Bacterial Vaginosis Prior to and During First Pregnancy in Kenyan Adolescent Girls and Young Women at Risk Of HIV

Lynda Myra Mboga Oluoch

A thesis
submitted in partial fulfillment of the requirements for the degree of
Master of Public Health

University of Washington
2021

Committee:
Alison Roxby
Nelly Mugo
Kenneth Ngure

Program authorized to offer degree:
Department of Global Health
Abstract

Bacterial Vaginosis Prior to and During First Pregnancy in Kenyan Adolescent Girls and Young Women at Risk Of HIV

Lynda Myra Mboga Oluoch MBChB, MSc

Chair of the Supervisory Committee: Alison Roxby MD, MSc, Associate Professor, Department of Global Health

Background: There is limited understanding of longitudinal changes brought about by bacterial vaginosis (BV) in the female genital tract of adolescent girls and young women (AGYW) at pregnancy. AGYW are at a high risk of HIV, STI and pregnancy soon after first sex. Hormonal shifts appear to influence vaginal dysbiosis; a pregnancy at a young age might disproportionately affect the vaginal environment to increase risk of HIV. The aim of this study is to determine the change in BV status of AGYW at preconception and during pregnancy. Using precisely collected longitudinal cohort data, this study examined how vaginal dysbiosis in recently sexually active young women is changing at the time of a first early pregnancy.

Methods: We conducted a secondary data analysis of the Girls' Health Study (GHS) cohort data from 2014 to 2020. Nugent scoring of vaginal Gram stains was used to diagnose BV. A score of 7 and above was considered positive for BV. Logistic regression models were used to analyze longitudinal trends in BV over time, and to examine whether there is increased risk of BV at visits during pregnancy compared to visits before pregnancy. Relevant covariates were adjusted for, including socioeconomic status, marital status, sexual history and reproductive history. Time-to-event analysis was used to describe timing of pregnancy. Additionally, Cox regression was used to assess associations between correlates and pregnancy.
**Results:** We enrolled 400 AGYW, aged 16-20 years, median age 18.6 years (17.6-19.4) into the study, and followed them for a median of 51 months (IQR: 27-57). At the end of follow-up, 306 (76%) had reported first penile-vaginal sex; median age of first sex was 18.9 years (Interquartile range (IQR): 18.3 - 19.9). Forty-two percent (127/306) of sexually active AGYW had a positive pregnancy test at least once during follow up. Contraception use among participants who ever reported pregnancy was low at 26% and only 9/127 (7.1%) reported condom use. The percentage of participants with BV before pregnancy was 38% (45/119) and during pregnancy 23% (24/105). The adjusted relative risk (aRR) of BV during pregnancy among AGYW who had experienced BV pre-pregnancy was 0.66 (95% CI : 0.48, 0.92; p value= 0.015). Factors that were associated with BV during pregnancy included history of CT infection (RR:4.13; 95%CI: 1.73-9.90; p value=0.001). Median number of sex acts increased during pregnancy: 8 sex acts (IQR 2-23) (timepoint defined as <=LMP+45 days) compared with 6 sexual acts pre-pregnancy (IQR:2-18) (timepoint defined as any visit >LMP + 45 days). Number of sex acts was not associated with increased BV in pregnancy.

**Conclusions:** BV was noted to be more prevalent pre-pregnancy compared to during pregnancy, and pregnancy was associated with a near 40% reduction in BV diagnosis. Further understanding of the vaginal environment and vaginal bacteria and before and during pregnancy is needed to assess the specific factors that might contribute to reduced BV risk among pregnant AGYW.
ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance and support of my committee: Alison Roxby, Nelly Mugo and Kenneth Ngure. Their commitment to addressing AGYW sexual and reproductive health issues including HIV prevention, through the advancement of research is inspiring, and it has been an honor to work with them. I will certainly utilize what I’ve learned from them during this experience in my future career. I would like to appreciate Dr. Anna Wald and Florian Hladik for their immense contribution to this study from its inception.

I am also very grateful to Bhavna Chohan, Kenneth Tapia and Stacy Selke from the University of Washington for their contribution to this work. Although not official members of my thesis committee, their input was invaluable. I am grateful to Dr. Irungu and the team at Partners in Health and Research Development (PHRD) for their collective work on this project. I thank the AGYW who keenly participated, provided data and samples for a duration of 5 years.

I, as well as my committee members, thank the International AIDS and Research Training Program (IARTP) for granting me scholarship to get this degree. I also want to thank the Department of Global Health at the University of Washington for providing me with the many enriching opportunities I have had, both inside and outside of the classroom. I feel proud to be graduating with a degree from this department and the values that it represents. Finally, to my family and friends for their unconditional support and encouragement – thank you.

FUNDING:

This research was funded by R01 HD091996-01 (AR) from NICHD, by P01 AI 030731 (AW) from NIAID, and by the Center for AIDS Research (CFAR) of the University of Washington/Fred Hutchinson Cancer Research Center AI027757. LO was funded by the Women and HIV program of the International AIDS Research and Training Program (IARTP) 2D43TW009783 from the Fogarty International Center. The funders had no role in study
design, data collection, and analysis, or preparation of the manuscript. Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Washington funded by UL1 TR002319, KL2 TR002317, and TL1 TR002318 from NCATS/NIH.
INTRODUCTION

BV is a common condition in women characterized by a shift from an optimal Lactobacillus-predominant vaginal microbiota to one characterized by high concentrations of diverse anaerobic species (J. M. Marrazzo et al. 2010). Globally, it is the most common cause of vaginal discharge and inflammation in women of reproductive age (Bagnall and Rizzolo 2017) and is highly prevalent among Sub-Saharan African women (Bukusi et al. 2006) (J. Marrazzo et al. 2019). BV increases risk for adverse reproductive health outcomes with increased risk for HIV infection, pelvic inflammatory disease and adverse pregnancy outcomes (McKinnon et al. 2019). Much of the data published on BV prevalence from Sub-Saharan Africa has been from cross sectional studies (Bayigga et al. 2019)

A risk factor for bacterial vaginosis (BV) is engaging in sexual activity (Marrazzo 2011) (Bradshaw et al. 2013) (Gazendam et al. 2020). Other known risk factors include Black race, vaginal washing, menses, presence of STI and sexual behaviors including condomless sex and new sexual partners (Marrazzo 2011) (Bradshaw et al. 2013). Additionally, African women have been noted to have lower level of protective Lactobacillus spp (Bayigga et al. 2019) (Dabee et al. 2019). High prevalence of BV among African women is concerning because of negative health consequences of BV. Meta-analysis done on prospective longitudinal studies show a strong association between BV and risk of HIV infection (Hilber et al. 2010). BV is also known to increase risk of pelvic inflammatory diseases (PID), with one landmark study showing an adjusted OR of 2.83 (95% CI, 1.81-4.42) (Ness et al. 2005).

While it has been established that BV is a major HIV risk factor among African women, to date most of the research has been among middle aged women and less is known about the youngest age group of AGYW (Bukusi et al. 2006). Women aged between 15- 24 years in Sub-Saharan Africa accounted for 26% of the incident 1.8 million HIV infections globally (ONUSIDA 2017). In Kenya, the statistics were similar with one third of all incident HIV
infections noted among AGYW (National AIDS Control Council 2018). An understanding of BV induced changes is needed to shed more light on how the vaginal microbiome influences HIV/STI risk and transmission in this cohort of young women (Bayigga et al. 2019) (van de Wijgert 2017). Studies to date have been conflicting; while some studies show lower BV prevalence in AGYW (25% of study participants) (Suzanna CarterFrancis et al. 2020); others show high levels of BV (41.1% (95% CI 32.3%-50.5%) among women aged 15–19 years and 44.2% (95% CI 35.5%-53.2%) among women aged 20–24 years) (Francis et al. 2018). Sexually active AGYW have been noted to have a more diverse vaginal microbiota, with only 37% of AGYW in one study demonstrating Lactobacilli dominant species compared to older women (Anahtar et al. 2015). In addition, presence of diverse vaginal microbiome is correlated with higher levels of inflammatory cytokines which increases risk of HIV acquisition (Anahtar et al. 2015) (Dabee et al. 2019). Overall, while these data show concern about high prevalence of BV among AGYW, these data are largely cross-sectional and were collected from teens who were already engaging in sex.

Prevalence of teenage pregnancy in Kenya is high at 20% as per the last Kenya Data and Health Survey 2014 Report (Dixon 2016). This translates to 1 in every 5 AGYW between 15-19 years either having given birth or being pregnant with her first child (Kahurani 2020). Around 60% of AGYW in 2014 who were sexually active and unmarried reported not using any contraceptive method (Kahurani 2020). BV in pregnancy increases the risk of adverse outcomes including preterm births, miscarriages, chorioamnionitis and low birth weight (Brabant 2016) (Woodman 2016) (McKinnon et al. 2019). It is hypothesized that vaginal dysbiosis at preconception is more likely to cause adverse pregnancy outcomes as opposed to vaginal dysbiosis during the pregnancy (Lokken et al. 2020). HIV acquisition risk doubles in pregnancy (Thomson et al. 2018). Given the high BV prevalence among African women, it is important to understand BV changes with pregnancy.
There is a scarcity in data published that focuses solely on AGYW and BV trends from first sex to pregnancy occurrence, with little understanding of longitudinal pregnancy-induced changes to BV status among AGYW who become pregnant. One approach that could be used to understand BV over time in AGYW would be to examine longitudinal cohort data. Such cohorts can elucidate the natural history of vaginal conditions before and during pregnancy, using well-defined measures of time of menarche, time of first sex and time of incident pregnancy.

We conducted secondary analysis of data from a cohort of low sexually naive, HIV negative, Kenyan AGYW. Our first objective was to assess changes in BV status from preconception to pregnancy. Our second objective was to look at correlates of increased risk of incident pregnancy. Our goal was to determine how BV status changes during pregnancy among AGYW who have recently become sexually active. Given the negative consequences of BV in pregnancy, we hypothesized that we might find predictors of BV in pregnant AGYW. Longitudinal analysis of BV trends over time enables a detailed view of the biological and environmental factors that contribute to the establishment of vaginal dysbiosis in AGYW, placing them at increased risk of BV and subsequently risk of HIV and other adverse reproductive health outcomes.

**METHODS**

*Ethical Approval:*

Human subjects approval was obtained from both the University of Washington and locally from the Kenya Medical Research Institute (KEMRI) Scientific Ethics and Review Unit. Research staff obtained written informed consent in a confidential area. Participants younger than 18 years of age were considered minors; their guardians provided written informed consents and participants provided written informed assents.
Study Setting, Population and Design:

Data was collected during the Girls’ Health Study (GHS), conducted between 2014 and 2020. This was a prospective observational cohort designed to determine if HSV-2 acquisition among young African women resulted in persistent genital inflammation.

To be eligible for enrollment, AGYW were required to be

- sexually naive or sexually inexperienced with one lifetime sexual partner by self-report
- aged 16-20 years
- confirmed HIV and HSV-2 seronegative
- willing to come to clinic every three months
- willing to undergo pelvic examinations.

AGYW were recruited from local communities in accordance with Good Community Participatory practices and oversight was provided by a community advisory group of local community members. The study was conducted at the Partners in Health Research and Development, KEMRI research clinic located in Thika, Kenya. The site has been collaborating with the University of Washington for the past 14 years on HIV and STI research projects. The University of Washington in Seattle served as a site for study preparation, data analysis and manuscript writing for this research.

Study Procedures

Participants were followed quarterly and pelvic examinations were done at every scheduled study visit. STI testing was done annually and on demand using nucleic acid testing (NAAT) of genital swabs for Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis (APTIMA, Hologic/Gen-Probe (Marlborough, MA). Lower vaginal swabs were assessed using Gram stain for bacterial vaginosis (BV). Nugent score was determined from vaginal Gram stains (Nugent, Krohn, and Hillier 1991) with a score of 7 and above considered positive for BV. Serum ELISA assays were used for HIV testing (Vironostika®
HIV Uni-Form II Ag-Ab (Biomerieux, Marcy-l’Etoile, France) and HSV-2 testing (HerpeSelect (Focus Diagnostics, Cypress, CA).

Data collection on last menstrual period (LMP) and contraception use was collected at every visit. Sexual activity was defined as penile-vaginal penetrative intercourse. Participants who were sexually active and not using any form of contraception were offered contraceptive counselling. Pregnancy testing (QuickVue hCG Urine Test) was done on request or upon report of missed LMP. If pregnancy test was positive, participants were counselled and linked to an antenatal clinic. Pregnant participants were encouraged to continue with quarterly visits and study procedures such as vaginal swab sampling; some participants opted out of pelvic examinations during pregnancy. Only first pregnancies were included in this analysis.

Data Collection

Our primary outcome, BV diagnosis, was defined as a Nugent score of 7 and above. For correlates of the primary outcome of BV, data was obtained from questionnaires, which included demographic data, medical history, and sexual and reproductive health history, including timing of first sex, number of sexual partners, frequency of sex, consistency of condom use, history of vaginal washing, and STI diagnoses. Number of sexual acts was measured pre-pregnancy by the number of reported sex acts in the 45 days before LMP; number of sex acts during pregnancy were measured as reported acts from LMP to 45 days later. Anonymized data in electronic form was entered and stored in a password-secured REDCap database.

Statistical analysis

Descriptive statistics were used to determine the baseline demographic characteristics of the AGYW cohort. Multivariate regression models using Generalized Estimating Equations were created to analyze longitudinal trends in BV over time, and to examine whether increased risk of BV was observed at visits during pregnancy compared to visits before pregnancy.
Treating presence of BV as a binary outcome, the relative risk of BV during pregnancy (compared to before pregnancy) was estimated using a log-binomial model (binomial error structure and a log link) with exchangeable working correlation and robust standard errors. Models were adjusted for covariates that included condom use, income, time from menarche to first sex, residence, history of CT, history of HSV-2 and frequent vaginal washing. Time-to-event analysis was used to visualize timing of pregnancy with Cox regression used to assess associations between correlates and pregnancy. Potential correlates of pregnancy include condom use, new partners, time from age of menarche to first sex and STI infection. Statistical analysis was performed using Stata version 16.0 (StataCorp, TX, USA); statistical significance was defined as 2-sided p value <0.05. For models, pre-pregnancy BV status was determined from BV testing collected 3 months prior to pregnancy; if not available, the closest BV result prior to pregnancy was used up to 12 months pre-pregnancy.

RESULTS

Study cohort characteristics:

The GHS study screened 610 AGYW, and 400 were enrolled (Figure 1). Study follow up spanned a period of up to 60 months, between 2014 and 2020. The study was concluded in June 2020 at the start of major disruptions from Covid-19. Median age of participants at enrollment was 18.6 years (IQR 17.6-19.4) and the median years of schooling was 12 (IQR10-12). Among newly enrolled participants, 322 (80.5%) reported no prior history of sex, while 78 (19.5%) reported one lifetime sexual partner. The median follow-up time for participants was 51 months (IQR: 27-57). Table 1 represents the sociodemographic characteristics of AGYW at enrollment.
Table 1: Baseline characteristics of adolescent girls and young women enrolled in the cohort.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (IQR) or N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at enrollment (years)</td>
<td>18.6 (17.6-19.4)</td>
</tr>
<tr>
<td>Education (years of school completed)*</td>
<td>12.0 (10.0-12.0)</td>
</tr>
<tr>
<td>Rural residence</td>
<td>245 (61%)</td>
</tr>
<tr>
<td>No prior sex</td>
<td>322 (80.5%)</td>
</tr>
<tr>
<td>One lifetime sexual partner</td>
<td>78 (19.5%)</td>
</tr>
<tr>
<td>Monthly income (KSH)</td>
<td>0 (0-1000)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; KSH, Kenya shillings. 110 KSH = 1 USD*N=399.

Sexual and reproductive history

Among the 400 enrolled participants, 306 AGYW reported sexual activity during study follow up (Table 2). The median age of menarche was 14 years (IQR: 13-15) and median age of first sex was 18.9 years (IQR: 18.3 - 19.9). Median reported years between menarche and first sex was 4.9 years (IQR: 3.7-6.1). Of AGYW reporting sexual activity, 42% (127/306) reported at least one pregnancy. Of the 127 with a first pregnancy, 23 participants went on to have a second pregnancy and 4 participants had a third. Contraception use among AGYW at first pregnancy was low at 26% (33/127) with only 9/127 (7.1%) reporting condom use.

Table 2: Reproductive health characteristics of adolescent girls and young women during longitudinal cohort follow up.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>Median (IQR) or N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproductive history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever sexually active</td>
<td>400</td>
<td>306 (77%)</td>
</tr>
<tr>
<td>Ever diagnosed BV</td>
<td>366</td>
<td>144 (39%)</td>
</tr>
<tr>
<td>Age at first sex (years)</td>
<td>306</td>
<td>18.9 (18.3-19.9)</td>
</tr>
<tr>
<td>(?Ever) Married AGYW</td>
<td>306</td>
<td>44 (14%)</td>
</tr>
<tr>
<td>Ever diagnosed with any STIs</td>
<td>306</td>
<td>169 (55%)</td>
</tr>
<tr>
<td>Ever became pregnant</td>
<td>306</td>
<td>127 (42%)</td>
</tr>
</tbody>
</table>
**Pregnancy (n=127)**

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
<th>Value (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first pregnancy (years)</td>
<td>127</td>
<td>20.5 (19.1-22.0)</td>
</tr>
<tr>
<td>Contraceptive use 3 months before confirmation of pregnancy</td>
<td>127</td>
<td>33 (26%)</td>
</tr>
<tr>
<td>Unintended pregnancies</td>
<td>127</td>
<td>96 (76%)</td>
</tr>
<tr>
<td>Screened for BV pre-pregnancy</td>
<td>127</td>
<td>119 (94%)</td>
</tr>
<tr>
<td>Screened for BV during pregnancy</td>
<td>127</td>
<td>105 (83%)</td>
</tr>
<tr>
<td>Screened for BV both pre and during pregnancy</td>
<td>127</td>
<td>100 (79%)</td>
</tr>
<tr>
<td>Diagnosed with BV 3 months before confirmation of pregnancy</td>
<td>119</td>
<td>45 (38%)</td>
</tr>
<tr>
<td>Diagnosed with BV during pregnancy</td>
<td>105</td>
<td>24 (23%)</td>
</tr>
<tr>
<td>Reported number of sexual acts in the 45 days prior to LMP</td>
<td>99</td>
<td>6 (2-18)</td>
</tr>
<tr>
<td>Reported number of sexual acts during pregnancy in the 45 days after LMP</td>
<td>106</td>
<td>8 (2-23)</td>
</tr>
</tbody>
</table>

*BV, bacterial vaginosis; IQR, interquartile range; AGYW, adolescent girls and young women; STIs, sexually transmitted infections; LMP, last menstrual period.*

**BV and Pregnancy**

During study follow-up, among 306 sexually active AGYW there were a total of 127 first pregnancies. Of these, 124 pregnancies were included in the BV data analysis, as 3 AGYW did not have vaginal samples collected. Among the 124 pregnant AGYW, 96% (119/124) had BV screening pre-pregnancy, while only 85% (105/124) had BV screening during pregnancy (Figure 2). The prevalence of BV pre-pregnancy was higher at 38% (45/119) compared to prevalence of BV during pregnancy, which was 23% (24/105). In total, 100 AGYW had BV testing done at both time points pre- and post-pregnancy (Figure 3).

Changes over time in BV status pre- and post-pregnancy are demonstrated in Figure 3. A multivariate model did not demonstrate that prior BV (3 months before conception) was associated with risk of BV while pregnant. The relative risk (aRR) of BV in pregnancy among AGYW, adjusted for income, residence and past history of STI was 0.63 (95% CI: 0.46, 0.87; p value= 0.005).

a) **Factors associated with risk of BV pre-pregnancy**

Sexually transmitted infections (CT and HSV-2) were associated with increased risk of BV pre-pregnancy. Having a CT infection resulted in an adjusted RR of 4.28 (95% CI:1.52-12.1;
p value=0.006). Frequent vaginal washing pre-pregnancy was not associated with increased risk of BV, although data were incomplete. AGYW who practiced vaginal washing more than 75% of the time had an adjusted RR of BV of 0.66 (95% CI:0.12-3.44; p value=0.623).

b) Factors associated with risk of BV during pregnancy

Shorter time from menarche to first sex was associated with a trend toward increased BV prevalence in pregnancy (aRR: 2.5; 95%CI: 0.82-7.82; p value=0.104) but was not statistically significant. Risk of BV in pregnancy was four times higher with history of past CT infection (aRR: 4.13; 95%CI: 1.73-9.90; p value=0.001). AGYW who reported frequent vaginal washing in pregnancy (>75% of the time) had reduced risk of BV, although not statistically significant (RR:0.63; 95% CI 0.17-2.34; p value=0.494).

Since BV is associated with sexual activity, we determined the median number of reported sex acts both before and during pregnancy, and found that AGYW were reporting more sexual activity while pregnant (median 8 sex acts [IQR:2-23]) compared to pre-pregnancy (median 6 sex acts [IQR:2-18]) (Figure 7). The median number of sexual acts pre-pregnancy was counted as 45 days before LMP, while the median number of sex acts during pregnancy was counted as 45 days after LMP.

Results of the Spearman correlation indicated that there was no correlation between BV diagnosis and median reported sexual activity in the 45 days prior to pregnancy ($r_s$ =0.03, p value= 0.714) and the median reported sexual activity during pregnancy ($r_s$ =0.09, p value =0.211).

A sensitivity analysis was performed for the 100 AGYW who had paired BV testing both pre and during pregnancy; the relative risk of BV was similar to that found with the larger cohort. We adjusted for income, residence and past history of STI and observed a nearly 40% reduction in BV during pregnancy (aRR of 0.66 (95%C.I:0.48-0.92; p value=0.015). BV in pregnancy was reduced compared to pre-pregnancy.
Table 3: Relative Risk of bacterial vaginosis (BV) among adolescent girls and young women (AGYW) (N=127) prior to pregnancy.

<table>
<thead>
<tr>
<th>Positive BV diagnosis</th>
<th>RR</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condomless sex in past 90 days</td>
<td>1.5</td>
<td>0.564</td>
<td>0.38-6.00</td>
</tr>
<tr>
<td>No regular source of income</td>
<td>1.02</td>
<td>0.968</td>
<td>0.32-3.31</td>
</tr>
<tr>
<td>Menarche to sexual debut &lt; 3 years</td>
<td>0.91</td>
<td>0.902</td>
<td>0.20-4.02</td>
</tr>
<tr>
<td>Urban Residence</td>
<td>2.48</td>
<td>0.067</td>
<td>0.94-6.56</td>
</tr>
<tr>
<td>History of CT</td>
<td>4.28</td>
<td>0.006</td>
<td>1.52-12.1</td>
</tr>
<tr>
<td>History of HSV-2</td>
<td>4.12</td>
<td>0.048</td>
<td>1.01-16.77</td>
</tr>
<tr>
<td>History of vaginal washing&gt; 75% of visits</td>
<td>0.66</td>
<td>0.623</td>
<td>0.12-3.44</td>
</tr>
</tbody>
</table>

BV, bacterial vaginosis; RR, relative risk; CT, Chlamydia trachomatis; HSV-2, herpes simplex virus type 2.

Logistic regression using Generalized Estimating Equations was created to analyze longitudinal trends in BV prior to pregnancy. Model included condom use, income, time from menarche to first sex, residence, history of CT, history of HSV-2) and frequent vaginal washing.

Table 4: Relative Risk of bacterial vaginosis (BV) among adolescent girls and young women (AGYW) during pregnancy, over 5 years.

<table>
<thead>
<tr>
<th>Positive BV diagnosis</th>
<th>RR</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condomless sex in past 90 days</td>
<td>1.26</td>
<td>0.767</td>
<td>0.28-5.74</td>
</tr>
<tr>
<td>Menarche to sexual debut &lt;3 years</td>
<td>2.5</td>
<td>0.104</td>
<td>0.82-7.82</td>
</tr>
<tr>
<td>History of CT</td>
<td>4.13</td>
<td>0.001</td>
<td>1.73-9.90</td>
</tr>
<tr>
<td>History of HSV-2</td>
<td>1.72</td>
<td>0.403</td>
<td>0.48-6.12</td>
</tr>
<tr>
<td>History of vaginal washing&gt;75% of visits</td>
<td>0.63</td>
<td>0.494</td>
<td>0.17-2.34</td>
</tr>
</tbody>
</table>

BV, bacterial vaginosis; RR, relative risk; CT, Chlamydia trachomatis; HSV-2, herpes simplex virus type 2

Logistic regression using Generalized Estimating Equations was created to analyze longitudinal trends in BV during pregnancy. Model included condom use, time from menarche to first sex, history of CT, history of HSV-2) and frequent vaginal washing.

Correlates of pregnancy

The probability of pregnancy increased from 0.9% at age 17 years to 7.8% at age 19 years. (Figure 4). After 3 months of study follow up, the probability of pregnancy was at 8.3%, by
month 60 the incident probability was at 52% (Figure 5). When asked, 96/127 (76%) reported that the pregnancy was unintended (Table 2).

History of sexual activity in the past 90 days was associated with a 3-fold higher risk of incident pregnancy (adjusted hazard ratio (aHR:3.30; 95%CI: 1.44- 7.43; p value=0.005). Condomless sex in the past 90 days was associated with 3-fold increase risk for incident pregnancy (aHR:3.01; 95% CI: 1.73 – 5.24; P<0.001). Shorter time between menarche and time of first sex (<3 years) was associated with 3-fold higher risk of incident pregnancy (aHR:2.70; 95% CI: 1.46 – 4.96; P value =0.001). Having a new sexual partner in the past 90 days (aHR:0.43; 95%CI:0.20-0.93; p value=0.031) was associated with lower risk of pregnancy; 31% (38/124) of AGYW reported to be in a monogamous marital relationship at the time of pregnancy. Absence of a regular source of income at time of pregnancy was associated with higher risk of incident pregnancy, but it was not statistically significant (aHR:1.50; 95%CI:0.92-2.45; p value=0.108)

Table 5: Multivariate Cox regression model of predictors for first pregnancy among adolescent girls and young women in a Kenyan reproductive health cohort.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard ratio</th>
<th>95% C.I.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at study visit</td>
<td>1.01</td>
<td>0.86-1.17</td>
<td>0.946</td>
</tr>
<tr>
<td>No source of income</td>
<td>1.5</td>
<td>0.92-2.45</td>
<td>0.108</td>
</tr>
<tr>
<td>Urban residence</td>
<td>0.68</td>
<td>0.46-1.02</td>
<td>0.062</td>
</tr>
<tr>
<td>Time from menarche to first sex &lt;3 years</td>
<td>2.7</td>
<td>1.46-4.96</td>
<td>0.001</td>
</tr>
<tr>
<td>Sexual activity in the past 90 days</td>
<td>3.3</td>
<td>1.44-7.43</td>
<td>0.005</td>
</tr>
<tr>
<td>No condoms used in past 90 days</td>
<td>3.01</td>
<td>1.73-5.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex with new partner in past 90 days</td>
<td>0.43</td>
<td>0.20-0.93</td>
<td>0.031</td>
</tr>
<tr>
<td>History of CT infection</td>
<td>1.28</td>
<td>0.79-2.08</td>
<td>0.31</td>
</tr>
<tr>
<td>History of GC</td>
<td>1.61</td>
<td>0.49-5.26</td>
<td>0.424</td>
</tr>
</tbody>
</table>

CI, confidence interval; LMP, last menstrual period; CT, Chlamydia trachomatis; NG, Neisseria gonorrhea
Cox regression analysis was performed to assess associations between correlates and pregnancy. Correlates adjusted for include age at study visit, income, residence, time from menarche to first sex, sexual activity in the past 90 days, new sexual partners, history of condomless sex, history of CT, and history of GC.
DISCUSSION

Using precisely collected longitudinal cohort data, this study examined how vaginal dysbiosis in recently sexually active young women is changing at the time of a first early pregnancy. This is one of few studies to concentrate solely on this age group and to have pre-pregnancy measurements of BV status with longitudinal follow up during pregnancy. Contrary to expectations, we found that there was a lower prevalence of BV during pregnancy compared to pre pregnancy, and we did not find evidence that BV in the 3 months before conception was associated with a higher risk of BV during pregnancy. In fact, BV diagnoses were 40% lower than prior to pregnancy. We further did not find a reason to explain this reduced risk; after controlling for known risk factors for BV including vaginal washing and sexual activity, the lowered risk of BV remained (Ranjit et al. 2018)(Verstraelen et al. 2010).

Our data are novel because of the longitudinal nature of the specimens, which include a pre-pregnancy specimen for most participants. We are not aware of other studies of BV in pregnancy which also have paired pre-pregnancy measurements. Much of the published literature discussing BV and pregnancy focuses on adverse outcomes of BV during and after pregnancy. (Afolabi, Moses, and Oduyebo 2016)(Juliana et al. 2020)(Shimaoka et al. 2019). There are multiple published studies of longitudinal BV patterns in women, but most sampled older and more sexually experienced women (Brotman et al. 2010) (Lambert et al. 2013) or AGYW who were more sexually active (Gosmann et al. 2018). Our study therefore represents a new look at the youngest and most vulnerable AGYW at the very start of their sexual lives.

Few studies have been done to analyze risk of dysbiosis in pregnant women (Romero et al. 2014)(Walther-António et al. 2014). The vaginal environment of pregnant women differs from that of non-pregnant women (Freitas et al. 2017) and has been found to be less diverse and richer in *Lactobacilli spp* which is protective from BV; but those studies are from small cohorts in North American and Europe and it is unclear if their findings are relevant to younger women in sub-Saharan Africa. Still, pregnancy has hormonal variations that could
stabilize the vaginal epithelium and promote a less-diverse microbial environment.

Production of estrogen during pregnancy is associated with increase in concentration of the protective *Lactobacilli* species (Boskey et al. 2001) (MacIntyre et al. 2015). This could be the reason we saw protection from BV in this cohort of pregnant AGYW.

In our study, known BV risk factors such as HSV-2 and *C. trachomatis* were associated with increased BV in the pre-pregnancy period (Koumans et al. 2007). We noted that during pregnancy, past CT infection was also associated with increased BV acquisition. We employed the use of Nugent scoring to diagnose BV, while CT was diagnosed via NAAT. However, as neither of these diagnostics are routinely used in low-resource settings (Bosu 1999)(Vuylsteke 2004)(White et al. 2008), attempts to improve the reproductive health of AGYW by addressing cofactors of BV will require better diagnostic testing that can be deployed in resource limited settings. Our data further adds to the evidence that STIs are importantly related to BV diagnoses in a way that is not possible to elucidate without serial measurements over time to determine sequence of events. Vaginal washing is also a significant risk factor for BV acquisition (Ness et al. 2002). We however lacked enough data on vaginal washing to make any significant statements about its role in this issue.

The pregnancy incidence in this cohort was high, with over 40% of participants who reported sexual activity having a pregnancy during follow up. Despite adequate counselling provided by the study clinic, contraception use was low with most pregnant AGYW reporting they were not using any contraception 3 months prior their pregnancy. It was noted that one-third of the pregnant AGYW reported being married, and contraception uptake may have been low as they may have desired to start a family. Increased frequency of sexual activity was not associated with BV diagnosis in pregnancy. We observed that pregnancy participants were reporting engaging in more sexual acts that they reported before pregnancy; non-pregnant women may have been attempting to reduce pregnancy risk, with no need for such limits in post pregnancy.
Our study had some limitations. Multiple key variables were collected by self-report including dates of LMP, menarche, and first sex. To improve accuracy, we amended the date of sexual activity if STI or pregnancy results indicated that sexual activity had occurred, and we also included revised retrospective reporting by participants. Our sample size was small (127 pregnancies reported) and findings may not be reflective of a larger AGYW population. Participants were slightly more likely to refuse genital sample collection during pregnancy, which may result in bias. However, 105 specimens representing 83% of pregnant women were collected, which should be adequate representations from specimens in pregnancy, and a sensitivity analysis with only paired specimens yielded similar results. Specimens were only collected every 3 months during pregnancy. Thirdly, questions about vaginal washing were added late in the study and the data collected was inadequate. Lastly, we had incomplete data on pregnancy outcomes.

CONCLUSION

Pregnant AGYW had fewer BV diagnoses during pregnancy then before pregnancy in this cohort with unique paired specimens from before and during pregnancy. This observation is unique as we are not aware of other studies with longitudinal assessment of BV status in AGYW from pre-conception to incident pregnancy. Pregnancy appears to be a time of lower risk of BV in these young women, for reasons that may relate to the hormonal milieu. Further molecular characterization of vaginal bacteria and biological factors that drive vaginal dysbiosis will be analyzed from this study to understand what specific factors might contribute to reduced BV risk during pregnancy.
Bibliography


FIGURES

Figure 1: Flow Diagram of Enrollment for adolescent girls and young women in the study

Total number of girls screened= 610

- 115 did not return for follow-up
- 31 had more than one sexual partner
- 19 screened out/not eligible
- 15 declined to join the study
- 8 unknown reasons
- 8 with HSV-2 positive by ELISA
- 5 uncomfortable with study procedures
- 3 investigator decision
- 3 younger than required age
- 2 unable to adhere to study follow up
- 1 HIV positive

400 enrolled
Figure 2: Flow diagram demonstrating BV pre and during pregnancy (N=127)
Figure 3: Change in individual participant Nugent score pre and during pregnancy (N=100).

Legend: Y-axis represents Nugent score. Each line shows a participant’s Nugent score over time. Scores $\geq 7$ were considered bacterial vaginosis.
Figure 4: Time to pregnancy in a cohort of AGYW from enrolment.

Kaplan-Meier estimates showing the probability of pregnancy after enrolment, over 5 years in Thika, Kenya. At 17 years, the probability of being pregnant was 0.9%. Median age of pregnancy (50% of pregnancy in this cohort) among 127 participants was at 20 years. Probability of pregnancy at each age is displayed.

*This data is inclusive of the 2\textsuperscript{nd} and 3\textsuperscript{rd} pregnancies. Incident pregnancies in this cohort was 127.

<table>
<thead>
<tr>
<th>Age at Study Follow up (years)</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants at risk</td>
<td>0</td>
<td>300</td>
<td>292</td>
<td>265</td>
<td>226</td>
<td>186</td>
<td>133</td>
<td>82</td>
<td>29</td>
</tr>
<tr>
<td>Pregnancy count*</td>
<td>0</td>
<td>3</td>
<td>41</td>
<td>16</td>
<td>24</td>
<td>22</td>
<td>19</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Probability of pregnancy</td>
<td>0</td>
<td>0.90%</td>
<td>2.30%</td>
<td>7.80%</td>
<td>16.40%</td>
<td>24.90%</td>
<td>33.30%</td>
<td>45.20%</td>
<td>51.40%</td>
</tr>
</tbody>
</table>
Descriptive statistics were done to calculate the number of sexual acts in this cohort pre and during pregnancy. Timepoint before pregnancy was defined as <= LMP + 45 days, and during pregnancy was any visit >LMP + 45 days. For sex activity prior to pregnancy, N=99; For sex activity during pregnancy, N=106; Median number of Sex acts prior to pregnancy was 6 (IQR:2-18). Median number of sex acts during pregnancy was 8 (IQR:2-23)