American Journal of Epidemiology
© The Author 2016. Published by Oxford University Press on behalf of the Johns Hopkins Bloomberg School of Public Health. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.
Vol. 183, No. 11
DOI: 10.1093/aje/kwv313
Advance Access publication:
May 8, 2016

Original Contribution

Herpes Simplex Virus Type 2 Seroprevalence and Ultrasound-Diagnosed Uterine Fibroids in a Large Population of Young African-American Women

Kristen R. Moore*, Jennifer S. Smith, Stephen R. Cole, Victor J. Schoenbach, Katherine Schlusser, Charlotte A. Gaydos, and Donna D. Baird*

* Correspondence to Dr. Donna D. Baird, Epidemiology Branch A3-05, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 (e-mail: baird@niehs.nih.gov); or Dr. Kristen R. Moore, Epidemiology Branch A3-05, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 (e-mail: kristen.moore@nih.gov).

Initially submitted September 1, 2015; accepted for publication November 5, 2015.

For decades reproductive tract infections (RTIs) have been hypothesized to play a role in uterine fibroid development. The few previous studies conducted used self-reported history of RTIs and had inconsistent findings. We investigated this hypothesis further using serological analysis, an immunological measure of past exposure. We focused on herpes simplex virus type 2 (HSV-2) because prior published data have suggested a possible association with fibroids, and serology for HSV-2 is much more sensitive than self-report. We used cross-sectional enrollment data from African-American women enrolled in a prospective study of fibroid incidence and growth (recruited 2010–2012) in the Detroit, Michigan, area. The women were aged 23–34 years and were screened for fibroids using a standardized ultrasound examination at their enrollment. Age- and multivariable-adjusted logistic regression models were used to estimate odds ratios. Of 1,696 participants, 1,658 had blood samples and HSV-2 serology results; 22% of participants with serology results had fibroids. There was no significant association between HSV-2 seropositivity and the presence of fibroids (multivariable-adjusted odds ratio = 0.94, 95% confidence interval: 0.73, 1.20), nor were there any associations with size of the largest fibroid, number of fibroids, or total fibroid volume. Our data provide no evidence for an influence of HSV-2 exposure on fibroid risk in young African-American women. Further study of other serologically measured RTIs is warranted.

herpes simplex virus type 2; seroprevalence; uterine fibroids

Abbreviations: DMPA, depot medroxyprogesterone acetate; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; RTI, reproductive tract infection; SELF, Study of Environment, Lifestyle, and Fibroids.

Uterine fibroids are among the most common gynecological conditions affecting women in the United States during their reproductive years (1). Based on ultrasound screening of randomly selected women, the estimated cumulative incidence of fibroid tumors by age 50 years is more than 80% for African-American women and close to 70% for white women (2). Symptoms resulting from fibroids (pain, severe bleeding, and reproductive problems) are the leading reason for hysterectomy in the United States, and the total annual costs of fibroids are as high as $34 billion (3).

Fibroids are hormone-dependent, benign tumors of the uterine smooth muscle. Their etiological causes are largely unknown, but established risk factors include African-American heritage (African Americans are 2–3 times as likely as white women to have clinically recognized fibroids (2)), older age (up to the age of menopause), younger age at menarche, and nulliparity (4–6). Three studies have found that progestin-only injectables (such as depot medroxyprogesterone acetate (DMPA)) are protective (5, 7, 8). Heavier alcohol use may be a risk factor, although the number of studies is small (9, 10).

A hypothesis was raised decades ago that reproductive tract infections (RTIs) may play a role in fibroid development (11). Both RTIs and fibroids disproportionately burden African-American women, and certain RTIs can lead to conditions such as chronic pelvic infection that could result in inflammatory reactions (11). This hypothesis is consistent

with another theorized mechanism of fibroid pathogenesis in which infection can stimulate an inflammatory immune response that can facilitate the initiation of tissue damage, resulting in tissue repair or regeneration (increased extracellular matrix, cell proliferation, decreased apoptosis), leading to the formation and growth of uterine fibroids (12, 13). Furthermore, fibroids were found to be associated with serologically determined Chagas disease (caused by a protozoan parasite) (14), and Epstein-Barr virus was found to infect smooth muscle cell tumors at sites outside of the uterus (15).

However, the limited data on associations between RTIs and fibroid risk have yielded inconsistent findings (4, 16, 17). One of the primary aims for undertaking the Study of Environment, Lifestyle, and Fibroids (SELF), a large study of fibroids in African-American women, was to fill this data gap (18). The data from prior studies suggested positive associations between fibroids and pelvic inflammatory disease (16), chlamydia (16), genital herpes (4), trichomonas (4), bacterial vaginosis (4, 17), and syphilis (4). We recently published self-reported RTI data from SELF that showed no strong associations with fibroids (17). We did find that women reporting a history of bacterial vaginosis had somewhat elevated odds of multiple fibroids (≥2) and larger total fibroid volume (≥2 cm^3), and women reporting a history of chlamydia tended to have fewer and smaller fibroids (17). 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 (19–22). An immunological measure of exposure, namely serological analysis (diagnostic identification of antibodies in the serum that remain after infection), would provide a more accurate assessment of exposure than self-reported RTI history.

Herpes simplex virus type 2 (HSV-2), the main cause of genital and neonatal herpes, is a common RTI in the United States (23). Compared with women of other racial/ethnic groups in the United States, African-American women have the highest seroprevalence of HSV-2 (50% for 14- to 49-year-olds between 2007 and 2010) (24). Other main risk factors for HSV-2 include higher numbers of sexual partners, heavy alcohol use, low socioeconomic status, being unpartnered rather than married or cohabiting, and lack of consistent condom use (24–28). Also, DMPA use may increase risk of HSV-2 seroconversion (29, 30), and age at menarche has been found to be associated with HSV-2 (31) and has been identified as a predictor of sexual behavior that is highly associated with HSV-2 (32, 33).

HSV-2 antibodies have been shown to persist for years after infection (34), and serological testing for HSV-2 can be done sensitively and specifically for past exposure to HSV-2 (35). Cell culture and polymerase chain reaction are the recommended laboratory tests for people who have active lesions or ulcers present (36). However, most HSV-2 infections are asymptomatic or unrecognized (in a study by Fanfair et al. (24), more than 85% of seropositive African-American women reported no history of diagnosis). Thus, seropositivity to HSV-2 is the best estimate of past, cumulative exposure (24, 25).

Building upon previous work, in which a positive association between self-reported HSV-2 and fibroids was found (4), 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, to our knowledge, the first to investigate the association of fibroids with HSV-2 exposure assessed serologically. We hypothesized that women seropositive for HSV-2 would have a higher prevalence of fibroids than women seronegative for HSV-2. We also explored the relationship between HSV-2 seropositivity and number, size, and total volume of fibroids.

METHODS

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 (18). In brief, from November 2010 to December 2012, the study recruited 1,696 African-American volunteers; all of the women were aged 23–34 years. 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, Sjögren syndrome, 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 the National Institute of Environmental Health Sciences (Research Triangle Park, North Carolina) and the Henry Ford Health System (Detroit, Michigan).

Fibroid assessment

Transvaginal ultrasound, the standard procedure for the detection and diagnosis of fibroids, was used in this study (37). Fibroids were assessed by study sonographers as described previously (18) at one of 3 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.

Outcome definitions

The primary outcome of this study was the presence of fibroids (yes or no) at the transvaginal ultrasound examination completed at enrollment. Fibroids were classified as "questionable" when at least 1 diameter could not be measured. Participants with at least 1 fibroid or questionable fibroid greater than or equal to 0.5 cm in diameter at the enrollment ultrasound were considered to have fibroids, and all women without a fibroid or questionable fibroid at least 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 × anterior-posterior × transverse × 0.5233) and

averaging across the 3 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 ($n = 6$) in whom at least 1 diameter was not measured.

HSV-2 assessment

HSV-2 antibody serostatus was assessed in the International Sexually Transmitted Diseases Research Laboratory at Johns Hopkins University (Baltimore, Maryland) using the Focus Diagnostics HerpeSelect 2 ELISA IgG test (Focus Diagnostics, Cypress, California) according to the package instructions (38). This type-specific assay (glycoprotein gG2) allows for the qualitative detection of HSV-2 immunoglobulin G antibodies with or without the presence of herpes simplex virus type 1 (HSV-1) (38). Antibody levels greater than 1.10 optical density units were categorized as seropositive and levels less than 0.90 were seronegative. Levels of 0.9 to 1.10 were considered indeterminate, and those samples 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 drawing performed 6 months after enrollment (18). Two aliquots from each of 42 specimens served as 84 blinded control samples. Forty of the 42 duplicate pairs had identical results. For each of the other 2 discordant pairs, 1 of the aliquots was considered indeterminate. The result for the other aliquot of each of these 2 discordant pairs matched the result found for the enrollment specimen from the same participant.

Statistical analyses

Due to the use of telephone and Web-based interview methods that did not allow participants to skip questions, there was a minimal amount of 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 on the basis of the distribution of the data and comparability with the literature. All analyses were completed using data from women who had blood samples taken at enrollment. Standard descriptive statistics were performed for all variables of interest. We also evaluated 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 using SAS, version 9.3 (SAS Institute, Inc., Cary, North Carolina).

Primary analyses. Logistic regression was used to compute odds ratios and 95% confidence intervals to evaluate the relationship between HSV-2 serostatus and the presence of fibroids. Although the relative odds overestimate the relative risk for associations with an outcome as common as fibroids, overestimation is minimal for weak associations and 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 (39) was used to provide a conceptual framework. Potential confounders included age in years (continuous), age at menarche (7–10 years or 11–19 years), alcohol consumption (low, moderate, or heavy), and use of DMPA (ever or never). The alcohol-consumption variable reflected the drinking level each woman reported for the age(s) at which she was drinking the most. Low alcohol consumption was defined as having had less than 10 alcoholic drinks in a year. Heavy drinkers were those who usually consumed 6 or more drinks on days when they imbibed alcohol or who consumed 4 or more drinks per sitting at least 2–3 times a month. All others were considered moderate drinkers. Variables of interest based on the literature included education (high school graduation, General Educational Development certificate, or less; some college, an associate degree, or technical education; or bachelor's, master's, or doctoral degree), and body mass index (15–24, 25–29, 30–34, or ≥35), which was calculated as weight (kg)/height $(m)^2$. Parity (nulliparous or 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 association between alcohol consumption and fibroids 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.

Secondary analyses. In 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. Median values 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). We used multinomial logistic regression models to estimate the odds ratios and 95% confidence intervals for the associations of HSV-2 serostatus with fibroid size, number, and total volume. Moreover, because the majority of HSV-2 infections are asymptomatic, self-reported infections are likely to be those that are symptomatic. Thus, in a secondary analysis, we evaluated the multivariable-adjusted odds ratios and 95% confidence intervals for the relationship 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 those included in the final model in the primary analysis (age, age at menarche, parity, and DMPA use).

Sensitivity analyses. To evaluate the robustness of our findings, we examined multivariable-adjusted odds ratios and 95% confidence intervals 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 (40). Thus, any HSV-2 associations we observed might have been attenuated. Therefore, we repeated the primary analyses after excluding those who reported cervical treatment (231 women excluded). To evaluate age as an effect modifier (due to possible decline in antibody levels over time),

Table 1. Selected Enrollment Characteristics of African-American Women Aged 23–34 Years According to Herpes Simplex Virus Type 2 Serostatus ($n = 1,658$), Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012

Covariate	HSV-2 Seropositive ($n = 789$)		HSV-2 Seronegative ($n = 869$)	
	No.	%	No.	%
Age, years				
23–26	191	24	319	37
27–30	284	36	286	33
31–35[a]	314	40	264	30
Education				
High-school graduate, GED certificate, or less	235	30	129	15
Some college, associate degree, or technical education	409	52	423	49
Bachelor's, master's, or doctoral degree	144	18	317	36
Missing	1		0	
Body mass index[b]				
15–24	164	21	164	19
25–29	157	20	186	21
30–34	141	18	177	20
≥35	327	41	342	39
Alcohol consumption[c]				
Low	198	25	240	28
Moderate	231	29	309	36
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
No. of sexual partners before age 20 years				
≤1[e]	154	19	268	31
2–5	399	51	413	48
≥6	234	30	187	21
Missing	2		1	
Age at first sexual intercourse, years				
≤14	284	36	196	23
15–16	274	35	287	33
≥17[e]	231	29	383	44
Missing	0		3	

Abbreviations: DMPA, depot medroxyprogesterone acetate; GED, General Educational Development; HSV-2, herpes simplex virus type 2.

[a] No persons over 34 years of age were recruited, but some 34-year-olds had turned 35 by the time they had their ultrasound examination.

[b] Body mass index was calculated as weight (kg)/height $(m)^2$.

[c] The alcohol-consumption variable reflected the drinking level each woman reported for the age(s) at which she was drinking the most. Low alcohol consumption was defined as having had less than 10 alcoholic drinks in a year. Heavy drinkers were those who usually consumed 6 or more drinks on days when they imbibed alcohol or who consumed 4 or more drinks per sitting at least 2–3 times a month. All others were considered moderate drinkers.

[d] Categorized to identify early age at menarche, the category associated with fibroids.

[e] Includes participants who reported never having had sex.

Table 2. Herpes Simplex Virus Type 2 Serostatus in Relation to Fibroids Among African-American Women Aged 23–34 Years ($n = 1,658$), Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012

HSV-2 Serostatus	No. of Women	Fibroids Detected		Age-Adjusted OR	95% CI	Multivariable-Adjusted[a] OR	95% CI
		No.	%				
Seronegative	869	195	22	1.00	Referent	1.00	Referent
Seropositive	789	170	22	0.82	0.64, 1.04	0.94	0.73, 1.20

Abbreviations: 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.

we evaluated the association between HSV-2 and fibroid presence in 2 age strata (23–29 years or 30–35 years).

To investigate temporality of HSV-2 exposure in relation to fibroid development, we assessed the association between HSV-2 and fibroids among strata of size of the largest fibroid (<2 cm vs. ≥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 with 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 years (0 or 1 vs. 2 or more). Those with more sexual partners before the age of 20 years 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.

RESULTS

The median age of our cohort at enrollment was 29 years, and the median age at menarche was 12 years. The majority of participants were parous, and more than 40% had 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 had more sexual partners before age 20 years, and to have been younger at first sex compared with those who were seronegative for HSV-2 (Table 1).

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

In primary analyses, the odds of fibroids were similar for those who were HSV-2-seropositive and those who were HSV-2-seronegative, in both age- and multivariable-adjusted models (multivariable-adjusted odds ratio = 0.94, 95% confidence interval: 0.73, 1.20) (Table 2). In secondary analyses, we evaluated size of the largest fibroid, number of fibroids, and total fibroid volume as the outcomes (Figure 1). There was no significant association between HSV-2 exposure and fibroid size, number, or total volume.

We also examined the relationship between symptomatic herpes and fibroid presence based on a combination of self-reported genital herpes and serology data (Table 3). Only 16% of women who were seropositive for HSV-2 reported having had a genital herpes diagnosis (sensitivity for self-reported

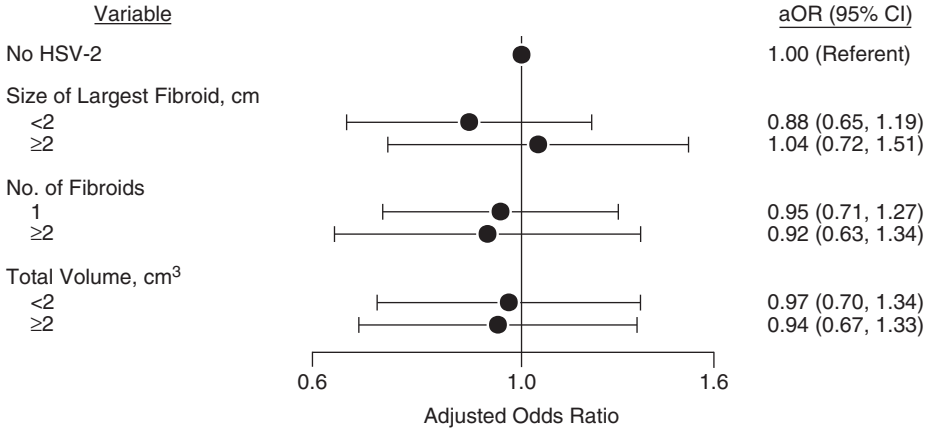

Figure 1. Size of largest fibroid, number of fibroids, and total fibroid volume in relation to herpes simplex virus type 2 (HSV-2) serostatus among young African-American women with fibroids ($n = 365$), Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012. The participants were aged 23–34 years. Odds ratios (aORs) and 95% confidence intervals (CIs) were adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.

Table 3. Symptomatic and Asymptomatic Serologically Detected Herpes Simplex Virus Type 2 in Relation to Fibroids Among African-American Women Aged 23–34 Years ($n = 1,641$), Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012

HSV-2 Status[a]	No. of Women	Fibroids Detected		OR[b]	95% CI
		No.	%		
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

Abbreviations: 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 reported genital herpes but were HSV-2-seronegative).

[b] Adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.

[c] Defined as HSV-2-seropositive participants who reported having been diagnosed with genital herpes ("Yes") or not having been diagnosed with genital herpes ("No").

HSV-2 exposure). However, 98% of those who were seronegative reported no diagnosis of genital herpes (specificity). Those who were seropositive and reported an HSV-2 diagnosis (our measure of symptomatic herpes) did not have higher odds of fibroids. Furthermore, the lack of an association between HSV-2 and fibroids remained consistent across the sensitivity analyses performed (Table 4).

Table 4. Sensitivity Analyses of the Association of Fibroids With Herpes Simplex Virus Type 2 Serostatus Among African-American Women Aged 23–34 Years, Study of Environment, Lifestyle, and Fibroids, Detroit, Michigan, 2010–2012

	No. of Women	OR[a]	95% CI
Full sample	1,658	0.94	0.73, 1.20
Exclusion of 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[b]			
<2	224	0.88	0.65, 1.18
≥2	141	1.06	0.73, 1.54
Stratified by no. of sexual partners before age 20 years[c]			
0–1	422	0.99	0.60, 1.64
≥2	1,233	0.91	0.68, 1.21

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for age, age at menarche, depot medroxyprogesterone acetate use, and parity.

[b] Referent was the group of 1,293 participants without fibroids.

[c] Three participants had missing data for number of sex partners before age 20 years.

DISCUSSION

Our study did not show an association between HSV-2 and the presence of fibroids or the size, number, or total volume of fibroids. Even among women who were seropositive and had genital herpes severe enough to warrant reporting 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 sexual partners before age 20 years.

Our findings are consistent with a previous study on this same population that evaluated several self-reported RTIs, showing no association of genital herpes with fibroids (17). Two other studies have investigated the association between self-reported genital herpes and the presence of fibroids (4, 16). A clinic-based case-control study of premenopausal women (aged 18–55 years) showed no association of genital herpes with clinically detected fibroids (16). The Uterine Fibroid Study (4), 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 (4). 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 Uterine Fibroid Study participants who had reported a history of sexually transmitted disease or multiple sexual partners were tested for viral DNA matching HSV-1, HSV-2, cytomegalovirus, human herpes virus (types 6, 7, and 8), and Epstein-Barr virus using polymerase chain reaction (4). None of these pathogens was detected in the tumor samples.

Our study had several limitations. It was a cross-sectional analysis. Thus, the timing of 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. More than half the women who self-reported genital herpes reported that their first diagnosis was before the age of 22 years, and approximately 45% of new HSV-2 infections in the United States are among persons aged 15–24 years (23). 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 (18, 41). Finally, the median amount of time between the first self-reported HSV-2 diagnosis and study enrollment was 6 years, and because antibody titers persist for years after infection (34), even exposure to HSV-2 multiple years prior to enrollment should have been captured.

We did not use the Western blot test, the gold standard method for HSV-2 serology (35, 42). However, the HerpeSelect 2 assay we used (38) has been found to perform very well for HSV-2 infections, with sensitivity between 96% and 100% and specificity between 97% and 98% (43, 44) in comparison

with the Western blot. The Western blot technique is not approved by the US Food and Drug Administration and is more complex, more costly, less time-efficient, and much less widely available than the enzyme-linked immunosorbent assay (45). In addition, we did not capture persons who had a 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 herpes simplex virus infection due to the omission of genital infections caused solely by HSV-1, which are increasing in incidence (46). However, because HSV-1 also causes orolabial infections, which are very prevalent (46), a large proportion of the population will have antibodies to HSV-1, and we would not be able to distinguish orolabial HSV-1 infection from genital HSV-1 infection. Thus, the value of the added information to be gained from measuring HSV-1 is unclear.

Finally, our sample was 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 United States (24). Furthermore, 22% of the women in our cohort had fibroids at ultrasound screening, which falls within the range of prior US studies that conducted ultrasound screening (2, 4, 41, 47).

Our study also had several strengths. This was, to our knowledge, 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, which enabled us 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 in the study than those without HSV-2; thus, there was limited potential for selection bias. Our study also had extensive data with which to assess potential confounding and minimal missing data. In addition, we conducted sensitivity analyses to evaluate potential bias.

Overall, based on our findings in a large cohort of young (ages 23–34 years) African-American women, HSV-2 seropositivity does not appear to be a risk factor for fibroids in this 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 of the relationship between RTIs and fibroid growth.

ACKNOWLEDGMENTS

Author affiliations: Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina (Kristen R. Moore, Jennifer S. Smith, Stephen R. Cole, Victor J. Schoenbach); Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina (Kristen R. Moore, Donna D. Baird); and Division of Infectious Diseases, Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, Maryland (Katherine Schlusser, Charlotte A. Gaydos).

This work was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (grant 10-E-N044), and American Recovery and Reinvestment Act funds designated for NIH research.

We thank Dr. Dirk Dittmer for help in design of the study and review of a draft of the manuscript and Drs. Lauren Wilson and Helen Meier for review of a draft of the manuscript. We also thank our collaborators and study staff at Social and Scientific Systems (Research Triangle Park, North Carolina) and the Henry Ford Health System (Detroit, Michigan).

Conflict of interest: none declared.

REFERENCES

1. Stewart EA. Uterine fibroids. *Lancet*. 2001;357(9252):293–298.
2. Baird DD, Dunson DB, Hill MC, et al. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. *Am J Obstet Gynecol*. 2003;188(1):100–107.
3. Cardozo ER, Clark AD, Banks NK, et al. The estimated annual cost of uterine leiomyomata in the United States. *Am J Obstet Gynecol*. 2012;206(3):211.e1–211.e9.
4. Laughlin SK, Schroeder JC, Baird DD. New directions in the epidemiology of uterine fibroids. *Semin Reprod Med*. 2010; 28(3):204–217.
5. Wise LA, Palmer JR, Harlow BL, et al. Reproductive factors, hormonal contraception, and risk of uterine leiomyomata in African-American women: a prospective study. *Am J Epidemiol*. 2004;159(2):113–123.
6. Marshall LM, Spiegelman D, Goldman MB, et al. A prospective study of reproductive factors and oral contraceptive use in relation to the risk of uterine leiomyomata. *Fertil Steril*. 1998;70(3):432–439.
7. Lumbiganon P, Rugpao S, Phandhu-fung S, et al. Protective effect of depot-medroxyprogesterone acetate on surgically treated uterine leiomyomas: a multicentre case–control study. *Br J Obstet Gynaecol*. 1996;103(9):909–914.
8. Harmon QE, Baird DD. Use of depot medroxyprogesterone acetate and prevalent leiomyoma in young African American women. *Hum Reprod*. 2015;30(6):1499–1504.
9. Wise LA, Palmer JR, Harlow BL, et al. Risk of uterine leiomyomata in relation to tobacco, alcohol and caffeine consumption in the Black Women's Health Study. *Hum Reprod*. 2004;19(8):1746–1754.
10. Nagata C, Nakamura K, Oba S, et al. Association of intakes of fat, dietary fibre, soya isoflavones and alcohol with uterine fibroids in Japanese women. *Br J Nutr*. 2009;101(10): 1427–1431.

11. Witherspoon JT, Butler VW. The etiology of uterine fibroids with special reference to the frequency of their occurrence in the Negro: a hypothesis. *Surg Gynecol Obstet*. 1934;58:57–61.
12. Leppert PC, Catherino WH, Segars JH. A new hypothesis about the origin of uterine fibroids based on gene expression profiling with microarrays. *Am J Obstet Gynecol*. 2006;195(2):415–420.
13. Wegienka G. Are uterine leiomyoma a consequence of a chronically inflammatory immune system? *Med Hypotheses*. 2012;79(2):226–231.
14. Murta EF, Oliveira GP, Prado FdeO, et al. Association of uterine leiomyoma and Chagas' disease. *Am J Trop Med Hyg*. 2002;66(3):321–324.
15. Jenson HB, Leach CT, McClain KL, et al. Benign and malignant smooth muscle tumors containing Epstein-Barr virus in children with AIDS. *Leuk Lymphoma*. 1997;27(3-4): 303–314.
16. Faerstein E, Szklo M, Rosenshein NB. Risk factors for uterine leiomyoma: a practice-based case-control study. II. Atherogenic risk factors and potential sources of uterine irritation. *Am J Epidemiol*. 2001;153(1):11–19.
17. Moore KR, Cole SR, Dittmer DP, et al. Self-reported reproductive tract infections and ultrasound diagnosed uterine fibroids in African-American women. *J Womens Health (Larchmt)*. 2015;24(6):489–495.
18. Baird DD, Harmon QE, Upson K, et al. A prospective, ultrasound-based study to evaluate risk factors for uterine fibroid incidence and growth: methods and results of recruitment. *J Womens Health (Larchmt)*. 2015;24(11):907–915.
19. Brown JL, Sales JM, DiClemente RJ, et al. Predicting discordance between self-reports of sexual behavior and incident sexually transmitted infections with African American female adolescents: results from a 4-city study. *AIDS Behav*. 2012;16(6):1491–1500.
20. Harrington KF, DiClemente RJ, Wingood GM, et al. Validity of self-reported sexually transmitted diseases among African American female adolescents participating in an HIV/STD prevention intervention trial. *Sex Transm Dis*. 2001;28(8): 468–471.
21. Hong Y, Fang X, Zhou Y, et al. Factors associated with sexually transmitted infection underreporting among female sex workers in China. *J Womens Health (Larchmt)*. 2011;20(1):129–136.
22. Niccolai LM, Kershaw TS, Lewis JB, et al. Data collection for sexually transmitted disease diagnoses: a comparison of self-report, medical record reviews, and state health department reports. *Ann Epidemiol*. 2005;15(3):236–242.
23. Centers for Disease Control and Prevention. *Incidence, Prevalence, and Cost of Sexually Transmitted Infections in the United States*. 2013. http://www.cdc.gov/std/stats/STI-Estimates-Fact-Sheet-Feb-2013.pdf. Published February 2013. Accessed June 20, 2013.
24. Fanfair RN, Zaidi A, Taylor LD, et al. Trends in seroprevalence of herpes simplex virus type 2 among non-Hispanic blacks and non-Hispanic whites aged 14 to 49 years—United States, 1988 to 2010. *Sex Transm Dis*. 2013;40(11):860–864.
25. Xu F, Sternberg M, Gottlieb S, et al. Seroprevalence of herpes simplex virus type 2 among persons aged 14–49 years—United States, 2005–2008. *MMWR Morb Mortal Wkly Rep*. 2010; 59(15):456–459.
26. Bauer GR, Khobzi N, Coleman TA. Herpes simplex virus type 2 seropositivity and relationship status among U.S. adults age 20 to 49: a population-based analysis. *BMC Infect Dis*. 2010;10:359.
27. Moss NJ, Harper CC, Ahrens K, et al. Predictors of incident herpes simplex virus type 2 infections in young women at risk for unintended pregnancy in San Francisco. *BMC Infect Dis*. 2007;7:113.
28. Martin ET, Krantz E, Gottlieb SL, et al. A pooled analysis of the effect of condoms in preventing HSV-2 acquisition. *Arch Intern Med*. 2009;169(13):1233–1240.
29. Grabowski MK, Gray RH, Makumbi F, et al. Use of injectable hormonal contraception and women's risk of herpes simplex virus type 2 acquisition: a prospective study of couples in Rakai, Uganda. *Lancet Glob Health*. 2015;3(8):e478–e486.
30. Kaushic C, Ashkar AA, Reid LA, et al. Progesterone increases susceptibility and decreases immune responses to genital herpes infection. *J Virol*. 2003;77(8):4558–4565.
31. Glynn JR, Kayuni N, Gondwe L, et al. Earlier menarche is associated with a higher prevalence of herpes simplex type-2 (HSV-2) in young women in rural Malawi. *eLife*. 2014;3:e01604.
32. Belsky J, Steinberg L, Houts RM, et al. The development of reproductive strategy in females: early maternal harshness → earlier menarche → increased sexual risk taking. *Dev Psychol*. 2010;46(1):120–128.
33. Downing J, Bellis MA. Early pubertal onset and its relationship with sexual risk taking, substance use and anti-social behaviour: a preliminary cross-sectional study. *BMC Public Health*. 2009;9:446.
34. Jerome K, Morrow RA. Herpes simplex viruses and herpes B virus. In: Versalovic J, Carroll K, Funke G, et al, eds. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: ASM Press; 2011:1530–1544.
35. LeGoff J, Péré H, Bélec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. *Virol J*. 2014;11:83.
36. Centers for Disease Control and Prevention. 2015 sexually transmitted diseases treatment guidelines: genital HSV infections. http://www.cdc.gov/std/tg2015/herpes.htm. Updated June 8, 2015. Accessed June 30, 2015.
37. Dueholm M, Lundorf E, Hansen ES, et al. Accuracy of magnetic resonance imaging and transvaginal ultrasonography in the diagnosis, mapping, and measurement of uterine myomas. *Am J Obstet Gynecol*. 2002;186(3):409–415.
38. Focus Diagnostics. *HerpeSelect 2 ELISA IgG*. Cypress, CA: Focus Diagnostics; 2011. http://www.focusdx.com/pdfs/pi/US/EL0920G.pdf. Published March 31, 2011. Accessed January 29, 2013.
39. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology*. 1999;10(1):37–48.
40. Moore KR, Smith JS, Laughlin-Tommaso SK, et al. Cervical neoplasia-related factors and decreased prevalence of uterine fibroids among a cohort of African American women. *Fertil Steril*. 2014;101(1):208–214.
41. Laughlin SK, Baird DD, Savitz DA, et al. Prevalence of uterine leiomyomas in the first trimester of pregnancy: an ultrasound-screening study. *Obstet Gynecol*. 2009;113(3): 630–635.
42. Strick LB, Wald A. Diagnostics for herpes simplex virus: is PCR the new gold standard? *Mol Diagn Ther*. 2006;10(1):17–28.
43. Ashley RL. Sorting out the new HSV type specific antibody tests. *Sex Transm Infect*. 2001;77(4):232–237.
44. Prince HE, Ernst CE, Hogrefe WR. Evaluation of an enzyme immunoassay system for measuring herpes simplex virus (HSV) type 1-specific and HSV type 2-specific IgG antibodies. *J Clin Lab Anal*. 2000;14(1):13–16.
45. Ashley RL. Performance and use of HSV type-specific serology test kits. *Herpes*. 2002;9(2):38–45.
46. Bradley H, Markowitz LE, Gibson T, et al. Seroprevalence of herpes simplex virus types 1 and 2—United States, 1999–2010. *J Infec Dis*. 2014;209(3):325–333.
47. Marsh EE, Ekpo GE, Cardozo ER, et al. Racial differences in fibroid prevalence and ultrasound findings in asymptomatic young women (18–30 years old): a pilot study. *Fertil Steril*. 2013;99(7):1951–1957.