REPRODUCTIVE TRACT INFECTIONS AND UTERINE FIBROIDS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

Chapel Hill
2016

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ABSTRACT

Kristen Renee Moore: Reproductive Tract Infections and Uterine Fibroids
(Under the direction of Jennifer S. Smith and Donna D. Baird)

For decades, it has been hypothesized that reproductive tract infections (RTIs) are risk factors for uterine fibroids. However, only two recent studies have been conducted. We aimed to investigate the relationship between RTIs and fibroids in a large study using ultrasound screening for fibroids. We used cross-sectional enrollment data from African-American women ages 23-34 years with no previous fibroid diagnosis. For Aim 1, RTI history was measured by self-report. For Aim 2 we used serology, an immunological measure of past exposure with a focus on herpes simplex virus type 2 (HSV-2) because prior published data have suggested a possible association, and serology for HSV-2 is much more accurate than self-report. For both aims, fibroid status was measured by standardized ultrasound. Secondary fibroid outcomes were size, number, and total volume. Age- and multivariable-adjusted logistic regression were used to estimate odds ratios (ORs).

In total, 1,656 women were included; 22% had fibroids. Self-reported bacterial vaginosis (BV) was associated with a 21% increased odds of fibroids [aOR 1.21, 95% confidence interval (CI) 0.93-1.58]. Self-reported chlamydia infection and pelvic inflammatory disease were associated with a 38% (aOR 0.62, 95% CI 0.40-0.97) and a 46% (aOR 0.54, 95% CI 0.25-1.17) reduced odds of having two or more fibroids, respectively. Those with a previous self-reported BV diagnosis had a 47% increased odds of having 2 or more fibroids (aOR 1.47, 95% CI 0.98-2.21) and a 41% increased odds of having a larger

total fibroid volume (aOR 1.41, 95% CI 0.98-2.04). There was no significant association between HSV-2 seropositivity and fibroid presence (multivariable-adjusted OR: 0.94 95% CI: 0.73, 1.20); nor were there any associations with size of largest fibroid, number of fibroids, or total fibroid volume. Our study was the first to investigate the association of fibroids with HSV-2 exposure assessed serologically. In addition, it was the first to explore the relationship between self-reported RTIs and fibroid size, number, and total volume. There appeared to be no strong associations between self-reported RTIs or serologically-measured HSV-2 seropositivity and the presence of fibroids.

To my loving husband and beautiful children who motivated me to press on. To my family and friends who supported me and never gave up on me; especially my parents and parents-in-love who stepped in when we needed them most. To my awesome nanny Martha who relieved a lot of stress and loved on my children during a very busy time. Most of all, to my heavenly Father, Jesus Christ and Holy Spirit, who proved faithful through it all.

ACKNOWLEDGEMENTS

I would like to thank Dr. Donna Baird whose incredible mentorship, wisdom and caring spirit guided me through. She also allowed me to use data from her awesome study which enabled me to conduct the research for this project. I am extremely grateful for Dr. Jennifer Smith for believing in me and providing sound advice and expertise for this project along with the rest of my dissertation committee, Drs. Victor Schoenbach, Stephen Cole and Dirk Dittmer.

I would also like to acknowledge Dr. Trevor Archer and the National Institute for Environmental Health Sciences (NIEHS) for granting me a fellowship to conduct my dissertation research and use their facilities and equipment. I would also like to thank Dr. Dale Sandler for approving my proposal for the herpes simplex virus-2 serological tests and the whole Epidemiology Department at NIEHS for all of their support.

I would like to thank the staff at Social and Scientific Systems that conducted the field work for the Study of Environment Lifestyle and Fibroids (SELF) and helped to facilitate the serology testing and data management, in particular, Dr. Christie Barker-Cummings, Deborah Cousins, Susan Baker, and Anna Jones. I would also like to thank Dr. Charlotte Gaydos and Katherine Schlusser at John's Hopkins for supervising and conducting the serology tests for this project, respectively.

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BMI	body mass index
BV	bacterial vaginosis
CAWI	computer-assisted web interviewing
CI	confidence interval
CT	*Chlamydia trachomatis*
DMPA	depot medroxyprogesterone acetate
GED	general education development
HFHS	Henry Ford Health System
HSV	herpes simplex virus
NAAT	nucleic acid amplification tests
NIEHS	National Institute of Environmental Health Sciences
OR	odds ratio
PID	pelvic inflammatory disease
PCR	polymerase chain reaction
RTI	reproductive tract infection
SELF	Study of Environment, Lifestyle and Fibroids
US	United States
UFS	Uterine Fibroid Study

CHAPTER 1: STATEMENT OF SPECIFIC AIMS

Uterine fibroids are one of the most common gynecologic conditions affecting women during their reproductive years [1], with estimated total annual costs (direct and indirect) in the United States (US) of up to $34 billion [2]. However, the cause(s) of fibroids is largely unknown. Many decades ago, a hypothesis was proposed that reproductive tract infections (RTIs) play a role in fibroid development [3]; however, this hypothesis has never been adequately tested.

More recently, two published studies [4, 5] have explored the relationship between RTIs and fibroids; both studies were based on self-reported infection histories. Results from these two studies were only partially consistent. The first was a clinic-based study of 318 cases and 394 controls [4] which found a positive dose-response association between self-reported diagnosis of pelvic inflammatory disease (PID) (and the number of PID episodes) and uterine fibroids in premenopausal women. No association was found between self-reported history of genital herpes or warts and fibroids, although there was a suggestion of an increased risk of fibroids for women with self-reported history of *Chlamydia trachomatis* (CT). The second, the Uterine Fibroid Study (UFS) [5], was a cross-sectional study of 1,364 women with ultrasound determination of fibroid status. This study found no association of uterine fibroids with self-reported diagnosis of PID in either African-American or White women. However, there were suggestions of positive associations with self-reported history of CT infection in White women, and with trichomonas, syphilis, and "other infections" in African-American women. Self-reported history of genital herpes had suggestions of a positive association with

uterine fibroids in both ethnic groups [5]. In both of these studies [4, 5], self-reported history of abnormal Pap smear was inversely associated with fibroids.

Building upon previous work which found suggestions of positive associations between a few self-reported RTIs and fibroids, our first aim was to investigate the association of fibroids with several self-reported RTIs in the largest sample to date. We also sought to explore the relationship between self-reported RTIs and the number, size, and total volume of fibroids which has not been previously done. Our second aim was to investigate the relationship between serological status of type-specific herpes simplex virus type 2 (HSV-2) and uterine fibroids (presence, number, size and total volume). The aforementioned studies of this often asymptomatic infection are limited by their use of self-reported exposure histories. Thus, we conducted a cross-sectional study in the ongoing Study of Environment, Lifestyle & Fibroids (SELF) based in the Detroit area among 1,696 African-American women. The primary outcome was fibroid status determined by ultrasound; the main exposures were self-reported histories of RTIs and seropositivity for HSV-2, measuring previous exposure to this pathogen. Participants with at least one fibroid ≥0.50 cm in diameter at enrollment ultrasound were considered to have fibroids, and all women without a fibroid ≥0.50 cm in diameter at enrollment ultrasound were considered not to have fibroids. Specifically, we aimed to:

1. Estimate the association of fibroids (presence, number, size of the largest fibroid and total fibroid volume) measured by ultrasound with the following RTIs assessed by self-report: PID, CT, genital herpes, gonorrhea, trichomonas, genital warts, and bacterial vaginosis (BV)

2. Estimate the association of fibroids with serologically-determined previous exposure to HSV-2

These aims were accomplished using enrollment questionnaire data, transvaginal ultrasound results and serologic findings from a cohort of 1,696 African-American women aged 23-34 years. We used laboratory testing of the blood specimens for type-specific antibodies to HSV-2 using standard serologic methods to determine past exposure to infection. We analyzed the study data using logistic regression.

This study was the largest to investigate the association of fibroids with several self-reported RTIs and the first to investigate HSV-2 exposure assessed serologically. We hypothesized that women with a history of RTIs would have a higher prevalence of fibroids than women without a history of RTIs. We also explored the relationship between self-reported RTIs and HSV-2 seropositivity and number, size, and total volume of fibroids.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1 Uterine Fibroids (myomas or leiomyomas)

Uterine fibroids, benign smooth muscle cell tumors of the uterus, are one of the most common gynecologic conditions affecting women during their reproductive years [1]. The Uterine Fibroid Study (UFS) of 1,364 randomly selected women 35 to 49 years of age screened by ultrasound found an estimated cumulative incidence of fibroid tumors by age 50 of >80% for African-American women and close to 70% for White women [6]. A pathology study conducted in New York showed comparable estimates; fibroids were found in 77 of the 100 sequential uteri inspected. The uteri were removed at hysterectomy (only 33 with pre-operative fibroid diagnosis) and were thinly sliced in 2-mm sections for analysis [7].

The symptoms experienced by an estimated 20-50% of women with fibroids include pelvic pain and pressure, reproductive problems (infertility and adverse birth outcomes), and severe bleeding that can result in anemia [1, 8-10]. These symptoms can negatively impact women's quality of life including their sexual life, performance at work, relationships and family [11, 12]. The primary treatment for fibroids is hysterectomy (the removal of the uterus) because it completely eliminates symptoms and the possibility of reemergence. Therefore, fibroids are the leading indication for hysterectomy in the US, accounting for 40% of hysterectomies or approximately 240,000 per year [13]. However, for women who have not completed childbearing or who want to keep their uterus, there are other treatment options such as myomectomy (surgical removal of fibroids with uteri intact), uterine artery embolization (minimally-invasive blockage of fibroid blood supply) and focused ultrasound

(non-invasive magnetic resonance imaging-guided sound waves that heat and destroy fibroid tissue) [1, 14]. However, with these treatments there is the possibility of recurrence. There is also a Food and Drug Administration approved short term therapy for reducing fibroid size and symptoms, gonadotropin-releasing hormone agonist. However, it can only be used for 3 to 6 months due to undesirable side effects (bone loss, hot flashes and depression), and once stopped, fibroids can rapidly return to their pre-treatment size [15].

Ultrasound is the clinical standard for diagnosis of fibroid tumors [16]. However, it is not routinely performed unless a woman is pregnant or experiencing symptoms. Thus, in order to estimate the true prevalence of fibroids, routine population-based ultrasound screening would have to be performed. The majority of previous studies have used hysterectomy, self-report and/or clinical diagnosis of fibroids to determine prevalence. However, these studies are more likely selecting women who have more severe symptoms, not those who may be asymptomatic. The majority of women with fibroids are asymptomatic.

As a result of the management and treatment of fibroids and their associated complications, the total estimated costs of fibroids in the US including direct (medical and surgical management) and indirect (lost work time and more complicated obstetric outcomes) are as much as $34 billion [2]. Thus the societal cost of fibroids in the US is more than breast cancer, colon cancer, or ovarian cancer and approximately one-fifth the annual cost of diabetes mellitus [2].

Surprisingly, although fibroids have a substantial cost and public health burden, their pathogenesis and etiologic cause(s) are largely unknown. Fibroids are very rarely malignant, and it is theorized that benign and malignant tumors have a different pathogenesis [1]. Fibroids

are typically classified by their size (diameter) and location- subserosal (outer uterine wall), submucosal (protruding into the uterine cavity) or intramural (within the uterine wall). Multiple tumors of various sizes (microscopic to very large) and locations are often found in the same uterus. Most tumors are monoclonal and thus develop independently from other leiomyomas in the same uterus [17]. Although their cause is unknown, fibroids are known to be hormonally responsive; both estrogen and progesterone can stimulate fibroid tissue growth [18]. Fibroids begin to develop after menarche [19] and regress after menopause [7]. However, what causes the initial transformation of muscle cells into abnormal muscle cells and then what causes them to proliferate and grow into clinically visible tumors is not clearly understood.

Selected risk factors that have been established for fibroids are African-American heritage, older age (up to the age of menopause), younger age at menarche, and nulliparity as well as earlier age at first birth and more years since last birth [5, 20-22]. Other factors such as body mass index (BMI), smoking, hormonal contraceptive use, and alcohol have been inconsistently associated with fibroid risk [5, 23, 24]. More recent studies have shown no association with fibroids and smoking [24]. Previous alcohol use appears to have a positive relationship [24, 25]. Oral contraceptives are prescribed to help control symptoms such as increased menstrual bleeding that may actually be due to fibroids [26]. Thus, whether oral contraceptives are a risk factor for fibroids is difficult to tease out. Studies have shown that progestin-only injectables (i.e. Depo-Provera) may be protective against fibroids [20, 27, 28]. Many decades ago, a hypothesis was proposed that reproductive tract infections play a role in fibroid development [3]; however, this hypothesis has never been adequately tested.

2.2 African-American Heritage and Fibroids

The disparate burden of fibroids among African-American women was acknowledged as early as the late 1800s. In Witherspoon et al. (1930), African-American women represented a substantial proportion of the fibroid cases (90% of 2,991 cases) at a local hospital although the total gynecological admissions were only slightly greater for African-Americans compared to Whites [3]. More recent estimates indicate that African-Americans are 2 to 3 times more likely to have fibroids than White women [6]. They have an earlier onset (~10-15 years), have more fibroids at a given age, and are more likely to have clinically relevant fibroids (based on size and location) than Whites [6, 29-33]. African-Americans are also more likely to report severe symptoms, interference with physical activities and relationships, and missed work days compared to White women [11, 34]. Additionally, a study of fibroid growth found that in contrast to White women, the growth rates of fibroids among African-American women did not tend to decline as age increases [35]. African-American women are also more likely to undergo hospitalization, hysterectomy and myomectomy (relative risk, 3.5, 2.4, 6.8, respectively) for fibroids compared to White women, consistent with more severe disease and an earlier age of onset [36, 37].

Studies on fibroids have primarily been conducted among women who have undergone hysterectomy or those self-reporting symptoms, and thus select for more severe fibroid cases and are not representative of the general population. However, in a recent pilot study of asymptomatic young women 18-30 years old, 26% of the 43 African-American and 7% of the 58 White women were ultrasound-diagnosed with fibroids [30]. Thus, even among young, asymptomatic women, African-Americans appear to have a higher burden of disease. It is not understood why African-Americans are so disproportionately impacted by fibroids. There is some evidence that environmental factors such as diet [38, 39], stress i.e. perceived

racial discrimination [40], childhood abuse [41] and vitamin D insufficiency [42] that may differ by race, may be linked to fibroids. Furthermore, genetic factors could explain some of the burden, but this requires further investigation [43, 44]. Witherspoon hypothesized in the 1930s that because both fibroids and RTIs disproportionately burden African-American women and certain RTIs can lead to conditions such as chronic pelvic infection that could result in inflammatory reactions, RTIs could play an etiological role [3]. However, since the 1930's very few studies have investigated this proposed relationship.

2.3 Infection and Fibroids

The two recently published studies [4, 5] that have explored the relationship between RTIs and fibroids were both based on self-reported infection histories. Results from these two studies were only partially consistent (Table 1). The first was a clinic-based case-control study of fibroid risk factors in the Baltimore metropolitan area [4]. Cases were 318 18-55 year-old pre-menopausal women with a first diagnosis of fibroids between 1990 and 1993 confirmed by histology or ultrasound. Controls were patients with intact uteri who had visited the same physicians as the cases for a routine checkup that included a pelvic exam without any reference to fibroids and no indication of fibroids in medical records. Cases and controls were frequency matched on 10 year age group, gynecologist and calendar period. The authors found a positive dose-response association between self-reported history of diagnosis of PID (and the number of PID episodes) and uterine fibroids. No association was found between self-reported history of genital herpes or warts and fibroids, although there was a suggestion of an increased risk of fibroids for women with self-reported history of CT.

The second was an analysis conducted using data from the UFS [5], which was a cross-sectional fibroid screening study of randomly selected members of an urban health plan aged 35-49 years. The analysis was limited to 1,016 premenopausal women with ultrasound

determination of fibroid status and complete fibroid and questionnaire data. RTI history was obtained by a mail-in questionnaire and analyses were conducted separately for African-Americans and Whites. Infections with <15 cases were excluded. This study found no association of uterine fibroids with self-reported diagnosis of PID in either African-American or White women. However, there were suggestions of positive associations with self-reported history of CT infection in White women, and with trichomonas, syphilis, and "other infections" [mainly BV] in African-American women. Self-reported history of genital herpes had suggestions of a positive association in both ethnic groups [5]. In both of these studies [4, 5], self-reported history of abnormal Pap smear was inversely associated with fibroids. This finding was also corroborated in our recent study that showed an inverse association between self-reported cervical treatment and fibroids [45].

Furthermore, in a small pilot study, fibroid tissue from 20 UFS participants who had reported a history of sexually transmitted disease(s) or multiple sex partners was searched for viral DNA to HSV-1 and HSV-2, cytomegalovirus, human herpes virus 6,7,8 and Epstein-Barr virus (EBV) [5] using polymerase chain reaction (PCR). They did not detect any of these pathogens in the tumor samples but did in positive controls. However, no serological diagnosis was done to measure past exposure to herpes infection.

In light of the previous studies conducted on RTIs and fibroids, the goal of this project was to add to the literature on self-report and more importantly to take the next step and use serology, an immunological measure of past infection, to better understand the relationship between these two conditions. We also explored the relationship between RTIs (self-reported and serologically measured) and fibroid characteristics (number, size and total volume), which has not previously been done.

Relatively few RTIs can be measured well serologically for epidemiologic purposes (mainly human immunodeficiency virus (HIV), herpes simplex virus, CT and syphilis) [46]. HIV and syphilis are rarer infections, with annual reported infection rates among young African-American women in the US of 64 per 100,000 (25-34 year-olds) for HIV in 2010 [47] and 13 per 100,000 (25-29 year-olds) for syphilis in 2013 [48] and thus are not likely to have a high enough seroprevalence for a well-powered analysis. Human papillomavirus (HPV) serology is only ~50% sensitive and the standard VLP ELISA can only measure 1 HPV type at a time [49] so it is not sufficiently feasible for measuring past exposure to HPV. In Aim 2 of this project we focus on serologically measured HSV-2 as the exposure. We will look at serology of CT in a future study.

Table 1. Two Recent Studies on Reproductive Tract Infections and Fibroids

Self-Reported History of Infection (Ever vs. Never)	*NIEHS UFS OR (95% CI)* [a]		*Faerstein, et al. OR (95% CI)* [b]
	African-American	White	
PID	0.9 (0.5-1.9)	1.2 (0.4-3.6)	1.8 (1.0-3.1)
No. of Episodes	----------	----------	
0	----------	----------	1.0
1	----------	----------	1.4 (0.7-2.9)
2	----------	----------	1.6 (0.5-5.2)
≥3	----------	----------	3.7 (0.9-15.9)
Genital herpes	1.6 (0.7-3.6)	1.4 (0.7-2.8)	0.8 (0.3-2.2)
Genital warts	1.1 (0.5-2.2)	1.0 (0.5-2.0)	1.0 (0.4-2.5)
Chlamydia	1.1 (0.6-2.4)	1.7 (0.6-4.9)	3.2 (0.8-13.7)
Gonorrhea	1.3 (0.7-2.2)	NR	----------
Trichomonas	3.1 (0.9-10.7)	NR	----------
Syphilis	2.2 (0.6-7.8)	NR	----------
Other (mainly BV)	2.1 (0.8–5.6)	1.1 (0.4–3.2)	----------
Abnormal Pap Smear	0.6 (0.4-1.0)	0.8 (0.4-1.4)	0.5 (0.3-1.0)

BV, bacterial vaginosis; CI, confidence interval; NIEHS, National Institute of Environmental Health Sciences; NR, not reported (<15 cases); OR, odds ratio; PID, pelvic inflammatory disease; UFS, Uterine Fibroid Study

[a] Adjusted for age, age of menarche, full-term pregnancies after age 24, and body mass index;

[b] Adjusted for clinic, ethnicity

2.4 Reproductive Tract Infections

Reproductive tract infections (RTIs) are a substantial public health problem in the US with an estimated 110 million prevalent infections and 20 million incident infections in 2008 [50]. RTIs also cost the US health care system close to $16 billion in health care costs every year. Youth 15-24 years of age account for half of all new RTIs, while representing only 25% of the sexually active population [50]. In addition, in women, these infections can result in reproductive complications; it is estimated that undiagnosed RTIs cause 24,000 women to become infertile each year [51]. Some of the risk factors for RTIs include young age, African-American race/ethnicity, lower socio-economic status (education, income, etc.), substance abuse, heavy alcohol use, single marital status, use of oral contraceptives and higher number of sexual partners (as well as other sexual behavior characteristics) [52-56]. A brief description of the self-reported RTIs of interest (Aim 1) follows. The special focus of this project was on the relationship between fibroids and serologically-determined HSV-2 (Aim 2); thus, this infection will be described in more detail.

2.4.1 Self-reported RTIs of Interest

Pelvic inflammatory disease is inflammation of the upper female reproductive tract caused by infection. Based on a nationally representative sample from 2006-2010, 5% of US women have reported being treated for PID in their lifetime [57]. It is a major concern because PID can result in infertility, ectopic pregnancy and chronic pelvic pain [58]. One in 8 women with a history of PID experience difficulties getting pregnant [59]. The majority of cases are due to sexually transmitted infections such as CT and gonorrhea as well as BV and *Mycoplasma genitalium* [58]. The clinical diagnosis of PID is based on the finding of pelvic organ tenderness along with signs of lower genital tract inflammation. Furthermore, women with potential PID should be tested for CT and gonorrhea. The treatment of PID involves the

use of antimicrobials to eliminate likely pathogens such as CT and gonorrhea, regardless of the results of testing [58]. Repeated episodes of pelvic inflammatory disease appreciably worsen reproductive outcomes [60, 61]. Thus, it is very important to continue to work to control CT and gonorrhea infection in order to prevent the development of PID.

CT is the most commonly reported bacterial RTI in the US with about 3 million new infections each year [50]. However, a large proportion of cases are asymptomatic and unrecognized. Thus, only a minority of cases are clinically reported. If symptoms do arise, they are commonly abnormal vaginal discharge, vaginal bleeding (including bleeding after intercourse), and painful urination. Young adults (<25 years of age) and African-Americans are disproportionately affected by CT infection (incidence among African-American women is estimated to be 7 times higher than among White women) [50].

CT can be treated and cured with antibiotics. However, untreated CT infections of the upper genital tract can result in long-term sequelae such as PID (~10-15% of CT cases), salpingitis, endometritis, and perihepatitis [62]. Salpingitis can lead to tubal scarring and reproductive complications such as tubal-factor infertility and ectopic pregnancy. Symptoms of these sequelae may include abnormal uterine bleeding, pelvic discomfort, or chronic abdominal pain [62] although these conditions are commonly "silent" with no or minimal signs or symptoms. In pregnant women, CT is also associated with preterm delivery and can be spread to newborns resulting in eye infection or pneumonia [63]. CT typically infects columnar or transitional epithelium of the urethra, cervix-extending to the endometrium, fallopian tubes, and peritoneum (membrane lining the abdominal cavity)- and rectum [64].

For years culture was the principal diagnostic method for CT. Many non-culture tests are now available, with the most sensitive being nucleic acid amplification tests (NAATs)

that are also able to test urine and thus do not require collection of specimens from the cervix or urethra [64]. Serologic testing as a means of diagnosis may be helpful for more complicated CT infections, such as lymphogranuloma venereum or neonatal pneumonia, and possibly as a screening test for tubal factor infertility; however, it is not often clinically utilized [62].

Gonorrhea is a common bacterial RTI with over 800,000 new infections annually; however, less than half are detected and reported [65]. Symptoms may include a painful or burning sensation when urinating, increased vaginal discharge, or vaginal bleeding between periods. Most cases are asymptomatic. Among women, the most common site of infection is the endocervical canal [66]. Similar to CT in women, gonorrhea can lead to salpingitis or PID and ectopic pregnancy [66]. In addition, if left untreated, it can spread to the blood and cause disseminated gonococcal infection, a systemic complication which can be life threatening [65]. Gonorrhea is typically diagnosed with a urine test (NAAT), by culture or nucleic acid hybridization tests, and can be treated with antibiotics; however, drug-resistant strains are becoming more prevalent [67].

Trichomoniasis is caused by infection with the protozoan parasite, *Trichomonas vaginalis,* and is the most common curable (non-viral) RTI in the US, with close to 4 million people infected [68]. It is more prevalent among women than men; and, unlike other RTIs, it is associated with older age among women [68, 69]. From 2001-2004, the prevalence of trichomoniasis in the US, among women 14-49 years of age, was 13% among African-Americans and only 3% overall [69]. It is also highly associated with other RTIs and is an important risk-factor for HIV [70]. Many infected women are asymptomatic; however, up to 50% may have symptoms ranging from mild irritation to vaginitis [70]. Some symptoms include itching, foul odor, yellow-green discharge, and bleeding after sex. Trichomoniasis

can be cured with a single dose of prescription metronidazole or tinidazole. However, after treatment, 1 in 5 people will be re-infected [68]. It is usually diagnosed clinically by microscopic examination of urine/discharge or by culture, which is the gold standard [70]. Furthermore, infection with trichomoniasis has been found to be associated with preterm delivery and low birth weight as well as PID [71, 72].

Genital warts are small external bumps or groups of bumps predominately (90%) caused by HPV type 6 and 11 [73]. They are usually asymptomatic, but at times may be painful or itchy. The four morphologic types of genital warts include: condylomata acuminata (cauliflower shaped), papular (flesh-colored round papules), keratotic (thick, crusty layer) and slightly raised, flat-topped papules [74]. They are usually located on the introitis, vulva, perineum, and perianal area in women although they can also be found on the cervix, vaginal wall, pubic area, upper thighs, and crural fold [74]. They are typically diagnosed clinically by visual inspection or biopsy and, if left untreated, may resolve naturally, get larger, or remain the same. Treatment (patient-applied or provider-administered) varies depending on the size, location, symptoms, and patient preference. Treatment only targets the warts, not the virus itself, although it may reduce the amount of infectious virus present and thus, the probability of transmission [74].

Bacterial vaginosis is a condition in women where the normal bacteria in vaginal fluid is replaced by an overgrowth of certain bacteria [75]. It is the most common cause of vaginal symptoms among women of childbearing age. It does not tend to cause inflammation or tissue damage and thus is more so a disturbance of the vaginal microflora [76]. Most women do not have symptoms; however, it is sometimes accompanied by white/gray discharge, fishy odor, pain, itching, or burning. The cause and route of transmission of BV is not clearly

understood. Thus, it may not be solely sexually transmitted, so women who are not sexually active are at risk. However, exposure to new or multiple sex partners has been shown to increase the incidence of BV, and younger sexual debut has been found to be associated with BV [76]. Intrauterine device use and douching have also been found to be risk factors [76]. Furthermore, BV has been shown to be associated with PID as well as low birth weight and premature delivery among pregnant women [76]. BV is diagnosed by microscopic examination and the presence of other signs (white discharge, vaginal fluid pH > 4.5 and fishy odor) and is usually treated with either metronidazole or clindamycin [76].

2.4.2 Herpes Simplex Virus-2 (HSV-2)

Herpes simplex virus-2 is the main cause of genital and neonatal herpes and one of the most common RTIs worldwide. In the US, 1 out of 6 people between the ages of 14 and 49 have genital herpes antibodies or clinical disease and women are more susceptible to infection than men [55, 77]. In addition, African-Americans are 3-4 times as likely to have HSV-2 as Whites [55]. According to National Health and Nutrition Examination Survey (NHANES) data from 2007-2010, the HSV-2 seroprevalence for 14-49 year-olds overall, among women, and among African-American women was 16%, 20%, and 50%, respectively [55]. People living with HSV-2 are also 2 times as likely to acquire HIV infection [78]. Other main risk factors for HSV-2 include: higher numbers of sexual partners, heavy alcohol use, low socioeconomic status, being unpartnered vs. married/cohabiting and lack of consistent condom use [55, 79-83]. Also, Depo-Provera use may increase risk of HSV-2 seroconversion [84, 85]. Early age at menarche has been found to be associated with HSV-2 [86] and has been identified as a predictor of early sexual behavior, a factor highly associated with HSV-2 [87, 88].

HSV-2 can lead to painful chronic infection, spontaneous abortion, and fatal infection in newborns (neonatal herpes). HSV-2 accounts for 70% of neonatal HSV cases. Neonatal HSV-2 is transmitted transplacentally (or congenitally) in about 5 to 10% of cases but the majority of cases occur as a result of perinatal contact with the maternal genital tract when a woman has a newly acquired primary infection (seronegative for HSV-2) close to delivery [89].

The symptoms of HSV-2 are primarily lesions (blisters that later ulcerate) at the site of infection which can be very mild to painful. Other symptoms of primary infection may include fever, painful urination, swollen or tender lymph nodes in the groin area, and a general sick feeling (malaise). However, some people may not have any symptoms. Transmission usually occurs from sexual contact with an infected but asymptomatic individual (in about 70% of cases) because HSV-2 can be excreted or shed in the absence of symptoms [90].

There is no treatment that can cure HSV-2; thus it is a lifelong companion. In fact, HSV-2 has a biologic property of staying latent in the dorsal nerve ganglia and reactivating, which can cause recurrent lesions at or near the initial site of infection or asymptomatic viral shedding. Generally, about 90% of symptomatic HSV-2 infected individuals will have one or more recurrences a year [91]. Symptoms of recurrent outbreaks are typically shorter in duration and less severe (shorter period of shedding and fewer lesions) than the first outbreak of HSV-2 [77]. HSV-2 is clinically diagnosed with culture or PCR of tissue during active infection when a blister is present. However, most HSV-2 infections are asymptomatic or unrecognized (over 85% of seropositive African-American women reported no history of diagnosis) [55]; thus, seropositivity to HSV-2 is the best estimate of cumulative past exposure [55, 79].

HSV-1 causes approximately 30% of genital herpes infections, but more recently proportions have been found of over 50% especially among college students [92]. Initial episodes of HSV-1 are clinically indistinguishable from HSV-2; however, it is characterized by fewer recurrences of lesions and less viral shedding than HSV-2 infection [93]. HSV-1 and HSV-2 have an intricate relationship that is still being explored.

2.5 Biological Mechanisms of Tumorigenesis Following Infection

Infectious agents have been linked to several neoplasms, with approximately 18% of cancers estimated to be due to infection (i.e. HPV, hepatitis B and C viruses, and *Helicobacter pylori*) [94]. Leiomyomas have also been linked to infection; one study found an increased risk of EBV-associated leiomyosarcoma and leiomyoma among children with HIV [95]. Large quantities of EBV were detected in the tumor cells. These tumors were not in the uterus, but the findings reveal that smooth muscle cells may have the ability to undergo virally-initiated tumorigenesis. There has also been an association reported between Chagas disease, a parasitic infection endemic in South America, and fibroids [96]. This parasite can also infect uterine smooth muscle cells. Thus, a biological mechanism for infection-related oncogenesis may also apply to the pathogenesis of fibroids [97].

HSV-2 has been isolated from the upper genital tract including the cervix [98] and endometrium [72, 99] and has also been found to infect the placenta [89, 100]. HSV-2 infection also induces an inflammatory response and can cause cervicitis which is highly associated with ulceration and necrosis [93]. Inflammation is an acute or chronic non-specific immune response the body uses to respond to infection and can be both beneficial and harmful [101]. For example, chronic inflammation is one of the primary mechanisms through which infections induce neoplastic lesions [94] usually through an increase in cell proliferation. Some infectious agents cause necrosis (premature cell death) which leads to a subsequent increase in cell

proliferation and tissue regeneration, and others may cause a decrease in apoptosis (programmed cell death) which increases cell proliferation [97].

One theorized mechanism for fibroid development is seen in Figure 1. Briefly, infection can stimulate an inflammatory immune response which can facilitate the initiation of tissue damage resulting in tissue repair/regeneration (increased extracellular matrix, cell proliferation, decreased apoptosis, etc.), leading to the formation and growth of uterine fibroids [102, 103]. The chronic inflammatory state can increase local estrogen production, which increases proliferation [104]. This mechanism is consistent with other theories of fibroid pathogenesis. Cramer et al. proposed that myometrial hyperplasia, areas of increased cellularity and nucleus/cell ratio compared to normal myometrium in the same uterus, is one of the pathogenic pathways of fibroid development [105]. Myometrial hyperplasia appears to originate as early as adolescence at the junctional zone of the uterus between the endometrium and myometrium [105] and can extend into the myometrium. Thus, paracrine signaling from infected endometrial cells to myometrial cells may also play a role. In addition, Leppert et al. proposed that fibroids develop as a result of an abnormal response to tissue repair (a lack of apoptosis which leads to chronically active cell proliferation and excess extracellular matrix) similar to abnormal wound healing [102], which could be initiated by infection.

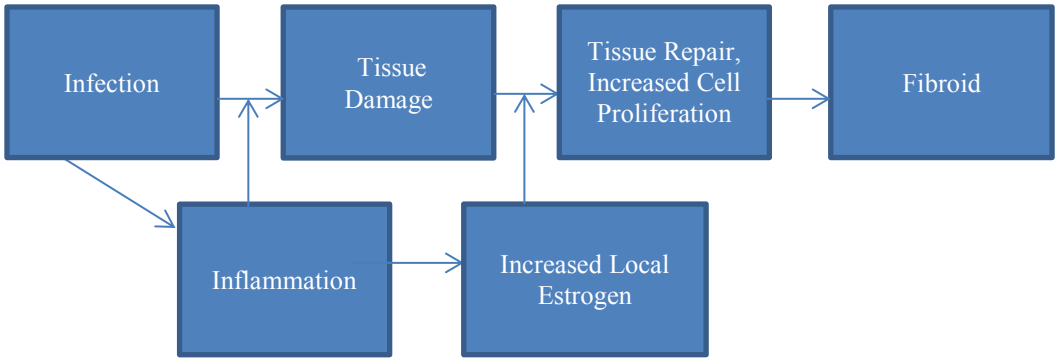


Figure 1. Theorized Mechanism for Infection and Fibroids (Adapted from Wegienka, 2012 [103])

CHAPTER 3: RESEARCH DESIGN AND METHODS

3.1 Overview

For this project, we used transvaginal ultrasound results, self-reported questionnaire data and blood samples collected from participants in the ongoing NIEHS SELF study based in the Detroit area. We used a cross-sectional study design with history of RTIs measured by self-report (Aim 1) and serology (Aim 2) as the exposures of interest. Fibroid status measured by enrollment ultrasound was the primary outcome. Fibroid characteristics i.e., size of the largest fibroid, the number of fibroids and the total fibroid volume, were also examined as secondary outcomes.

3.2 Study Participants and Data Collection

SELF is a prospective cohort study of fibroid development. From November 2010 to December 2012, the study recruited a volunteer sample of 1,696 African-American women ages 23–34 without a prior diagnosis of fibroids. Recruitment was designed to saturate the recruitment area (Detroit, Michigan and the surrounding area) with information about the study. Materials included a website (detroitself.org); flyers; brochures at health care clinics; local radio, television, newspaper, and magazine advertisements; information booths at community events, and letters to women who had been seen in the past year by a doctor at the Henry Ford Health System (HFHS). HFHS is a large medical provider in the Detroit area and a collaborating institution. The letters were sent to women listed as 23–34 years of age, with stratification by age to help achieve equal recruitment by age. This age group was

chosen on the basis of ultrasound screening data [29], to capture women early enough to have a sizeable proportion without fibroids.

Women who were interested in learning more about the study contacted the study by phone or e-mail. Prospective participants were screened for eligibility by phone. Women were not eligible for SELF if they had previously been diagnosed with uterine fibroids; had a hysterectomy; had ever taken medication to treat lupus, Grave's disease, Sjogren's scleroderma, or multiple sclerosis; or ever had any type of cancer treated with radiation or chemotherapy. Eligible prospective participants received detailed information about the study during an orientation session. Those who chose to enroll after the orientation gave informed consent and completed self-administered questionnaires and a telephone interview, and had a standardized research ultrasound examination to screen for the presence of fibroids. Some women had fibroids at enrollment of which they were unaware.

Women pregnant at recruitment were delayed enrollment until 4 months after delivery so that pregnancy did not interfere with ultrasound assessment of fibroids. SELF participants will be followed for at least 5 years after enrollment, with subsequent ultrasound examinations every 20 months. Women who screened negative at enrollment will be followed for fibroid development; women who screened positive at enrollment and those who develop incident fibroids will be followed for development of additional fibroids and fibroid growth. The study was approved by the institutional review boards of the NIEHS and HFHS.

3.3 Outcome Assessment

Ultrasound is the standard procedure for the detection and diagnosis of fibroids [16] and it is as accurate as magnetic resonance imaging for women with no more than four fibroids [16]. If there were problems with transvaginal visualization, an abdominal scan was also

completed. Focal fibroids of 0.50 cm diameter or greater were recorded. A data-collection form designed for the study was completed by the sonographer for each examination, which included documentation of the number, size and location of the fibroids. The diameters of the 6 largest fibroids were measured in cm longitudinally, anterior-posteriorly and transversely three separate times (thus, a participant with at least 6 fibroids ≥0.50 cm will have 18 fibroid measurements). Questionable fibroids were those where at least 1 diameter could not be measured. Sonographers recorded the number of fibroids visualized (up to 10) as well as the number of questionable fibroids visualized (up to 10). If more than 10 fibroids were seen, the number 10 was recorded on the form. All ultrasounds were conducted by sonographers at one of three HFHS clinics. To ensure the quality of these ultrasound data, study sonographers were required to have at least 3 years of experience in gynecologic ultrasound, receive formal training for the study, and be individually monitored by the head sonographer during their first 10 study examinations. A video clip of the uterus and still images of all measurements were archived for each ultrasound examination, and a random selection of 8% of each sonographer's ultrasound examinations, stratified to oversample participants with fibroids, was checked by comparing the data collected with the archived images.

3.3.1 Outcome Definitions

The primary outcome of this study was fibroid presence (yes/no) at the transvaginal ultrasound examination completed at enrollment. Thus, participants who had at least one fibroid or questionable fibroid ≥0.5 cm in diameter at enrollment ultrasound (22%; n=377) were considered to have fibroids, and all the women who did have a fibroid or questionable fibroid ≥0.5 cm in diameter at enrollment ultrasound (n=1,319) were considered not to have fibroids. The secondary outcomes were size of the largest fibroid, total fibroid volume and

the number of fibroids at enrollment ultrasound. The size of the largest fibroid was determined by averaging the maximum dimension of each set of fibroid measurements for each participant. Total fibroid volume (cm^3) was measured by computing the volumes of each of the 3 sets of measurements (longitudinal diameter [L], anterior-posterior [A] diameter and transverse [T] diameter) using the ellipsoid formula (L x A x T x 0.5233), taking the average volume of the 3 and then summing the average volumes from each of the fibroids.

3.4 Exposure Assessment

For specific information on exposure assessment for each Aim, please refer to Chapters 4 and 5 below.

3.5 Covariates

Extensive data on demographic characteristics, medical history, medication use and personal history was collected at enrollment. Variables of interest assessed at the enrollment telephone interview include: age at menarche, parity, type of contraceptive used and cervical treatment history. Weight and height used to calculate body mass index (weight [kg]/height $[m]^2$) were measured at the enrollment clinic visit. The web-based questionnaire at enrollment included questions on education, employment, income, alcohol and sexual behavior variables such as age at first intercourse, and number of sexual partners before age 20.

3.6 Statistical Analyses

3.6.1 Data Preparation

In order to prepare the data for analysis, each variable of interest was renamed in a standard manner to enable easy recognition. In addition, each variable was explored for impossible or unusual values. We also performed cross-tabulations between variables to

check for consistency and logic. For example, did participants who reported never being sexually active also report "No" for ever being diagnosed with an RTI? Because the majority of questions were asked via telephone interview or web-based interview where participants had to respond to a question before proceeding, there was very minimal missing data. However, participants were able to respond "prefer not to answer." Any "prefer not to answer" response was coded as missing and subjects with missing values were excluded from relevant statistical analyses. In addition, different coding schemes/methods of collapsing multi-level variables were evaluated based on the distribution of the data and comparability with the literature.

3.6.2 Descriptive Analyses

Standard descriptive statistics were reviewed for all variables of interest (exposures, outcomes and covariates). For categorical variables, proportions at each level were described. For continuous variables (age, age at menarche, BMI, etc.) the shape of the distribution was described graphically with means (standard deviations), medians (interquartile ranges), ranges and spread (skewness and kurtosis) to confirm that values were plausible. All continuous covariates were assessed for linearity of their association with the outcome. The assumption of linearity in the logit was evaluated by plotting logits and examining the incremental OR of the variable-outcome relationship. If the assumptions were violated, other coding schemes such as common referent indicator variable coding, category scores (using the median value for each category), and power transformations (quadratic, cubic, etc.) were explored. BMI is a continuous variable but was categorized by common cut-points for comparison with the literature.

Correlations between sociodemographic covariates, education and income, were assessed by evaluating the bivariable distributions and strength of age-adjusted ORs (95% CIs). Because these socioeconomic variables were, we elected to use only education, which had the stronger association with the outcome. In addition, although we did not have reason to believe that sexual behavior is related to fibroids except through the RTI pathway, we evaluated the relationship between each RTI and the sexual behavior variables (number of sexual partners, age at first intercourse, etc.) for comparison with the literature. Age of menarche has not been found to be directly associated with RTIs; however, it has been identified as a predictor of sexual behavior which is highly associated with RTIs [87, 88].

Potential confounders and variables of interest were determined based on a review of the literature, and a directed acyclic graph [106] was used to provide a conceptual framework (see Appendix Figure 1). Potential confounders included: age in years (continuous), age at menarche (7 to 10, 11 to 19 years), alcohol (low, moderate, heavy), and use of Depo-Provera (ever, never). The alcohol variable reflected the drinking level each woman reported for the age(s) when she was drinking the most. Low drinkers were those who never had 10 or more drinks in any one year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others. Variables of interest based on the literature included education (high school/general education development or less, some college/associates/technical, bachelors/masters/doctorate) and BMI (15-24, 25-29, 30-34, $\geq$35) in kg/m^2; parity (nulliparous, parous) was included to increase precision because of the consistent association between parity and fibroids in the literature.

Please see chapters 4 and 5 below for more specific information on statistical analyses.

3.6.12 Power Calculations

Because we knew the number of total fibroid cases (n=377) and the number of non-cases (n=1,319), we calculated power based on an unmatched case-control design using Episheet (2012) [107]. The table below shows the ORs detectable with 80% power, two-sided alpha=0.05, a 3 to 1 ratio of controls to cases, and an exposure prevalence in the source population ranging from 5% to 50%.

Table 2. Detectable Odds Ratio with 80% Power

Power	*Exposure Prevalence of Non-Diseased*									
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
80%	1.79	1.57	1.47	1.42	1.39	1.37	1.36	1.35	1.35	1.35

In SELF, ever being diagnosed with chlamydia and BV was reported by 37%, trichomonas by 32%, gonorrhea by 19%, genital herpes by 8, genital warts by 7% and PID by 10%. Thus, we had 80% power to detect odds ratios of 1.4 to 1.8 with the self-reported data. The estimated seroprevalence of HSV-2 among African-American females is 50% [79]. Thus, for Aim 2 with an estimated seroprevalence of > 20% for HSV-2 in the source population, we had 80% power to detect an OR of 1.4.

CHAPTER 4: SELF-REPORTED REPRODUCTIVE TRACT INFECTIONS AND ULTRASOUND DIAGNOSED UTERINE FIBROIDS IN AFRICAN-AMERICAN WOMEN[1]

4.1 Introduction

Uterine fibroids, benign smooth-muscle-cell tumors of the uterus, are one of the most common gynecologic conditions affecting women during their reproductive years [1]. By age 50, the estimated risk of developing fibroids is >80% for African-American women and close to 70% for White women [6]. The majority of women with fibroids are asymptomatic; however, an estimated 20-50% of women experience symptoms, i.e., pelvic pain and pressure, severe bleeding and reproductive problems [1, 8-10]. The primary treatment for fibroids is hysterectomy and they are the leading indication in the US, accounting for 40% of all procedures. The total estimated costs of fibroids in the US are as much as $34 billion annually [2].

Although fibroids are responsible for substantial morbidity and public health burden, their pathogenesis and etiology are largely unknown. Fibroids are hormonally responsive [18]; they develop after menarche [19] and tend to regress after menopause [7]. However, what causes the initial transformation of muscle cells into abnormal muscle cells and then their proliferation and growth into clinically visible tumors is not understood.

Risk factors that have been established for fibroids are African-American heritage (African-Americans are 2 to 3 times as likely to have clinically recognized fibroids as White

[1]This whole chapter was taken from: Moore KR, Cole SR, Dittmer DP, et al. Self-Reported Reproductive Tract Infections and Ultrasound Diagnosed Uterine Fibroids in African-American Women. *Journal of Women's Health* 2015; 24(6):489-95.

women [6]), older age (up to the age of menopause), younger age at menarche, and nulliparity [5, 20-22]. Other factors such as BMI, smoking, and hormonal contraceptive use have been inconsistently associated with fibroid risk [5, 23, 24]. More recent studies have shown no association with fibroids and smoking [24]. Two studies have reported that progestin-only injectables (i.e. Depo-Provera) may be protective [20, 27]. Alcohol use may be a risk factor, but the number of studies is small [24, 25].

In the 1930s, Witherspoon [3] hypothesized that RTIs play an etiological role in fibroid development. Both RTIs and fibroids disproportionately burden African-American women and certain RTIs can lead to conditions, i.e., chronic pelvic infection that could result in inflammatory reactions. Rates of reportable RTIs, i.e., CT and gonorrhea, are 6 and 14 times as high among African-American women compared to White women, respectively [108]. In addition, rates of CT and gonorrhea are highest among younger women ages 15-24 years which is, in most cases, the period before the diagnosis of fibroids [50]. This hypothesis is consistent with another theorized mechanism of fibroid pathogenesis involving tissue damage and aberrant tissue repair/regeneration (increased extracellular matrix, cell proliferation, and decreased apoptosis), leading to the formation and growth of uterine fibroids [102, 103].

Yet, very few studies have investigated the effect of RTIs on fibroid development. The two recently published studies have only partially consistent results [4, 5]. The first was a clinic-based case-control study of fibroid risk factors in the Baltimore metropolitan area with fibroids confirmed by histology or ultrasound [4]. A positive dose-response association was observed between the number of self-reported PID episodes and uterine fibroids. No association was found between self-reported history of genital herpes or warts and fibroids,

although there was a suggestion of an increased odds of fibroids for women with self-reported history of CT [4].

The UFS [5] was a cross-sectional study of randomly selected members of an urban health plan screened for fibroids with ultrasound. No association of fibroids was found with self-reported diagnosis of PID. However, there were suggestions of positive associations with self-reported history of CT in White women, and with trichomonas, syphilis, and "other infections" [mainly BV] in African-American women. Self-reported history of genital herpes had non-significant elevated ORs in both ethnic groups [5]. In both studies [4, 5] self-reported history of abnormal Pap smear was inversely associated with fibroids. This finding was also corroborated in our recent study that showed an inverse association between self-reported cervical treatment and fibroids [109].

A small study of 20 UFS participants examined fibroid tissue for evidence of HSV-1 and HSV-2, cytomegalovirus, human herpes virus 6,7,8, EBV and CT [5] using PCR and histology. They did not detect any evidence of these pathogens in the tumor samples.

The goal of this study was to further investigate the relationship between self-reported RTIs and fibroids in a large study of African-American women with ultrasound screening for fibroids. We also explored the relationship between self-reported RTIs and number, size and total volume of fibroids which has not previously been done.

4.2 Methods

We used transvaginal ultrasound results and self-reported questionnaire data from participants in the ongoing NIEHS SELF study based in the Detroit area. We used cross-sectional enrollment data with history of RTIs measured by self-report as the exposures of interest. Fibroid status measured by ultrasound at enrollment was the outcome. Secondary outcomes were size of the largest fibroid, number of fibroids, and total fibroid volume.

4.2.3 Study Participants and Data Collection

SELF is a prospective cohort study of fibroid development. Enrollment and data collection have been described previously [109]. In brief, from November 2010 to December 2012, the study enrolled a volunteer sample of 1,696 African-American women ages 23–34 without a prior diagnosis of fibroids in the Detroit, Michigan area. Women were not eligible for SELF if they had previously been diagnosed with uterine fibroids; had a hysterectomy; had ever taken medication to treat lupus, Grave's disease, Sjogren's scleroderma, or multiple sclerosis; or ever had any type of cancer treated with radiation or chemotherapy.

During 2010 to 2012, recruitment materials, primarily composed of targeted letters, media announcements and flyers, were distributed throughout the Detroit area with the aim of informing the target population about the study. Interested volunteers called the study number and began the enrollment process including an orientation that thoroughly described study activities. Those that completed all enrollment questionnaires and a clinic visit with ultrasound were enrolled. Participants gave written informed consent. The study was approved by the institutional review boards of NIEHS and HFHS, a large medical provider in the Detroit area and a collaborating institution.

4.2.4 Fibroid Assessment

Transvaginal ultrasound is the standard procedure for the detection and diagnosis of fibroids [16]. It is as accurate as magnetic resonance imaging for women with no more than four fibroids [16]. Fibroids were assessed by study sonographers as described previously [110] at one of three HFHS clinics. Focal fibroids of 0.5 cm diameter or greater were measured in triplicate. For each measurement, the 3 perpendicular diameters (longitudinal [L], anterior-posterior [A] and transverse [T]) were recorded.

4.2.5 Outcome Definitions

The primary outcome of this study was fibroid presence (yes/no) at the transvaginal ultrasound examination completed at enrollment. Questionable fibroids were those where at least 1 diameter could not be measured. Participants who had at least one fibroid or questionable fibroid ≥0.5 cm at enrollment ultrasound (22%; n=377) were considered to have fibroids, and all women who did not have a fibroid or questionable fibroid ≥0.5 cm in diameter at enrollment ultrasound (n=1,319) were considered to not have fibroids. The secondary outcomes were size of the largest fibroid, number of fibroids and total fibroid volume. The size of the largest fibroid was determined by averaging the maximum diameter (L, A or T) of each of the triplicate fibroid measurements. Fibroid volume (cm^3) was measured by computing the volumes of each of the triplicate fibroid measurements using the ellipsoid formula (L x A x T x 0.5233), and averaging across the three volumes. Total fibroid volume (cm^3) was calculated by adding the average volumes from each of a woman's fibroids. Total fibroid volume was not computed for women with only questionable fibroids (n=6) because at least 1 diameter was not measured.

4.2.6 Reproductive Tract Infection Assessment

At enrollment, SELF participants responded to questions regarding their history of RTIs via a self-administered computer-assisted web interviewing (CAWI) questionnaire. Each question on the CAWI required an answer before the next question was presented. If no answer was recorded, the same question was presented again, but an additional response category was offered: "prefer not to answer."

The questions of interest used for this study were: 1. Has a doctor or other health professional ever told you that you had...? 2. How old were you when you were first

diagnosed with...? 3. In total, how many times have you been diagnosed with...? These questions were asked for PID, CT, BV, gonorrhea, trichomonas, genital herpes and genital warts.

For each RTI, those exposed were the women who reported "Yes" to ever being diagnosed with that particular RTI. The unexposed were those who reported "No" to ever being diagnosed with that particular RTI. We also created a variable, "any RTI", representing whether the participant had been diagnosed with any of the aforementioned RTIs. The exposed group included those who self-reported at least one RTI diagnosis and the unexposed group comprised those who did not self-report any RTI diagnosis. Two participants were not included because they responded "prefer not to answer" for at least 1 RTI and reported "No" for other RTIs; thus, we could not determine their "any RTI" status.

4.2.7 Statistical Analyses

Because the majority of questions were asked via telephone or web-based interview where participants had to respond to a question before proceeding, there was minimal missing data. Any "prefer not to answer" response (0.06% to 0.12%) was coded as missing, and complete case analysis was performed. Variables were categorized based on the distribution of the data and comparability with the literature. All analyses were completed on women who reported ever being sexually active and who did not self-report a diagnosis of HIV or use of HIV medications. Standard descriptive statistics were performed for all variables of interest. For categorical variables, proportions at each level were described. For continuous variables, medians (interquartile ranges) were computed. Although sexual behavior variables are not confounders, we described the relationship between each RTI and

number of sexual partners and age at first intercourse for comparison with the literature. All analyses were conducted with SAS 9.3.

4.2.7.1 Primary Analyses: Association between RTIs and Fibroid Presence

Logistic regression models were used to compute ORs and 95% CIs to evaluate the associations between the self-reported RTI related variables (PID, CT gonorrhea, trichomonas, BV, genital herpes and genital warts) and fibroid presence. Because fibroids are common, the relative odds will overestimate the relative risk, but it provides a valid method of testing for statistically significant differences between those with and without a self-reported RTI diagnosis. Potential confounders and variables of interest were determined based on a review of the literature and a directed acyclic graph, and were included in the full model: age in years (continuous), age at menarche (7 to 10, 11 to 19 years), parity (nulliparous, parous), education (high school/general education development (GED) or less, some college/associates/technical, bachelors/masters/PhD), BMI (15-24, 25-29, 30-34, ≥35) in kg/m^2, alcohol (low, moderate, heavy), and use of Depo-Provera (ever, never). The alcohol variable reflected the drinking level each woman reported for the age(s) when she was drinking the most. Low drinkers were those who never had 10 or more drinks in a year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others.

We evaluated the association of "any RTI", and each self-reported RTI with fibroids in both age- and multivariable-adjusted models. To control for confounding of an RTI-fibroid association by another RTI, all RTIs were included in the multivariable-adjusted model together (with the exception of PID which was modeled separately because it can share causal pathways with other RTIs (e.g., CT can cause PID, so only the proximate factor, PID,

would be included in a model investigating effects of PID)). Spearman partial correlations (adjusted for age) among the other RTIs were assessed to assure they were not highly correlated (Suppl. Table 1).

4.2.7.2 Secondary Analyses

As a secondary analysis we examined the association between RTIs and 3 outcomes: size of the largest fibroid, number of fibroids and total fibroid volume. Medians rounded to the nearest whole number were used to determine category cutpoints for size of the largest fibroid and total fibroid volume (2 cm and 2 cm^3, respectively). To estimate the ORs and 95% CIs for the association of "any RTI" and each RTI and fibroids, we used multinomial logistic regression with the same potential confounders as in the primary analysis. All RTIs were included in the models together (with the exception of PID which was modeled separately).

As a secondary analysis to examine the association between RTI exposure severity and fibroid presence, we evaluated age- and multivariable-adjusted ORs of the association between the number of times diagnosed with each RTI and the presence of fibroids using 2 indicator variables (1 vs. none; 2+ vs. none). Because of limited numbers of women reporting multiple infections, each separate RTI was modeled without adjusting for other RTIs. In addition, we looked at whether self-reporting more than 1 type of infection increased the odds of fibroids using 3 indicator variables (1 vs. none; 2 vs. none; 3+ vs. none).

4.2.7.3 Sensitivity Analyses

Syphilis was reported by too few women to analyze separately (n=15), but we repeated the primary analyses after excluding them to make sure they were not influential. Cervical treatment is an indicator of cervical lesions secondary to persistent HPV infection

and was found to be inversely associated with fibroids in our previous study[109]. Thus, the RTI associations we were investigating might be attenuated. Therefore, we repeated the primary analyses after excluding those who reported cervical treatment (n=229).

4.3 Results

Of the 1,696 women enrolled, 40 were excluded (34 reported never having sex and 6 reported having HIV). Seventy percent (70%) of participants reported at least 1 RTI diagnosis (Table 3). Those with a prior history of an RTI, compared to women without one, tended to be older, more educated, heavier drinkers, parous, to have ever used Depo-Provera, to have more sex partners before age 20 years, and to have been younger at first sex (Table 3). The prevalences of specific self-reported RTI diagnoses were: 10% for PID, 38% for CT and BV, 32% for trichomonas, 20% for gonorrhea, 9% for genital herpes, and 7% for genital warts (Suppl. Table 2). The median time since first diagnosis of any RTI was 9 years (IQR: 5-13 years) (Supp1. Table 3).

Twenty-two percent (22%) of women had fibroids discovered at ultrasound screening (Table 4). Of those with fibroids, the size of the largest fibroid was <2 cm for 62%; 63% had only 1 fibroid. The total fibroid volume was <2 cm^3 for 52% of participants with fibroids.

In primary analyses, age-adjusted and multivariable adjusted estimates were very similar (Table 4). Self-reported BV had a positive association with fibroids though not significant [aOR: 1.21 95% CI (0.93-1.58)]. In secondary analyses we examined size of the largest fibroid, number of fibroids and total fibroid volume as the outcomes (Figure 3). Women who reported any RTI had an elevated odds of a small fibroid [aOR: 1.31 95% CI (0.93-1.84)]. There was a 38% reduction in the odds of having 2 or more fibroids for CT [aOR: 0.62 95% CI (0.40-0.97)] and a 46% reduction for PID [aOR: 0.54 95% CI (0.25-1.17)]. Those with a previous diagnosis of BV had a 47% increase in the odds of having 2 or

more fibroids [aOR: 1.47 95% CI (0.98-2.21)] and a 41% increase in the odds of having a larger total fibroid volume (≥2 cm^3) [aOR: 1.41 95% CI (0.98-2.04)].

In the secondary analyses that examined "severity" of RTI based on number of different RTIs we saw an increase in odds of fibroids for those with 2 RTIs [aOR: 1.41 95% CI (0.99-2.01)], but the odds was not elevated for those with 3 or more RTIs (Suppl. Table 4). Multiple diagnoses of the same RTI were not associated with higher odds of fibroids (Suppl. Table 4).

The first sensitivity analysis, exclusion of those with a self-reported diagnosis of syphilis (n=15), resulted in little change in associations between RTIs and fibroid presence (data not shown). However, the second sensitivity analysis, removal of those with a self-reported diagnosis of cervical treatment (n=229), resulted in a somewhat stronger association for genital warts [aOR: 1.29 95% CI (0.76-2.18)]. Other RTI associations were essentially unchanged (data not shown).

4.4 Discussion

In this large study of young African-American women, there was little overall support for an association between women's self-reported histories of RTIs and subsequent fibroid development. Even those having a history of three or more different RTIs or multiple diagnoses of the same RTI showed no indication of elevated odds of fibroids. However, our results for BV and CT are suggestive of possible associations with fibroids. Women reporting a history of BV had somewhat elevated odds of both small and large fibroids. The associations were of borderline significance for 2 or more fibroids and for larger total fibroid volume (≥2 cm^3). Although speculative, the tendency for increased odds with BV might be influenced by its chronic nature [111, 112]. For those with a previous CT diagnosis, the odds of having multiple fibroids (≥2) was significantly reduced. However, any mechanism for

protection by CT is unknown. In summary, our study results do not corroborate any of the suggestive increased odds of fibroids associated with self-reported histories of PID, CT, trichomonas or herpes previously described in smaller studies [4, 5].

This study has several limitations. It was a cross-sectional analysis with self-reported RTI diagnoses. Though the onset of fibroid development is unknown, over half of the women reported their first RTI diagnosis before the age of 20, while fibroid development in African-Americans appears to be infrequent before the mid-20s [29]. Also, most of the fibroids were small suggesting relatively recent development. Finally, the median time between first RTI diagnosis and study enrollment was 9 years, again supporting the likelihood that exposure occurred before disease onset.

Self-reported data on history of RTI diagnosis may be subject to recall error [113-116]. However, the frequencies of RTIs in our sample are generally similar to those reported in other studies of African-American women [117, 118]. Perhaps a more important problem is that the majority of RTIs can often be asymptomatic, so even those who have not had a previous diagnosis may still have had or currently have an RTI. Some women also may have been tested and were positive but never received their results, did not understand them or just did not report them (due to confidentiality concerns or social desirability bias). Because questions were asked via a self-administered CAWI questionnaire, social desirability bias should have been reduced [119, 120]. The validity of our self-report data is supported by our observation that both lower age at first sex and higher numbers of partners were associated with increased reporting of infection. Finally, our sample is not a representative population-based sample of women. However, because it captures early fibroid development (most of the women with fibroids had small fibroids), our sample was a relevant one for investigating

the possible role of RTIs in the hypothesized fibroid pathogenesis involving aberrant tissue repair [102, 103].

Our study has several strengths. We used enrollment data from an ongoing prospective study with a standardized measure of fibroid status based on systematic ultrasound screening rather than fibroids clinically detected because of symptoms. The number, diameter and volume of the fibroids were systematically measured, so we were able to examine associations with these separate characteristics. Twenty-two percent (22%) of our cohort of young African-American women had fibroids at ultrasound screening, a prevalence which falls within the range of prior US studies that conducted ultrasound screening [5, 6, 29, 30]. Our sample size was sufficient to provide good precision for the main hypotheses. We also had extensive data to assess potential confounding, very minimal missing data, and we conducted sensitivity analyses to evaluate potential bias.

4.5 Conclusions

Overall, the studies of RTIs and fibroids, including ours, reveal no strong associations. Our study was the first to look at the relationship between RTIs and fibroid size, number and total volume, but most studies have been limited by the use of self-reported exposure histories of these often asymptomatic infections. The small study that looked for pathogens in fibroid tissue did no serological assessment to biochemically measure past exposure to infection [5]. Future studies are needed to take the next step and use serology to better investigate associations between RTIs and fibroids.

Table 3. Distribution of Covariates by Self-Reported Reproductive Tract Infection Status

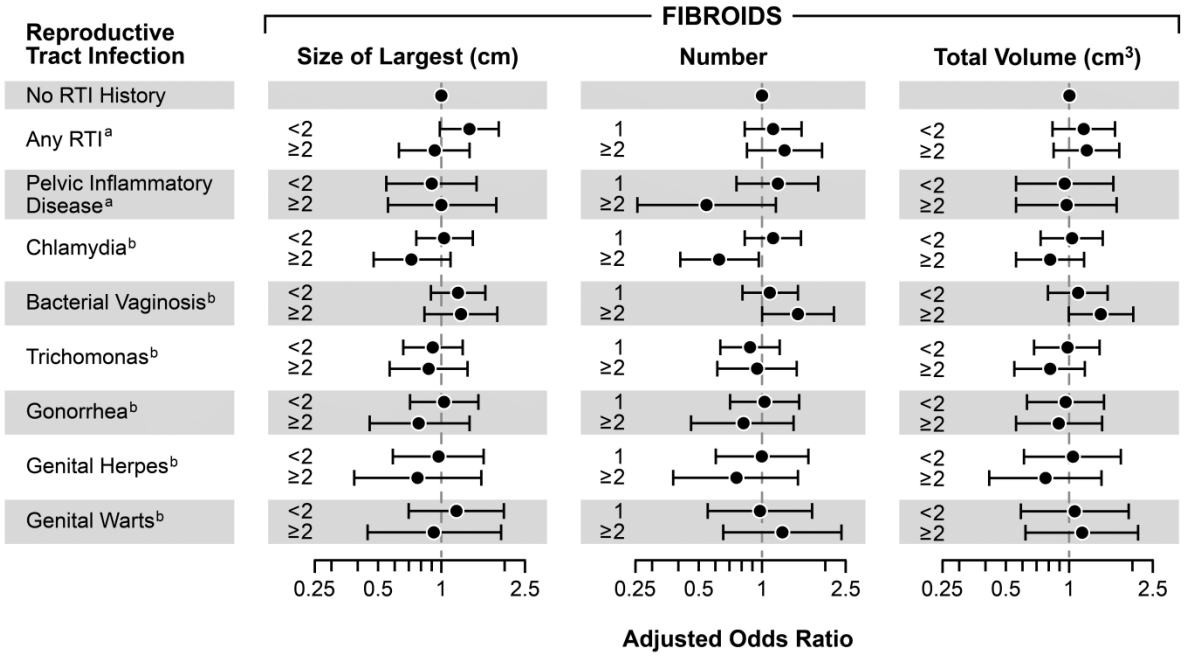
	Any Reproductive Tract Infection[a]	
Covariate	*Yes* n = *1,172* n (%)	*No* n = *482* n (%)
Age		
23-26	343 (29)	157 (33)
27-30	396 (34)	170 (35)
≥31	433 (37)	155 (32)
Education		
≤ High school/GED	229 (20)	133 (28)
Some college or technical	639 (55)	201 (42)
≥ Bachelors	303 (26)	148 (31)
Missing	1	
BMI (kg/m^2)		
15-24	212 (18)	111 (23)
25-29	266 (23)	78 (16)
30-34	236 (20)	84 (17)
≥ 35	458 (39)	209 (43)
Alcohol at age when drinking the most[b]		
Low	255 (22)	172 (36)
Moderate	386 (33)	157 (33)
Heavy	531 (45)	153 (32)
Parity		
Nulliparous	409 (35)	218 (45)
Parous	763 (65)	264 (55)
Depo-Provera		
Never used	638 (54)	300 (62)
Ever used	534 (46)	182 (38)
Age at menarche		
7-10	206 (18)	96 (20)
11-19	966 (82)	386 (80)
Sex partners before age 20		
≤1	203 (17)	190 (39)
2-5	588 (50)	237 (49)
≥ 6	379 (32)	55 (11)
Missing	2	
Age at 1st sex		
≤14	407 (35)	83 (17)
15-16	426 (36)	143 (30)
≥ 17	338 (29)	254 (53)
Missing	1	2

BMI, body mass index; GED, general education degree; IQR, interquartile range
[a]Includes pelvic inflammatory disease, chlamydia, bacterial vaginosis, trichomonas, gonorrhea, genital herpes, and genital warts; 2 participants not included because they responded "prefer not to answer" for at least 1 RTI and reported "No" for other RTIs; thus, their "any RTI" status could not be determined.
[b]Low drinkers were those who never had 10 or more drinks in a year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others.

Table 4. Adjusted Odds Ratios for Fibroids According to Self-Reported History of Reproductive Tract Infections

	Fibroids		*Age-adjusted*	*Multivariable-adjusted*
Reproductive Tract Infections	*Yes* n = *363* n (%)	*No* n = *1,293* n (%)	*OR (95% CI)*	*OR (95% CI)*
Any RTI[a]				
Yes	262 (72)	910 (70)	1.05 (0.80-1.36)	1.15 (0.87-1.52)[b]
No	101 (28)	381 (30)		
Missing		2		
PID				
Yes	34 (9)	128 (10)	0.90 (0.60-1.35)	0.94 (0.62-1.43)[b]
No	329 (91)	1,164 (90)		
Missing		1		
Chlamydia				
Yes	125 (34)	503 (39)	0.85 (0.66-1.09)	0.92 (0.70-1.21)[c]
No	238 (66)	788 (61)		
Missing		2		
Bacterial Vaginosis				
Yes	147 (41)	477 (37)	1.14 (0.89-1.45)	1.21 (0.93-1.58)[c]
No	216 (60)	815 (63)		
Missing		1		
Trichomonas				
Yes	116 (32)	414 (32)	0.90 (0.70-1.16)	0.90 (0.68-1.20)[c]
No	247 (68)	878 (68)		
Missing		1		
Gonorrhea				
Yes	62 (17)	261 (20)	0.81 (0.60-1.11)	0.94 (0.67-1.33)[c]
No	301 (83)	1,031 (80)		
Missing		1		
Genital Herpes				
Yes	31 (9)	112 (9)	0.93 (0.61-1.42)	0.89 (0.58-1.39)[c]
No	332 (91)	1,180 (91)		
Missing		1		
Genital Warts				
Yes	28 (8)	85 (7)	1.10 (0.70-1.73)	1.09 (0.68-1.74)[c]
No	335 (92)	1,207 (93)		
Missing		1		

CI, confidence interval; OR, odds ratio; PID, pelvic inflammatory disease; RTI, reproductive tract infection
[a]Includes pelvic inflammatory disease, chlamydia, bacterial vaginosis, trichomonas, gonorrhea, genital herpes, and genital warts
[b]Logistic regression model adjusted for age (continuous), education, body mass index, alcohol, menarche, parity, Depo-Provera use
[c] Logistic regression model adjusted for age (continuous), education, body mass index, alcohol, menarche, parity, Depo-Provera use, and other RTIs except PID and the RTI being evaluated



RTI, reproductive tract infection
[a]Adjusted for age, education, body mass index, alcohol, menarche, parity, and Depo-Provera use
[b]Adjusted for age, education, body mass index, alcohol, menarche, parity, Depo-Provera use, and all other RTIs except PID and the RTI being evaluated

Figure 2. Adjusted Odds Ratios for Size of the Largest Fibroid, Number of Fibroids and Total Fibroid Volume According to Self-Reported History of Reproductive Tract Infections

CHAPTER 5: HERPES SIMPLEX VIRUS-2 SEROPREVALENCE AND ULTRASOUND DIAGNOSED UTERINE FIBROIDS IN A LARGE POPULATION OF YOUNG AFRICAN-AMERICAN WOMEN[2]

5.1 Introduction

Uterine fibroids are one of the most common gynecologic conditions affecting women in the US during their reproductive years [1]. Based on ultrasound screening of randomly selected women, the estimated cumulative incidence of fibroid tumors by age 50 is >80% for African-American women and close to 70% for White women [6]. Symptoms resulting from fibroids (pain, severe bleeding, reproductive problems) are the leading reason for hysterectomy in the US, with the total annual costs of fibroids as high as $34 billion [2].

Fibroids are hormonally dependent benign tumors of the uterine smooth muscle. Their etiologic causes are largely unknown, but established risk factors include African-American heritage (African-Americans are 2 to 3 times as likely to have clinically recognized fibroids as White women [6]), older age (up to the age of menopause), younger age at menarche, and nulliparity [5, 20, 21]. Three studies have reported that progestin-only injectables (i.e. depot medroxyprogesterone acetate (DMPA)) are protective [20, 27, 28]. Heavier alcohol use may be a risk factor, although the number of studies is small [24, 25].

A hypothesis was postulated decades ago that RTIs may play a role in fibroid development [3]. Both RTIs and fibroids disproportionately burden African-American women and certain RTIs can lead to conditions, e.g., chronic pelvic infection, that could result in

[2]This whole chapter was taken from: Moore KR, Smith JS, Cole SR, et al. Herpes Simplex Virus-2 Seroprevalence and Ultrasound Diagnosed Uterine Fibroids in a Large Population of Young African-American Women. *American Journal of Epidemiology*; In press.

inflammatory reactions [3]. This hypothesis is consistent with another theorized mechanism of fibroid pathogenesis where infection can stimulate an inflammatory immune response which can facilitate the initiation of tissue damage resulting in tissue repair/regeneration (increased extracellular matrix, cell proliferation, decreased apoptosis), leading to the formation and growth of uterine fibroids [102, 103]. Furthermore, fibroids were found to be associated with serologically-determined Chagas disease (caused by a protozoan parasite) [96], and EBV was found to infect smooth muscle cell tumors at sites outside of the uterus [95, 96].

However, the limited data that examine associations between RTIs and fibroid risk [4, 5, 121], have yielded inconsistent findings. One of the primary aims for undertaking a large study of fibroids in African-American women, the SELF study was to fill this data gap [122]. The data from prior studies suggested positive associations between fibroids and PID [4], CT [4], genital herpes [5], trichomonas [5], BV [5, 121] and syphilis [5]. We recently published self-reported RTI data from SELF, showed no strong associations with fibroids (16). We did find that women reporting a history of bacterial vaginosis had somewhat elevated odds of multiple fibroids (>2) and larger total fibroid volume ($\geq$2 cm^3) and women reporting a history of CT tended to have fewer and smaller fibroids [121]. However, all of these previous reports measured RTI history with questionnaire data, which can be plagued by recall error as well as misclassification due to the asymptomatic nature of most RTIs [113-116]. An immunological measure of exposure namely serology (diagnostic identification of antibodies in the serum that remain after infection), would provide a more nearly accurate assessment of exposure than self-reported RTI history.

HSV-2, the main cause of genital and neonatal herpes, is a common RTI in the US [50]. Compared to women of other racial/ethnic groups in the US, African-American women have

the highest seroprevalence of HSV-2 (50% for 14-49 year-olds between 2007-2010) [55]. Other main risk factors for HSV-2 include: higher numbers of sexual partners, heavy alcohol use, low socioeconomic status, being unpartnered vs. married/cohabiting and lack of consistent condom use [55, 79-83]. Also, DMPA use may increase risk of HSV-2 seroconversion [84, 85], and age at menarche has been found to be associated with HSV-2 [86] and has been identified as a predictor of sexual behavior which is highly associated with HSV-2 [87, 88].

HSV-2 antibodies have been shown to persist for years post-infection [123, 124], and serologic testing for HSV-2 can be done sensitively and specifically for past exposure to HSV-2 [125]. Cell culture and polymerase chain reaction are the recommended laboratory tests for people who have active lesions or ulcers present [126]. However, most HSV-2 infections are asymptomatic or unrecognized (over 85% of seropositive African-American women reported no history of diagnosis) [55]; thus, seropositivity to HSV-2 is the best estimate of past, cumulative exposure [55, 79].

Building upon previous work which found a positive association between self-reported HSV-2 and fibroids [5], our primary aim was to investigate the relationship between HSV-2 and fibroids in a large cohort with ultrasound screening for fibroids and serological measurement of exposure. This study was the first to investigate the association of fibroids with HSV-2 exposure assessed serologically. We hypothesized that women seropositive to HSV-2 would have a higher prevalence of fibroids than women seronegative to HSV-2. We also explored the relationship between HSV-2 seropositivity and number, size, and total volume of fibroids.

5.2 Methods

5.2.1 Study Participants and Data Collection

We used transvaginal ultrasound results, self-reported questionnaire data and stored frozen serum specimens from participants in SELF, an ongoing study based in the Detroit, Michigan area. SELF is a prospective cohort study of fibroid development. Enrollment data and specimen collection protocols have been described previously [122]. In brief, from November 2010 to December 2012, the study recruited 1,696 African-American women volunteers ages 23-34. Women were not eligible for SELF if they had previously been diagnosed with uterine fibroids; had had a hysterectomy; had ever taken medication to treat lupus, Grave's disease, Sjogren's scleroderma, or multiple sclerosis; or had ever had any type of cancer treated with radiation or chemotherapy. The study was approved by the institutional review boards of NIEHS and HFHS.

5.2.2 Fibroid Assessment

Transvaginal ultrasound, the standard procedure for the detection and diagnosis of fibroids, was used in this study [16]. Fibroids were assessed by study sonographers as described previously [122] at one of three clinics. Focal fibroids of 0.5 cm diameter or greater were measured in triplicate. For each measurement, the 3 perpendicular diameters (longitudinal, anterior-posterior and transverse) were recorded.

5.2.3 Outcome Definitions

The primary outcome of this study was fibroid presence (yes/no) at the transvaginal ultrasound examination completed at enrollment. Fibroids classified as “questionable” were those where at least one diameter could not be measured. Participants with at least one fibroid or questionable fibroid ≥0.5 cm in diameter at enrollment ultrasound were considered to have fibroids, and all women without a fibroid or questionable fibroid ≥ 0.5 cm in

diameter were considered not to have fibroids. The secondary outcomes for this analysis were size of the largest fibroid, number of fibroids, and total fibroid volume. The size of the largest fibroid was estimated by averaging the maximum diameter (longitudinal, anterior-posterior or transverse) of each of the triplicate fibroid measurements. Fibroid volume (cm^3) was measured by computing the volumes of each of the triplicate fibroid measurements using the ellipsoid formula (longitudinal x anterior-posterior x transverse x 0.5233), and averaging across the three volumes. Total fibroid volume (cm^3) was calculated by adding the average volumes from each of a woman's fibroids. Total fibroid volume was not computed for women with only questionable fibroids (n=6) because at least one diameter was not measured.

5.2.4 HSV-2 Assessment

HSV-2 antibody serostatus was assessed by the International Sexually Transmitted Diseases Research Laboratory at Johns Hopkins University using the Focus Diagnostics HerpeSelect® 2 Enzyme-Linked Immunosorbent Assay (Focus Diagnostics, Cypress, California) per package instructions [127]. This type-specific assay to glycoprotein gG2 allows for the qualitative detection of HSV-2 immunoglobulin G antibodies with or without the presence of HSV-1 [127]. Antibody levels of >1.10 optical density units were categorized as seropositive and levels <0.90 were seronegative. Levels ≥0.9 and ≤1.10 were indeterminate and were retested. The level of antibody response cannot be used to determine active infection or recency of initial infection.

For quality control, we included blinded duplicate samples. Aliquots were created from unused serum samples collected from SELF participants during a special blood draw six months after enrollment [122]. Two aliquots from each of 42 specimens served as 84 blinded

controls. Forty of the 42 duplicate pairs had identical results. For each of the other two discordant pairs, one of the aliquots was considered indeterminate. The result for the other aliquot of each of these two discordant pairs matched the result found for the enrollment specimen from the same participant.

5.2.5 Statistical Analyses

Due to the use of telephone and web-based interview methods which did not allow participants to skip questions, there was minimal missing data on covariates. However, participants were able to respond "prefer not to answer." This response (<1%) was coded as missing, and complete case analysis was performed. Variables were categorized based on the distribution of the data and comparability with the literature. All analyses were completed on women who had blood samples taken at enrollment. Standard descriptive statistics were performed for all variables of interest. We also described the relationship between HSV-2 serostatus and number of sexual partners and age at first intercourse for comparison with the literature. All analyses were conducted with SAS 9.3 (SAS Institute, Inc., Cary, NC).

5.2.5.1 Primary Analyses

Logistic regression was used to compute ORs and 95% CIs to evaluate the relationship between HSV-2 serostatus and fibroid presence. Although the relative odds will overestimate the relative risk for associations with an outcome as common as fibroids, overestimation is minimal for weak associations and in any case does not affect statistical tests. Potential confounders and variables of interest were determined based on a review of the literature, and a directed acyclic graph [106] was used to provide a conceptual framework. Potential confounders included: age in years (continuous), age at menarche (7 to 10, 11 to 19 years), alcohol (low, moderate, heavy), and use of DMPA (ever, never). The alcohol variable

reflected the drinking level each woman reported for the age(s) when she was drinking the most. Low drinkers were those who never had 10 or more drinks in a year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others. Variables of interest based on the literature included education (high school/general education development or less, some college/associates/technical, bachelors/masters/doctorate) and body mass index (15-24, 25-29, 30-34, ≥35) in kg/m^2; parity (nulliparous, parous) was included to increase precision because of the consistent association between parity and fibroids in the literature. We included age, age at menarche, parity, and DMPA use in a full model *a priori*. The alcohol variable was not included *a priori* because the amount of literature supporting an alcohol and fibroid association is small. After adding alcohol and the additional variables of interest, we used backwards elimination and the 10% change in estimate approach to determine the minimally adjusted final model. Only the covariates included *a priori* remained in the final model.

5.2.5.2 Secondary Analyses

As a secondary analysis we examined the association between HSV-2 and fibroid characteristics: size of the largest fibroid, number of fibroids and total fibroid volume. Medians rounded to the nearest whole number were used to determine category cut points for size of the largest fibroid and total fibroid volume (2 cm and 2 cm^3, respectively). To estimate the ORs and 95% CIs for the association of HSV-2 serostatus and fibroid size, number and total volume, we used multinomial logistic regression. Moreover, because the majority of HSV-2 infections are asymptomatic, self-reported infections are likely to be those that are symptomatic. Thus, as a secondary analysis, we evaluated the multivariable-adjusted

ORs and 95% CIs between HSV-2 and fibroid presence using a 3-level exposure variable: 0=seronegative, 1=not symptomatic (seropositive with no self-reported diagnosis), and 2=symptomatic (seropositive with self-reported diagnosis). For both of these secondary analyses we adjusted for the same confounders as in the final model in the primary analysis (age, age at menarche, parity, and DMPA use).

5.2.5.3 Sensitivity Analyses

To evaluate the robustness of our findings, we examined multivariable-adjusted ORs and 95% CIs for the association of HSV-2 with fibroids in a series of sensitivity analyses by restricting or stratifying the sample. Cervical treatment is an indicator of cervical lesions secondary to persistent human papillomavirus infection and was found to be inversely associated with fibroids in a previous study [109]. Thus, any HSV-2 associations we observed might be attenuated. Therefore, we repeated the primary analyses after excluding those who reported cervical treatment (n=231 excluded). To evaluate age as an effect modifier (due to possible decline in antibodies over time), we looked at the association between HSV-2 and fibroid presence within two age strata (23 to 29 years, 30 to 35 years).

To investigate temporality of HSV-2 exposure in relation to fibroid development, we looked at the association between HSV-2 and fibroids among strata of size of the largest fibroid (<2 cm and ≥2 cm). Those with smaller fibroids are more likely to have developed fibroids more recently and thus, may be more likely to have had exposure to HSV-2 before fibroid development compared to women with larger fibroids. In addition, we looked at the association of HSV-2 and fibroid presence within 2 strata of number of sexual partners before age 20 (0 to 1 sex partner, 2 or more sex partners). Those with more sexual partners

before the age of 20 would have been more likely to be exposed to HSV-2 prior to fibroid development.

A total of 1,696 women were enrolled in the SELF study. HSV-2 serology was conducted for the 98% of participants with available enrollment blood samples (n=1,662). Four of the samples had indeterminate results that were excluded. Therefore, our analyses were performed on the 1,658 participants with HSV-2 serology results.

5.3 Results

The median age of our cohort at enrollment was 29 years, the median age at menarche was 12 years, the majority were parous, and over 40% had ever used DMPA. Forty-seven percent (n=869) of the participants were seropositive for HSV-2. HSV-2 seropositive women tended to be older, to be less educated, to be heavier drinkers, to be parous, to be more likely to have ever used DMPA, to have more sex partners before age 20, and to have been younger at first sex compared to those who were seronegative for HSV-2 (Table 5).

Twenty-two percent of women had fibroids discovered at ultrasound screening (Table 6). Of those with fibroids, the size of the largest fibroid was <2 cm for 61%; 63% had only 1 fibroid. The total fibroid volume was <2 cm^3 for 51% of participants with fibroids.

In primary analyses, the odds of fibroids was similar for those who were HSV-2 seropositive and seronegative, in both age- and multivariable-adjusted models (multivariable-adjusted, aOR: 0.94 95% CI: 0.73, 1.20) (Table 6). In secondary analyses we examined size of the largest fibroid, number of fibroids, and total fibroid volume as the outcomes (Figure 4). There was no significant association with HSV-2 exposure and fibroid size, number or total volume.

We also examined symptomatic herpes and fibroid presence based on a combination of self-reported genital herpes and serology data (Table 7). Only 16% of those who were

seropositive for HSV-2 actually self-reported having had a genital herpes diagnosis (sensitivity for self-reported HSV-2 exposure). However, 98% of those who were seronegative self-reported no diagnosis of genital herpes (specificity). Those who were seropositive and self-reported an HSV-2 diagnosis (our measure of symptomatic herpes) did not have a higher odds of fibroids. Furthermore, the lack of an association between HSV-2 and fibroids remained consistent across the sensitivity analyses performed (Table 8).

5.4 Discussion

Our study did not show an association between HSV-2 and fibroid presence, size, number or total volume. Even among women who were seropositive and had genital herpes severe enough to self-report a clinical diagnosis, there was no association with fibroids. In addition, the lack of an association was consistent across various sensitivity analyses: excluding women with cervical treatment, and stratifying by size of the largest fibroid, age, and number of sex partners before age 20.

Our findings are consistent with a previous report in this same population looking at several self-reported RTIs, which showed no association of genital herpes with fibroids [121]. Two other studies have investigated the association between self-reported genital herpes and fibroid presence [4, 5]. A clinic-based case-control study of 18-55 year-old pre-menopausal women showed no association of genital herpes with clinically detected fibroids [4]. The UFS [5], a cross-sectional study that used ultrasound to screen randomly- selected members of an urban health plan aged 35-49 years for fibroids, found suggestions of a positive association of self-reported genital herpes with fibroids in both African-American and White women [5]. However, these findings were not precise enough to rule out associations due to chance. A small pilot study investigated whether pathogens were present in fibroid tissue; specimens from 20 UFS participants who had reported a history of sexually transmitted disease or

multiple sex partners were tested for viral DNA to HSV-1 and HSV-2, cytomegalovirus, human herpes virus 6,7,8 and EBV [5] using PCR. None of these pathogens was detected in the tumor samples.

Our study has several limitations. It was a cross-sectional analysis. Thus, the acquisition of HSV-2 infection in relation to fibroid development is unknown. However, it is likely that exposure occurred before disease onset for most women. Over half of the women who self-reported genital herpes reported that their first diagnosis was before the age of 22 and approximately 45% of new HSV-2 infections in the US are among 15-24 year olds [50]. Also, most of the fibroids were small suggesting relatively recent development, and fibroid development in African-Americans appears to be infrequent before the mid-20s [29, 122]. Finally, the median time between first self-reported HSV-2 diagnosis and study enrollment was 6 years, and because antibody titers persist for years post-infection [123, 124], even exposure to HSV-2 multiple years prior to enrollment should be captured.

We did not use the Western blot, the gold standard method for HSV-2 serology [125, 128]. However, the HerpeSelect® 2 assay we used [127] has been found to perform very well for HSV-2 infections, with sensitivity between 96-100% and specificity between 97-98% [129, 130] compared to the Western blot. The Western blot is not US Food and Drug Administration approved and is more complex, costly, less time efficient and much less widely available compared to the enzyme-linked immunosorbent assay [131]. In addition, we did not capture those who have very recent infection because it can take weeks to months after infection for immunoglobulin G antibodies to be detected. However, because we seek to measure past exposure, ideally at the time of fibroid development, low sensitivity for very recent infections did not jeopardize our assessment of cumulative exposure to HSV-2.

In addition, HSV-2 seroprevalence alone underestimates the prevalence of genital HSV infection due to the omission of genital infections caused solely by HSV-1 which are increasing [132]. However, because HSV-1 also causes orolabial infections which are very prevalent [132], a large proportion of the population will have antibodies to HSV-1 and we would not be able to distinguish orolabial from genital HSV-1 infection. Thus, the value of the added information to be gained from measuring HSV-1 is unclear.

Lastly, our sample is a volunteer sample of women. However, the seroprevalence of HSV-2 in our cohort (47%) was very similar to the seroprevalence of 50% for African-American women in the US [55]. Furthermore, 22% of our cohort had fibroids at ultrasound screening, which falls within the range of prior US studies that conducted ultrasound screening [5, 6, 29, 30].

Our study also had several strengths. This was the first study to investigate the relationship between HSV-2 and fibroids using an immunological measure of exposure. Our blinded quality control samples demonstrated low measurement error in HSV-2 serostatus. Previous studies have used only self-reported diagnosis of genital herpes as the exposure measurement, which is problematic due to the high prevalence of asymptomatic infection. Furthermore, we used a standard and valid measure of fibroid status based on systematic ultrasound screening rather than fibroids clinically detected because of symptoms or incidental detection. The number, diameter, and volume of the fibroids were systematically measured, so we were able to examine associations with these separate characteristics. Our sample size was sufficient to provide good precision for the main hypotheses. In addition, most women with a history of HSV-2 are unaware of their exposure status making it very unlikely that women without fibroids who had a history of HSV-2 exposure would be more

likely to enroll than those without HSV-2; thus, there is limited potential for selection bias. Our study also had extensive data to assess potential confounding, minimal missing data, and sensitivity analyses to evaluate potential bias.

Overall, based on our findings in a large cohort of African-American women, HSV-2 seropositivity does not appear to be a risk factor for fibroids in this 23-34 year-old age group. However, this does not suggest that other RTIs do not play a role in fibroid development. Further study of other serologically measured RTIs is still warranted, as are prospective studies on the relationship between RTIs and fibroid growth.

Table 5. Herpes Simplex Virus Type 2 Serostatus, Stratified by Selected Enrollment Characteristics of 1,658 23-34 Year-Old African-American Women in the Study of Environment Lifestyle and Fibroids, Detroit Metropolitan Area, Michigan, 2010-2012

	HSV-2 Seropositive N=1,658			
	Yes n = 789		*No n = 869*	
Covariate	*n*	*%*	*n*	*%*
Age, years				
23-26	191	24	319	37
27-30	284	36	286	33
31-35[a]	314	40	264	30
Education				
≤ High school/GED	235	30	129	15
Some college or technical	409	52	423	49
≥ Bachelors	144	18	317	36
Missing	1			
BMI[b]				
15-24	164	21	164	19
25-29	157	20	186	21
30-34	141	18	177	20
35-80	327	41	342	39
Alcohol at age when drinking the most[c]				
Low	198	25	240	28
Moderate	231	29	309	35
Heavy	360	46	320	37
Parity				
Nulliparous	238	30	412	47
Parous	551	70	457	53
DMPA				
Never used	383	49	566	65
Ever used	406	51	303	35
Age at menarche, years				
7-10[d]	157	20	142	16
11-19	632	80	727	84
Sex partners before age 20				
≤1[e]	154	19	268	31
2-5	399	51	413	48
≥ 6	234	30	187	21
Missing	2		1	
Age at 1st intercourse, years				
≤14	284	36	196	23
15-16	274	35	287	33
≥ 17[e]	231	29	383	44
Missing	0		3	

Abbreviation: BMI, body mass index; DMPA, depot medroxyprogesterone acetate; GED, general education development; HSV-2, herpes simplex virus type 2.

[a] No one over 34 was recruited, but some 34 year-olds had turned 35 by the time they had their ultrasound examination.

[b] BMI given as kg/m^2

[c]Low drinkers were those who never had 10 or more drinks in a year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4+ drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others.
[d]Categorized to identify early age at menarche, the category associated with fibroids.
[e]Includes participants who reported never having sex.

Table 6. Herpes Simplex Virus Type 2 Serostatus in Relation to Fibroids among 1,658 23-34 Year-Old African-American Women in the Study of Environment, Lifestyle and Fibroids, Detroit, MI Metropolitan Area, 2010-2012: Odds Ratios and 95% Confidence Intervals

	N	*Have Fibroids*		*Age-Adjusted OR*	*95% CI*	*Multivariable-Adjusted*[a] *OR*	*95% CI*
		n	%				
HSV-2 Seropositive							
No	869	195	22	1.00	referent	1.00	referent
Yes	789	170	22	0.82	0.64, 1.04	0.94	0.73, 1.20

Abbreviation: CI, confidence interval; HSV-2, herpes simplex virus type 2; OR, odds ratio.
[a]Adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.

Table 7. Symptomatic and Asymptomatic Serologically-Detected Herpes Simplex Virus Type 2 in Relation to Fibroids among 1,658 23-34 Year-Old African-American Women in the Study of Environment, Lifestyle and Fibroids, Detroit, Michigan Metropolitan Area, 2010-2012: Odds Ratios and 95% Confidence Intervals

HSV-2[a]	*N*	*Have Fibroids*		*OR*[b]	*95% CI*
		n	%		
Seronegative	853	189	22	1.00	referent
Symptomatic[c]					
No	664	146	22	0.99	0.76, 1.29
Yes	124	24	19	0.77	0.47, 1.26

Abbreviation: CI, confidence interval; HSV-2, herpes simplex virus type 2; OR, odds ratio.
[a]Limited to participants with data on HSV-2 symptomatology; 17 participants had missing data: 1 with missing data on self-reported genital herpes and 16 who self-reported genital herpes but were HSV-2 seronegative.
[b]Adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.
[c]Defined as participants who were HSV-2 seropositive who self-reported having been diagnosed with genital herpes ("Yes") or self-reported not having been diagnosed with genital herpes ("No").

Table 8. Sensitivity Analyses of the Association of Fibroids with Herpes Simplex Virus Type 2 Serostatus among 1,658 23-34 Year-Old African-American Women in the Study of Environment, Lifestyle and Fibroids, Detroit, Michigan Metropolitan Area, 2010-2012: Odds Ratios and 95% Confidence Intervals

	N	*OR*[a]	*95% CI*
Full sample	1,658	0.94	0.73, 1.20
Excluding women with prior cervical treatment	1,426	0.95	0.72, 1.24
Stratified by age, years			
23-29	934	0.84	0.58, 1.22
30+	724	1.01	0.72, 1.41
Stratified by size of largest fibroid, cm			
<2 vs. none	224 vs. 1,293	0.88	0.65, 1.18
≥2 vs. none	141 vs. 1,293	1.06	0.73, 1.54
Stratified by number of sex partners before age 20[b]			
0 to 1	422	0.99	0.60, 1.64
2+	1,233	0.91	0.68, 1.21

Abbreviation: CI, confidence interval; OR, odds ratio.
[a]Adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.
[b]3 participants had missing data for number of sex partners before age 20.

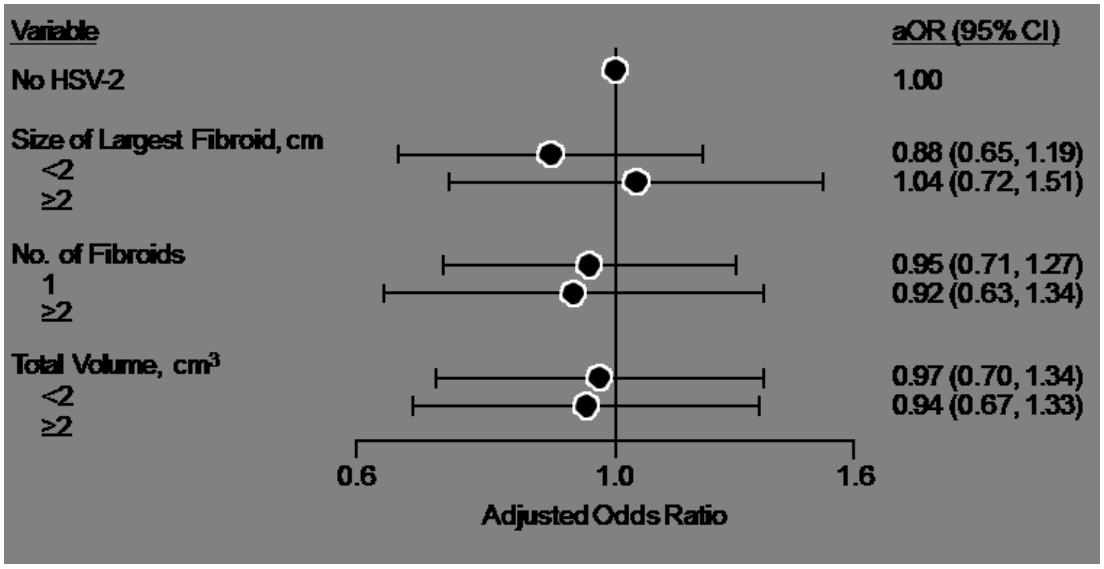


Figure 3. Size of largest fibroid, number of fibroids, and total fibroid volume in relation to herpes simplex virus type 2 (HSV-2) serostatus among 365 23-34 year-old African-American women with fibroids in the Study of Environment Lifestyle and Fibroids, Detroit, Michigan metropolitan area, 2010-2012: odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.

CHAPTER 6: CONCLUSIONS

In summary, we investigated possible associations of uterine fibroids with past exposure to self-reported RTIs and with HSV-2 measured with standard serological methods. We used enrollment data from an ongoing prospective study with a standardized measure of fibroid status based on systematic ultrasound screening. We had extensive data to assess potential confounding, minimal missing data and conducted sensitivity analyses to evaluate potential bias. The study for Aim 2 was the first to investigate the association of uterine fibroids with RTI exposure assessed serologically. The results of these studies revealed no strong evidence for a relationship between RTIs and fibroids.

6.1 Limitations

This project had several limitations. The two studies have a cross-sectional design, thus the timing of both infection and fibroid development was unknown. However, in this study population, over half of the women reported their first diagnosis of any RTI before age 20 and genital herpes before the age of 22 while fibroid development in African-Americans appears to be infrequent before the mid-20s. Also, the median time between first RTI diagnosis and study enrollment was 9 years. In addition, most of the fibroids were small suggesting relatively recent development. All of these factors support the likelihood that exposure occurred before disease onset.

For Aim 1 of our study, we used self-reported exposure data on history of RTI diagnosis which could be subject to recall error [113-116]. The majority of RTIs are asymptomatic so even those who have not had a previous diagnosis may still have had or

currently have an RTI. In addition, some women may have been tested and were positive but never received their results, did not understand them or just did not report them (due to confidentiality concerns or social desirability bias). Therefore, misclassification due to underreporting could result. However, the misclassification should be non-differential because it should not differ by fibroid status (no one was diagnosed with fibroids prior to the study and, in most cases, the questions on RTIs were administered before enrollment ultrasound). In addition, the questions were asked via a self-administered computer assisted web interviewing (CAWI) questionnaire; therefore, an interviewer was not present, which should help to minimize social desirability bias and improve reporting [119, 120].

For Aim 2, serological detection of infection as a measure of lifetime exposure has its limitations. Type-specific HSV-2 seroprevalence alone underestimates the prevalence of genital HSV infection due to the omission of genital infections caused solely by HSV-1 (30-50%) [132, 133]. There has been an increase in genital HSV-1 infections over time which is possibly due to declines in early childhood acquisition of HSV-1 [132] leading to more young people who are susceptible to genital HSV-1 and/or increases in oral-genital contact among adolescents [133].

As stated previously, HSV-1 and HSV-2 have an intricate relationship. Symptomatic HSV-2 disease is more likely among persons lacking HSV-1 antibodies and acquisition of HSV-1 among persons with prior HSV-2 infection is rare [93]. Prior oral labial HSV-1 appears to protect against the acquisition of genital HSV-1 disease. However, genital or oral HSV-1 does not protect against HSV-2 acquisition [93]. Furthermore, because HSV-1 also causes orolabial infections, a large proportion of the population would have antibodies to HSV-1 and we would not be able to distinguish orolabial from genital infection. If persons

are seronegative for HSV-2 but self-report a diagnosis of genital herpes, a seropositive HSV-1 result could indicate genital HSV-1 infection, incorrect diagnosis, incorrect recall of diagnosis, HSV-2 assay error (false negative) or lack of HSV-2 antibody persistence. Thus, given the complexity of the relationship and our lack of prospective data on infection, the value of the added information to be gained from measuring HSV-1 is unclear.

We also did not have clinical measures of symptomatic infection or frequency of recurrences for HSV-2 which could all be important factors leading to more inflammation. However, for HSV-2, we did explore whether those who were seropositive and self-reported a diagnosis of infection had an elevated OR compared to those who are only seropositive because those who self-report diagnosis may be more likely to have symptomatic infection. In regards to HSV-2, the more symptomatic the primary infection, the more recurrences and the more symptomatic recurrences [91]. In addition, we looked at the self-reported information on the number of times diagnosed with infection and the number of RTIs, which could shed some light on the relationship between reinfection or recurrences and fibroid risk.

This was also a volunteer sample of women; we did not have a representative population-based sample, thus there is potential for selection bias. All of these women had never been diagnosed with fibroids and thus may have a more asymptomatic condition. However, the symptoms related to fibroids especially heavy menstrual bleeding and pain overlap with other conditions and some women may not know that these symptoms signal any condition other than being a woman. Thus, these women are not necessarily more asymptomatic but may not have sought care or treatment for their symptoms. However, we may have selected for women with more asymptomatic fibroids because they have not been diagnosed. We found null associations while other studies found suggestions of positive

associations. The past studies were among older women who had larger fibroids and the Faerstein et al.[4] study only looked at clinically recognized fibroids which are most likely larger and more symptomatic; in contrast, most fibroids in our population were small; thus, the past studies could be looking at a different aspect of the relationship between RTIs and fibroids such as growth vs. initiation. Because our sample captures early fibroid development (most of the women with fibroids had small fibroids), it was most relevant for investigating the role of RTIs in fibroid initiation. However, our study did include 51 (13% of those with fibroids) women with large fibroids (>=4 cm), and we did look at the association between RTIs and size of fibroid and total fibroid volume and did not see any suggestions of positive associations.

In addition, because women had to have never been diagnosed with fibroids to enter the SELF study, there could have been women that did not enter the study because they had a previous diagnosis of fibroids and these women may have been more likely to have been exposed to HSV-2. Thus, below is a scenario based on an estimate of the number of women who would have been diagnosed with fibroids before being offered participation in the SELF study. We sought to determine what proportion of them would have had to be seropositive for HSV-2 in order for their inclusion to have changed the null association we observed to a strong enough positive association to change our conclusion.

A prior ultrasound screening study indicated that approximately 30% of black women ages 25-34 years with ultrasound-confirmed fibroids report a prior fibroid diagnosis[134]. Based on that data, we assumed that 30% of women in our target population who had fibroids (denoted y) were not included in the observed SELF dataset (n=365 women with undiagnosed fibroids in the data set). Given this assumption, the expected total number of

women with fibroids in SELF if this study sample had included women with a prior fibroid diagnosis (denoted x) would be equal to 365 + y. We solved for x and y:

- x-x(0.30)=365
- x=521, representing the expected number of women with fibroids in SELF if the study sample had included women with previously-diagnosed fibroids and 30% were missing due to the exclusion
- x-365=y
- y=156, representing the expected number of women with prior clinically-diagnosed fibroids that were excluded from entry into SELF

The calculations above were similar to those in the sensitivity analysis in Upson et al., 2015[135].

In order to determine what percentage of these 156 women would have had to be seropositive for HSV-2 in order for us to have found a strong enough positive association between HSV-2 and fibroid status to change our conclusion, we computed the crude odds ratio (OR) and 95% CIs for varying prevalences of seropositivity, ranging from 50% to 100% (Table 9).

Table 9. Effect of Potential Selection Bias Due to Exclusion of Women Previously Diagnosed with Fibroids. Crude Odds Ratios for Fibroids According to HSV-2 Serostatus assuming Varying HSV-2 Seroprevalence among an estimated 156 Women with Fibroids Excluded from Entry into SELF

HSV-2 seroprevalence in 156 women with previously-diagnosed fibroids	*OR for fibroids in relation to HSV-2 serostatus*	*95% CI*
50%	0.99	0.81-1.21
55%	1.05	0.85-1.28
60%	1.12	0.91-1.36
65%	1.18	0.96-1.45
70%	1.26	1.02-1.54
75%	1.34	1.09-1.64
80%	1.42	1.15-1.73

CI, confidence interval; HSV-2, herpes simplex virus type 2; OR, odds ratio

Based on the above crude calculations, at least 70% of the women who were excluded due to having been previously diagnosed with fibroids would have had to be seropositive for HSV-2 in order to have an OR of 1.3 or higher. Given that this proportion is much higher than the seroprevalence of HSV-2 among African-American women in the US (50%)[55], it is unlikely that exclusion of women with previously diagnosed fibroids resulted in selection bias strong enough to produce the observed result. Also, these estimates are not adjusted for age which is a confounder because increasing age is a risk factor for both prevalence of fibroids and HSV-2 seropositivity (among women without fibroids) in SELF. Thus, the women excluded because they already had a fibroid diagnosis would most likely be, on average, older than the women in the study and be more likely to have been exposed to HSV-2, so the adjusted estimates would be closer to the null.

6.2 Strengths

This project had several strengths. We used enrollment data from an ongoing prospective study with a standardized measure of fibroid status based on systematic ultrasound screening rather than fibroids clinically detected because of symptoms. The diameter and volume of the fibroids were systematically measured, so we were able to examine the association with small as well as large fibroids and total volume. In addition, we measured our exposure using standard serological methods (for Aim 2) rather than self-report and our sample size was sufficient to provide good precision for the main hypotheses. Twenty-two percent (22%) of our cohort of young African-American women had fibroids at ultrasound screening, a prevalence which falls within the range of prior US studies that conducted ultrasound screening [5, 6, 29, 30]. We also had extensive data to assess potential confounding. Our population is comprised of young, African-American women. Thus, our population inherently accounts for some of the biggest confounders (African-American heritage and age), so we did not have to adjust for them. We also had information on other potential confounders such as Depo-Provera and alcohol use which the previous studies did not adjust for. In addition, we had minimal missing data and conducted sensitivity analyses to evaluate potential bias.

6.3 Public Health Implications

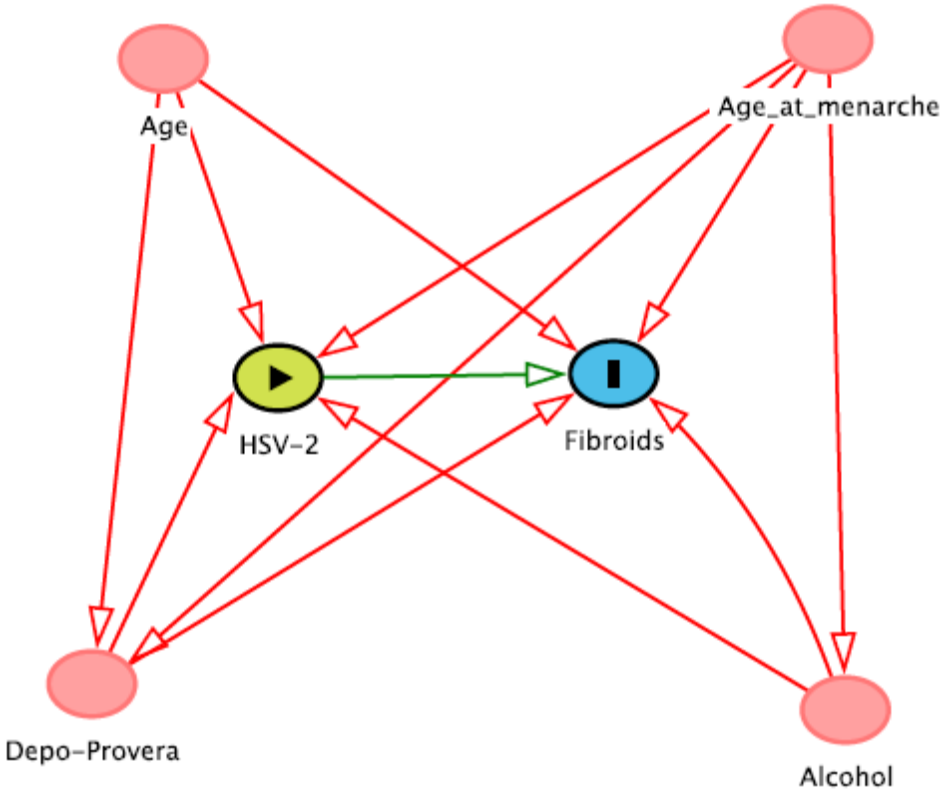
Overall, based on our findings in a large cohort of African-American women, HSV-2 seropositivity does not appear to be a risk factor for fibroids in this 23-34 year-old age group. Thus, HSV-2 serostatus does not help to predict women at high risk of fibroids and any systemic or uterine inflammation associated with HSV-2 infection does not appear to increase risk of fibroids. Furthermore, we did not find any strong associations between self-

reported RTIs and fibroid presence, number, size or total volume. However, our findings for CT and BV warrant further investigation.

6.4 Future Research

Further study of other serologically measured RTIs is still warranted, as are prospective studies of the impact of RTIs on fibroid growth. We are currently in the process of collecting serology data on CT in the SELF study cohort in order to further investigate the relationship with fibroids similar to Aim 2 in this project. In addition, the SELF study is a prospective study. Almost 80% of the participants did not have fibroids at enrollment, and because vaginal swabs, urine and blood were collected at enrollment, there is potential to investigate RTIs as a risk factor for fibroid incidence in this cohort as well.

APPENDIX 1: DIRECTED ACYCLIC GRAPH



Appendix Figure 1. Directed Acyclic Graph of the Relationship between Herpes Simplex Virus-2 and Fibroids

APPENDIX 2: CHAPTER 2 SUPPLEMENTAL TABLES

Supplemental Table 1. Age-Adjusted Correlation Coefficients among Self-Reported Reproductive Tract Infections

Spearman Partial Correlation Coefficients N = 1,653						
Reproductive Tract Infections[a]	Chlamydia	Bacterial Vaginosis	Trichomonas	Gonorrhea	Genital Herpes	Genital Warts
Chlamydia	1					
Bacterial Vaginosis	0.20	1				
Trichomonas	0.24	0.24	1			
Gonorrhea	0.31	0.13	0.23	1		
Genital Herpes	0.08	0.12	0.08	0.09	1	
Genital Warts	0.05	0.07	0.07	0.04	0.11	1

[a]PID is not included because it can be a mediator in associations between the above listed RTIs and fibroids, thus precluding it's inclusion in a model with them

Supplemental Table 2. Percent Distributions of Covariates by Self-Reported Reproductive Tract Infection Status

	PID	*Chlamydia*	*Bacterial Vaginosis*	*Trichomonas*	*Gonorrhea*	*Genital Herpes*	*Genital Warts*
Covariate	*Yes* n = *162* %	*Yes* n = *628* %	*Yes* n = *624* %	*Yes* n = *530* %	*Yes* n = *323* %	*Yes* n = *143* %	*Yes* n = *113* %
Age (years)							
23-26	24	33	28	25	29	26	20
27-30	41	33	35	34	36	34	37
≥31	36	34	37	41	35	40	43
Education							
≤ High school/GED	27	19	15	20	25	18	16
Some college or technical	56	57	54	58	56	47	49
≥ Bachelors	17	24	31	22	19	36	35
BMI (kg/m^2)							
15-24	20	18	20	14	21	15	18
25-29	20	25	25	19	25	33	24
30-34	18	20	22	20	20	21	26
≥ 35	43	37	33	47	35	32	33
Alcohol at age when drinking the most[a]							
Low	21	21	18	17	20	18	18
Moderate	22	31	36	30	31	41	36
Heavy	57	48	46	53	50	41	46
Parity							
Nulliparous	30	33	36	32	30	43	40
Parous	70	68	64	68	70	57	60
Depo-Provera							
Never used	48	52	56	50	46	52	53
Ever used	52	48	44	50	54	48	47
Age at menarche (years)							
7-10	22	19	17	20	18	20	15

	PID	*Chlamydia*	*Bacterial Vaginosis*	*Trichomonas*	*Gonorrhea*	*Genital Herpes*	*Genital Warts*
Covariate	*Yes* n = *162* %	*Yes* n = *628* %	*Yes* n = *624* %	*Yes* n = *530* %	*Yes* n = *323* %	*Yes* n = *143* %	*Yes* n = *113* %
11-19	78	81	83	80	82	80	85
Sex partners before age 20							
≤1	14	12	17	13	12	16	17
2-5	49	48	49	50	43	51	42
≥ 6	38	40	34	38	45	33	42
Age at 1st sex (years)							
≤14	43	38	35	41	42	40	33
15-16	32	38	37	34	36	25	34
≥ 17	25	24	28	25	23	35	34

BMI, body mass index; GED, general education degree; IQR, interquartile range; PID, pelvic inflammatory disease

[a]Low drinkers were those who never had 10 or more drinks in a year. Heavy drinkers were those who usually drank 6 or more drinks on days when they drank or drank 4 or more drinks per sitting at least 2 to 3 times a month. Moderate drinkers were all others.

Supplemental Table 3. Years since First Diagnosis of Reproductive Tract Infections

Reproductive Tract Infection	*Fibroids*		*Total*
	Yes	*No*	
	Median Years (IQR)		*Median Years (IQR)*
Any RTI[a]	10 (6-14)	8 (5-12)	9 (5-13)
PID	8.5 (5-12)	7 (4-12)	7 (4-12)
Chlamydia	11 (7-14)	8 (5-12)	9 (5-13)
Bacterial Vaginosis	6 (3-10)	6 (3-9)	6 (3-10)
Trichomonas	8 (3-12)	7 (4-11)	7 (4-11)
Gonorrhea	12 (8-15)	8.5 (5-12)	9 (6-13)
Genital Herpes	7 (5-12)	5 (2-9)	6 (2-10)
Genital Warts	6 (2-11.5)	10 (6-12.5)	10 (6-13)

IQR, interquartile range; PID, pelvic inflammatory disease; RTI, reproductive tract infection
[a]Includes pelvic inflammatory disease, chlamydia, bacterial vaginosis, trichomonas, gonorrhea, genital herpes and genital warts

Supplemental Table 4. Adjusted Odds Ratios for Fibroids According to Self-Reported History of Number of Different Reproductive Tract Infections and Number of Diagnoses for Each Infection

	Fibroids		*Age-adjusted*	*Multivariable-adjusted*[a]
Reproductive Tract Infections	*Yes* n = *363* n (%)[a]	*No* n = *1,293* n (%)	*OR (95% CI)*	*OR (95% CI)*
Number of Different RTIs Reported				
0	101 (28)	381 (30)	1 (ref)	1 (ref)
1	103 (28)	350 (27)	1.08 (0.79-1.49)	1.19 (0.86-1.64)
2	85 (23)	238 (18)	1.32 (0.94-1.85)	1.41 (0.99-2.01)
3+	74 (20)	320 (25)	0.82 (0.58-1.15)	0.91 (0.63-1.30)
Missing		4		
PID				
Diagnoses (n)				
0	329 (91)	1,164 (90)	1 (ref)	1 (ref)
1	28 (8)	104 (8)	0.94 (0.60-1.47)	0.98 (0.62-1.55)
2+	6 (2)	24 (2)	0.75 (0.30-1.88)	0.79 (0.31-2.02)
Missing		1		
Chlamydia				
Diagnoses (n)				
0	238 (66)	788 (61)	1 (ref)	1 (ref)
1	80 (22)	339 (26)	0.82 (0.61-1.09)	0.86 (0.64-1.16)
2+	45 (12)	164 (13)	0.92 (0.63-1.32)	1.02 (0.70-1.50)
Missing		2		
Bacterial Vaginosis				
Diagnoses (n)				
0	216 (60)	815 (63)	1 (ref)	1 (ref)
1	50 (14)	164 (13)	1.22 (0.85-1.74)	1.23 (0.85-1.78)
2+	97 (27)	313 (24)	1.10 (0.83-1.45)	1.11 (0.83-1.49)
Missing		1		
Trichomonas				
Diagnoses (n)				
0	247 (68)	878 (68)	1 (ref)	1 (ref)
1	76 (21)	276 (21)	0.91 (0.68-1.23)	0.93 (0.68-1.27)
2+	40 (11)	138 (11)	0.88 (0.60-1.30)	0.90 (0.60-1.35)
Missing		1		
Gonorrhea				
Diagnoses (n)				
0	301 (83)	1,031 (78)	1 (ref)	1 (ref)
1	41 (11)	190 (15)	0.74 (0.51-1.07)	0.81 (0.56-1.19)
2+	21 (6)	71 (6)	1.01 (0.61-1.69)	1.21 (0.71-2.04)
Missing		1		

CI, confidence interval; OR, odds ratio; PID, pelvic inflammatory disease; RTI, reproductive tract infection
[a]Logistic regression model adjusted for age (continuous), education, body mass index, alcohol, menarche, parity, and Depo-Provera use
Genital herpes was not included because the number of times diagnosed was not applicable;
Genital warts was not included because only 9 participants were diagnosed more than once

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