	**Incident cases (n)**	**Rate per 100 person years (95%CI)**	**Person years**	**Incident cases (n)**	**Rate per 100 person years (95%CI)**
CT	311	39	13 (9-17)	241	29	12 (8-19)
NG	318	6	2 (1-4)	244	10	4 (2-8)
TV	315	20	6 (4-10)	246	12	5 (3-9)
BV	266	193	72 (63-83)	206	163	79 (68-92)

4.6 Survival time outcomes

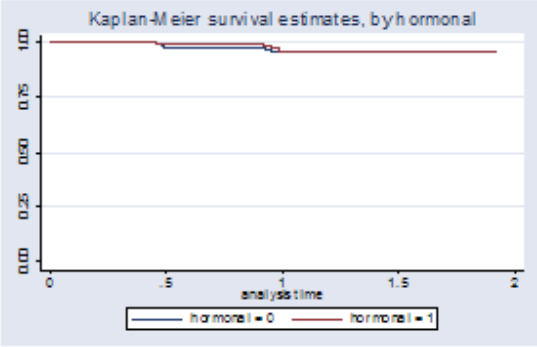
Kaplan Meier graphs for each infection comparing hormonal contraception uses and non hormonal contraception uses at visit 1 or visit 2 are illustrated in figure 2. The log rank test for equality shows no significant difference in survival time for any of the infections and the group infections (table 4.3).



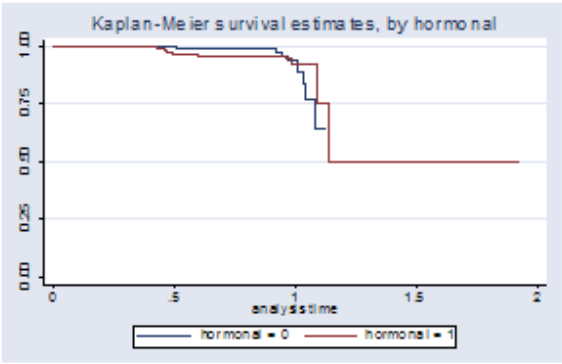
Incident CT infections



Incident BV infections



Incident NG infections



Incident TV infections

Figure 2 Kaplan Meier Curves for survival to STI infection at either visit 1 or visit 2 in a cohort of women in Soweto 2002 to 2004

Table 4.3 Log rank test for equality of survival times measured for incident STIs in a cohort of women in Soweto 2002 to 2004.

Variable	*Chi square*	*p value*
CT	0.07	0.79
TV	0.2	0.66
NG	2.21	0.14
BV	0.92	0.34

4.7 Univariate and multivariate analysis

4.7.1 *C. trachomatis* infections

The univariate analysis for the association between the use of hormonal contraception and the acquisition of CT infection describes a HR of 1.07 (95% CI, 0.66 to 1.72; p= 0.794). The introduction of possible confounders into the Cox regression model, as per the entry criterion of p<0.1 in the univariate model, suggested confounding by:

Partner type, where women in not cohabiting partnerships had an increased risk of infection, HR 1.79 (95% CI, 0.81 to 3.92; p=0.1) compared to women who were not cohabiting with their sexual partners.

Age group, where women aged 20 to 24 had an increased risk of infection, HR 2.56 (95% CI, 0.91-7.18; p=0.075) compared to women younger than 20.

Level of education, women who had secondary school education or who had more than secondary school education had a lower risk than those who did not complete secondary school; HR 0.44 (95% CI 0.22 to 0.87; p=0.018) and HR 0.52 (95% CI 0.28 to 0.99; p= 0.049) respectively.

When these potential confounders were entered into the multivariate model, the HR for the relationship between the use of hormonal contraception and CT infection demonstrated a slightly increased risk of 1.12 (95% CI, 0.69 to 1.82; p0.657) (Table 4.4). The effect modifier of vaginal cleansing did not fit the criteria for inclusion in to a multivariate model for interaction (p= 0.933) for this association.

4.7.2 *N. gonorrhoea* infections

The univariate analysis for the association between the use of hormonal contraception and the acquisition of NG infection demonstrated a HR of 0.47 (95% CI, 0.17 to 1.30; p=0.147). Introduction of possible confounders into univariate model did not show any significant confounding nor did any of the possible confounders fit the entry criterion for inclusion into a multivariate model (Table 4.4). Likewise vaginal cleansing did not meet the inclusion criterion for inclusion in the model (p= 0.874).

4.7.3 *T. vaginalis* infections

The unadjusted HR for TV infections was 1.18 (95% CI 0.57 to 2.43; p= 0.059). The introduction of confounders suggested confounding by:

Number of partners; women who had more than one partner in the last three months had a significantly increased risk of TV infections HR 5.19 (95% CI, 1.47 to 18.37; p=0.011) compared to women who had only one sexual partner.

Age group; Women aged 20 to 24 had a decreased risk of infection HR 0.28 (95% CI, 0.09 to 0.86; p=0.027) compared to women aged less than 20 years.

When these confounders were included into a single model the HR for the association between hormonal contraception and TV infection decreased slightly (adjusted HR 1.06 (95% CI 0.48 to 2.34; p=0.89) (Table 4.4). The effect modifier of vaginal cleansing did not fit the entry criterion for inclusion in the model as a test for interaction (p0.812).

4.7.4 Bacterial Vaginosis infections

For the association between hormonal contraception and BV the only possible confounder that fitted the criteria for inclusion into a multivariate model was the partnership classification of having no regular partner. When this was included into a model the univariate HR of 0.90 (95% CI. 0.73 to 1.12; p 0.350) changed very little (AHR 0.90 (95% CI, 0.73 to 1.11; p0.34). The univariate consideration of the effect of vaginal cleansing suggested that women who practiced vaginal cleansing were 2 times more likely to acquired BV, HR 2.03 (95% CI, 0.87 to 4.76; p 0.102). This was then assessed as an effect modifier in the final model. The inclusion of these two possible confounders or effect modifiers (no regular partner and BV infection) into

the multivariate model resulted in the use of hormonal contraception being protective for BV infection (AHR 0.27 (95% CI 0.04 to 1.2; p0.081)(Table 4.4).

Table 4.4: Univariate and multivariate analysis results for incident STI infection in a cohort of women in Soweto 2002 to 2004.

	Unadjusted HR		***Adjusted HR***	
	Hazard Ratio (95% confidence interval)	P value	Hazard Ratio (95% confidence interval)	P value
CT	1.07 (0.66-1.72)	0.794	1.12 (0.69-1.82)	0.657
NG	0.47 (0.17-1.30)	0.147	0.47 (0.17-1.30)	0.147
TV	1.18 (0.57-2.34	0.059	1.06 (0.48-2.34)	0.890
BV	0.90 (0.73-1.12)	0.350	0.27 (0.05-1.53)	0.140

4.8 Comparison of models

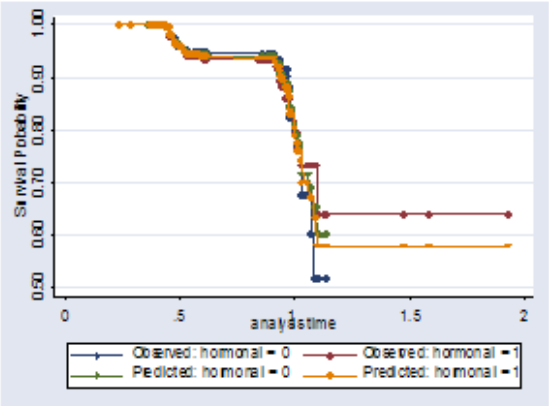
Models were compared by assessing the p value for the model and the log likelihood value for the model (Table 4.5). Although the log likelihood is less for each of the multivariate models, the only significant model is the multivariate model for TV infections.

Table 4.5: Comparison of univariate and multivariate models to assess risk of acquiring STIs in a cohort of women in Soweto, 2002 to 2004.

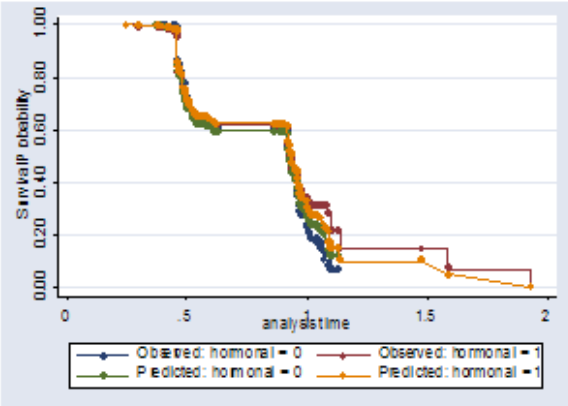
	Univariate model		***Multivarate model***	
	Log likelihood	p value	Log likelihood	p value
CT	-381	0.794	-374	0.100
NG	-96	0.139	-96	0.139
TV	-172	0.657	-165	0.044
BV	-2047	0.350	-2045	0.172

4.9 Test for assumption of Cox regression model

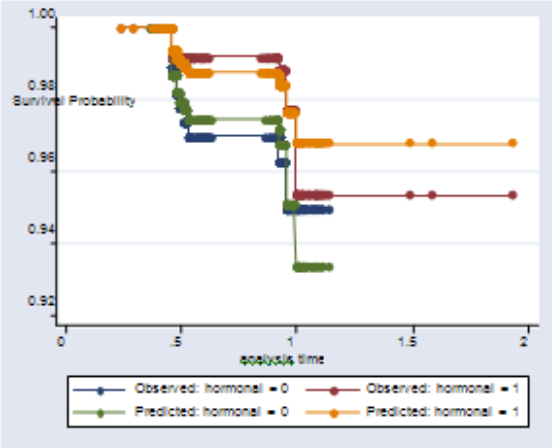
A test for the only assumption of Cox regression model that the proportion of hazards is constant over time was done in STATA® version 8. The graphs illustrated that the assumption holds in the main part for each of the outcomes. This is seen in the lines of the graphs being parallel (Figure 3).



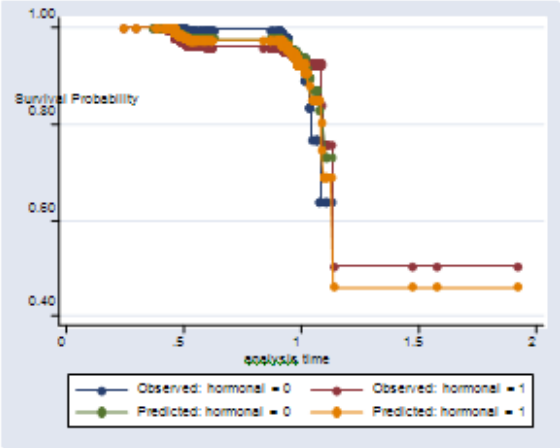
Test for proportional hazard (CT)



Test for proportional hazard (BV)



Test for proportional hazard (NG)



Test for proportional hazard (TV)

Figure 3 Test for the assumptions of Cox Regression Model applied to the Cox models for the assessment of risk in a cohort of women, Soweto 2002 to 2004.

5.0 DISCUSSION

This analysis did not reveal any significant associations between the use of hormonal contraception and the acquisition of STIs; however the trends in risk mostly mirror the published data (24, 25). Increased risk is suggest with CT infection (HR 1.12) but would not be considered clinically significant. No association is seem for TV infection (HR 1.06). Protective effects are seen with GC infection: women using hormonal contraception being 53% less likely to acquire GC infection. A much larger protective effect is seen for BV infection with women using hormonal contraption being 73% less likely to have BV.

Baseline sexually transmitted infection prevalences are similar to those seen in other studies (24, 25), although CT (4% vs 11%) and BV (39% vs 53%) infections were more prevalent in this cohort. This comparison is interesting as the Kenyan cohort consisted of commercial sex workers as opposed to women drawn from the general population as in this cohort (25). However, despite this different composition the risk profiles for STIs is significantly raised in both cohorts. The high baseline prevalence of BV is alarming as BV is considered a risk factor for HIV acquisition (13). BV, a change in the normal vaginal flora, is associated with multiple sexual partners and vaginal cleansing. Although treatment for BV is effective BV often reoccurs making it difficult to sustain a cure (45). The prevalence of NG is lower than other infections and may reflect adequate primary health care treatment of NG by syndromic

management, although this may have changed in the time since this study was completed due to the emergence of NG resistance to Ciprofloxacin. NG infections are generally less prevalent in the general population than in high risk groups, such as CSW, where frequent partner changes maintain a higher prevalence. Infections with CT are more prevalent in this cohort than other infections which may be due in part to the asymptomatic nature of the infection which means than women may not seek treatment so sustaining a pool of infection in the community. Because CT infections are associated with serious complications such as pelvic inflammatory disease and infertility the higher prevalence is concerning. Although there are some challenges with the development of bedside tests for CT, the use of these tests may be considered as an option for pathogen specific testing at a primary health level.

Morrison et al (2004), in his study based in the USA, reported significant associations between the use of oral contraception and the acquisition of cervical infections. The American cohort had more enrolled women than this study (1003 vs 752) which may account for the significant results seen in that study as compared to this analysis (24). It is difficult to make a direct comparison between this study and the study by Morrison et al, USA, and Baetan et al (2001) in Kenya because the Morrison study combined CT and NG infections as a single outcome of cervical infection, so increasing the number of incident infections. Both studies by Morrison and Baetan divided the hormonal exposure into OC and DMPA which may assign

different risks to the slightly different exposure of combined oral contraception (oestrogen and progesterone) and the progesterone only DMPA (24, 25).

The apparent protective effect of hormonal contraception use on the acquisition of gonorrhea (57% decrease in risk) has been reported in other studies including cross sectional studies (26). The confidence interval around the hazard ratio is wide in this analysis and the true population figure may lie anywhere from 0.41 to 1.32. So it is not possible to exclude whether contraception was associated with an increased risk of NG infection in this study. Although there is little data on the acquisition of TV infections, most studies have reported protective effects of contraception (26). This analysis shows a slightly increased risk. A hazards ratio of 1.06 which is not clinically significant and wide confidence intervals suggesting that true HR may lie anywhere from 0.48 to 2.34 mean that from this analysis we are not able to make an association between the use of hormonal contraception and TV infections. The small protective effect seen in the acquisition of BV, where women who use hormonal contraception are 73% less likely to acquire BV, AHR 0.27 (0.05 to 1.53) is reflected in the Kenyan cohort with both oral contraception (HR 0.8) and DMPA (HR 0.7) users demonstrating a decreased risk of acquiring BV infection (25). This protective effect is encouraging in a high HIV prevalence setting.

Vaginal cleansing or douching was considered as an effect modifier for this analysis. Vaginal cleansing has been associated with changes in the vaginal environment such as the loss of lactobacilli which is also described as an effect caused by the

use of DMPA (27-30). When the variable douching was introduced into models for the various STI the only association that met the p< 0.1 criteria was for BV where women who use hormonal contraception and who practiced douching were two times more likely to acquire BV HR 2.03 (0.87 to 4.76; p=0.102). Although not significant this association is expected as vaginal cleansing is considered a risk factor for the presence of BV (13).

5.1 Biological plausibility:

Although there are biological changes documented both macroscopically and microscopically in the genital tract in the presence of OC and DMPA (27-30) these changes do not reflect in a significant increased risk of infection in this analysis. Additional studies suggest a pathogenic effect on causative organisms by hormones (25, 31, 32). Again this suggested effect of hormones on pathogens does not result in an observed risk in this cohort. In the Morrison cohort study cervical ectopy, which has been considered an important mediator for the acquisition of cervical infections, was not associated with infection risk (24). The ability to describe the confounding influence of cervical ectopy may have added to this analysis.

5.2 Strengths of the study

5.2.1 Definition of end points

This study used laboratory based testing for the definition of outcomes; these techniques included PCR testing for CT and NG, Nugent scoring for BV and culture

for TV. These techniques all report high sensitive and specificity. Sensitivity and CT test on cervical swabs range from 91 to 100% compared with culture sensitivity of 73% to 93%, and specificity of > 99% for both methods (42). The alternative to PCR for GC is gram stain which is not considered sensitive or specific in women (42). Although BV can be diagnosed with a simple bedside test the use of Nugent scoring on a gram stain allows quantification of the result. The diagnosis of TV is made by microscopy on a wet mount, by culture in Diamonds medium followed by microscopy or more recently by PCR. In a study of 200 women wet mount recorded a prevalence of 7%, culture prevalence of 10.5% and PCR 11%. Meaning culture and PCR performed similarly (43).

5.2.2 General population, study design and local setting

This prospective cohort study increases the ability of the study to describe the association between exposure and outcomes. The local general population cohort could provide information relevant to South African policy makers.

5.2.3 Limitations of the study

5.2.3.1 Inhibition of PCR and missing lab results

Inhibition of PCR is a recognised difficulty with this technique. Inhibition of PCR testing for CT and NG ranges from 5-9% with 64% of these resolving on repeat testing (40). Accurate PCR testing seems to be dependent on sampling methods, transport conditions and laboratory preparation (42). During the conduct of this study

the high inhibition rates were noted (in excess of 10% of tests). Some tests were repeated and retraining of staff was conducted. There are far fewer results missing for TV (4-6%) and BV (4-6%), but the loss of incident infections is a possibility. This kind of missing data would be considered random as the women who had missing results were not necessarily different from those who did not, however bias cannot be excluded and statistical methods to deal with missing data may be considered for further analysis. For example, multiple imputation methods could be applied (46).

5.2.3.2 Definition of exposure

The study did not collect data to define the length of time that a woman was exposed to hormonal contraception. The study did not exclude or collect information on women who may recently have used hormonal contraception and stopped. There was no biological confirmation of hormonal levels to measure consistent use of hormonal contraception or recent exposure to hormonal contraception. No assessment was made in this analysis of women who may have switched contraceptive methods or stopped contraceptive methods during the course of the study. This may have underestimated the effect of hormonal contraception on the risk of acquiring STIs.

The numbers of women using either oral contraception or injectable contraception were small (Injectable 315 (74%) and OC 110(26%)) requiring that these were combined into a single analysis. The literature does suggest that the different hormonal exposure may result in different risk (24, 25). This measurement may have

lead to either over estimation of the exposure (if many women had recently started or changed hormonal contraception) or underestimation of exposure if women were not consistent on their use of hormonal contraception.

5.2.3.3 No test for cure

Women were treated at baseline for laboratory and syndromically diagnosed infections, however no confirmation of cure was documented. A small number of infections may not have been adequately treated resulting in some misclassification of outcomes. This may have lead to over estimation of incident infections.

5.2.3.4 Number of incident cases

In the case of some of the infections, for example NG infections, the number of incident infections is low (n=16, single failure infections) which reflects in some of the wide confidence intervals and may have underestimated the measure of effect.

The large number of recurrent BV infections (n=123) may have been better analyzed in a repeated measures model to allow for more than one incident infection in a woman. The Cox regression model with single endpoint may result in standard errors that are too small resulting in narrow confidence intervals and smaller p values.

5.2.3.5 Measurement of confounders:

Some potential confounders, for example cervical ectopy, were not measured in this study and so were not adjusted for in the analysis. Due to the relatively small sample size in this cohort, STIs in women who were pregnant or HIV positive at clinical visits were included in the analysis. Pregnancy may have changed the risk of acquiring STIs due to the genital tract decrease in immune system function (32, 33).

5.2.3.6 Cohort structure and loss to follow up

The cohort was constructed from volunteers who may have been different to women who did not volunteer to participate. Similarly women who did not complete follow up may have been different to those who did complete follow up. Both these factors may lead to bias in the results.

5.3 Conclusion

There has been wide spread and prolonged interest in association between the use of hormonal contraception, either oral or injectable, and the acquisition of sexually transmitted infections. The evidence for a significant association is visible in the increasing number of cohort studies in different population groups. Because the use of hormonal contraception is the foundation of effective family planning worldwide a shift in policy would need epidemiological evidence that can assign causality. The evidence to support causality would only come from a randomized controlled trial with hormonal and non hormonal interventions. To date most scientists have felt that randomizing women to contraception would be difficult and unacceptable to patients.

However in a study conducted in Jamaica and South Africa women were asked if they would consider participating in a trial and would accept randomization: 70% of the participants indicated that they would consider randomization to either DMPA or an intrauterine device (44). With this concern aside there may be place for a randomized controlled trial to finalise the debate.

In the interim, public health planning for family planning may consider other contraception options like progesterone implants which are a lower dose of hormone. The reintroduction of the intra uterine contraceptive device (IUD) may add an additional option for women. Health care providers should continue to conduct risk assessments with women attending family planning clinics and advise women on the need for barrier methods in addition to hormonal contraception to protect against STI and HIV acquisition.

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