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Original Contribution

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

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Reproductive tract infections have long been hypothesized to be risk factors for development of uterine fibroids, but few studies have investigated the issue. In our 2016 cross-sectional analysis from the Study of Environment, Lifestyle and Fibroids (2010–2018), a large Detroit, Michigan, community-based cohort study of 23- to 35-year-old African-American women with ultrasound fibroid screening, we found no association between a very prevalent reproductive tract infection, herpes simplex virus type 2 (HSV-2), and fibroids. With prospective data from the cohort (ultrasounds performed every 20 months over 5 years), we examined HSV-2's associations with fibroid incidence (among 1,208 women who were fibroid-free at baseline) and growth (among women with fibroids at baseline or diagnosed during the study). Using Cox proportional hazards models, we computed adjusted hazard ratios and 95% confidence intervals for fibroid incidence comparing HSV-2–seropositive women with HSV-2–seronegative women. The influence of HSV-2 infection on growth was assessed on the basis of the difference in fibroid size between successive ultrasounds (1,323 growth measures) using a linear mixed model, estimating the percent difference in growth scaled to 18 months. HSV-2 seropositivity was not associated with fibroid incidence (adjusted hazard ratio = 0.88, 95% confidence interval: 0.69, 1.12) or growth (estimated growth difference = 3.1%, 95% confidence interval: −5.8, 13.0). Women can be reassured that HSV-2 infection is unlikely to increase their risk of fibroid-related health problems, given these longitudinal measures.

herpes simplex virus type 2; incidence; seroprevalence; tumor growth; uterine fibroids

Abbreviations: CI, confidence interval; HR, hazard ratio; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; SELF, Study of Environment, Lifestyle and Fibroids.

Uterine fibroids are common benign smooth-muscle–cell tumors and are the leading indication for hysterectomy in the United States (1). For decades, reproductive tract infections have been hypothesized to play a role in uterine fibroid development (2). Reproductive tract infections could plausibly increase the risk of fibroids by inducing an inflammatory environment, leading to cell proliferation, increased extracellular matrix production, and decreased apoptosis, and ultimately the formation and growth of fibroids (3, 4). However, of the few studies on this topic, most have used self-reported reproductive tract infection status (5). In 2016, we investigated this hypothesis further using serological testing (6), a much more sensitive and specific measure of infection status. We focused on herpes simplex virus type 2 (HSV-2) because 1) it is one of the most prevalent reproductive tract infections in the United States (7); 2) one of the 2 prior published studies suggested an association (8); and 3) serological testing for HSV-2 is a highly sensitive and specific (9) measure of cumulative past exposure (10, 11). In our previous cross-sectional analysis from a large cohort study with ultrasound screening for fibroids (6), we found no evidence of a relationship between HSV-2 seropositivity and the presence, size, number, or total volume of prevalent fibroids.

However, given the cross-sectional design of our prior analysis (6), the timing of fibroid onset in relation to HSV-2 acquisition was unknown. We now have prospective data from the same cohort with which to investigate the association between HSV-2 serological status and subsequent

ultrasound-documented fibroid development. We analyzed fibroid incidence from a 5-year period of ultrasound follow-up among women who were fibroid-free at their baseline examination, as well as fibroid growth among women who had fibroids present at baseline or developed them during the study.

METHODS

We used data from the Study of Environment, Lifestyle, and Fibroids (SELF), a prospective study of fibroid development in a community-based volunteer sample of 23- to 35-year-old African-American women ($n = 1{,}693$) in the Detroit, Michigan, area recruited between 2010 and 2012. Women were not eligible for SELF if they had previously been diagnosed with uterine fibroids. Four clinic visits were conducted approximately every 20 months over 5 years. At each visit, participants provided questionnaire data and nonfasting blood samples, and standardized study ultrasound examinations were conducted. The recruitment strategy and specimen collection protocols have been described previously (12). Baseline HSV-2 serological test results were available for 1,655 (98%) participants, as previously described (6), and this group had the same follow-up rates as the entire sample; 95% attended at least 1 follow-up visit, and 90% attended the final 5-year visit. Enrollment occurred in 2010–2012, and women were invited back for a second visit scheduled to occur about 20 months after the first, a third visit about 40 months after the first, and a fourth visit about 60 months after the first. Thus, study intervals were staggered among participants by enrollment date.

The study protocol 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), and all participants gave written informed consent.

HSV-2 measurement

HSV-2 serostatus was assessed by the Johns Hopkins University's International STI, Respiratory Diseases, and Biothreat Research Laboratory (Baltimore, Maryland) using the Focus Diagnostics HerpeSelect 2 enzyme-linked immunosorbent assay (Focus Diagnostics, Cypress, California) (13). This type-specific assay qualitatively detects HSV-2 immunoglobulin G antibodies with or without the presence of herpes simplex virus type 1 (HSV-1). Antibody levels with an absorbance greater than 1.10 were categorized as seropositive, and levels with absorbance less than 0.90 were seronegative. Antibody levels with absorbance of 0.9–1.10 were considered indeterminate, and such samples were retested. Further details were published previously (6). Baseline questionnaires also provided data on self-reported clinical diagnosis of genital herpes.

Fibroid measurement

Fibroids were assessed at each visit by certified sonographers with specific training in the standardized study protocol (12) for transvaginal ultrasonography, the standard clinical procedure for the detection of fibroids (14). The 3 diameters (longitudinal (L), anterior-posterior (A), and transverse (T)) of fibroids greater than or equal to 0.5 cm in any diameter were measured in triplicate and recorded by the sonographers. Fibroid volume (cm^3) was calculated by computing the volumes of each of the triplicate measurements using the ellipsoid formula ($L \times A \times T \times 0.5233$) and averaging across the 3 volumes. For these analyses, examinations with ultrasound quality that may have impaired the ability to properly identify fibroids (transabdominal ultrasound only or sonographer-identified imaging difficulty) were excluded. The ultrasonographic methods used have previously been described in detail (12).

Covariates

Age, time since last use of the injectable contraceptive depot medroxyprogesterone acetate (never use, <4 years before, or ≥4 years before), and time since last birth (<3 years, ≥3 years, or no birth) were included as adjustment factors a priori, because of the strong protective associations we found with fibroids in this study population (15); income ($0–$20,000, >$20,000–$50,000, or >$50,000 per year) was included as a measure of socioeconomic status a priori. These variables were updated at each study visit. The information on births was anchored to the end of each study interval as an efficient way to capture the strong effects of births occurring right before or during an interval for fibroid development (16). We examined additional factors based on the fibroid literature and included those resulting in a 10% change in the risk estimate (i.e., a 10% change in the adjusted hazard ratio in the incidence analysis and a 10% change in the exponentiated β coefficient for the difference in growth) (17). The additional factors of interest were: current smoking (yes, no), current use of oral contraceptives (yes, no), current alcohol consumption (low/moderate, heavy), body mass index (weight (kg)/height $(m)^2$; 18.5–24.9, 25.0–29.9, 30.0–34.9, 35.0–39.9, or ≥40), educational attainment (high school or less, more than high school), employment (yes, no), and number of cumulative births (0, 1–2, or ≥3)—all updated at each follow-up visit—and age at menarche, recorded at baseline (modeled on an ordinal scale: ≤10, 11, 12, 13, or ≥14 years).

Assessment of incident fibroids. Of the 1,655 study participants with HSV-2 serological results, 1,208 (73%) were fibroid-free at baseline and had at least 1 follow-up ultrasound visit with quality ultrasound data. The association between incident fibroids and HSV-2 serostatus was determined using a Cox proportional hazards model (hazard ratios and 95% confidence intervals) with age as the time scale. Only the a priori adjustment factors were included in the final model (time since last use of depot medroxyprogesterone acetate, time since last birth, and income).

Two sensitivity analyses were conducted to reduce the likelihood for misclassification of HSV-2 serostatus due to seroconversion after the baseline HSV-2 assay. First, we restricted the analysis to the first follow-up visit to shorten the time period of potential seroconversion. Second, we restricted the analysis to women aged ≥30 years at baseline

because the prevalence of seroconversion tends to level off after age 30 years (18).

Given that 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 adjusted hazard ratios and 95% confidence intervals for the relationship between HSV-2 and fibroid incidence using a 3-level exposure variable: seronegative, not symptomatic (seropositive with no self-reported diagnosis), and symptomatic (seropositive with a self-reported diagnosis).

Fibroid growth. Fibroid growth was estimated for individual fibroids that could be identified as the same fibroid across 2 successive ultrasounds based on the fibroid's position in the uterus and/or by assessments of the archived ultrasound videos and images by the head sonographer. There were 1,323 growth measurements from 421 women with HSV-2 results available.

We examined the influence of baseline HSV-2 serostatus on fibroid growth over the 3 follow-up intervals using a linear mixed model (the GLIMMIX procedure in SAS 9.4; SAS Institute, Inc., Cary, North Carolina) with a random intercept for participant and fibroid, to account for the potentially correlated growth among fibroids from the same woman and among separate intervals of growth for the same fibroid, as previously described (19). For normalization, we used the natural log transformation of the fibroid volumes, and we modeled the change over the interval of the natural log-volume scaled to 18 months (model outcome). For ease of interpretation, we rescaled the growth estimate back to a volume scale by using the resulting β coefficient from the regression and applying the formula $(\exp(\beta) - 1) \times 100$ to calculate the estimated percent growth difference for the seropositive women compared with the seronegative. As an example, an estimated percent growth difference of 10% indicates that the average volume change for fibroids over the course of 18 months in exposed women was approximately 10% greater than that for fibroids from unexposed women. We adjusted a priori for fibroid-related factors (20): fibroid volume (<0.52, 0.52–4.18, 4.19–14.09, or ≥14.10 cm^3) and number of fibroids on an ordinal scale (1, 2, 3, or ≥4). In addition, only the a priori adjustment factors were included in the final model (age, time since last use of depot medroxyprogesterone acetate, time since last birth, and income).

In 3 sensitivity analyses, we 1) excluded statistical outliers defined as studentized residuals greater than ±3 (19); 2) restricted the analysis to the first follow-up visit; and 3) restricted the analysis to participants aged ≥30 years.

We also conducted the secondary analysis using the 3-level exposure variable (seronegative; seropositive, not symptomatic; or seropositive, symptomatic).

All analyses were carried out with SAS 9.4.

RESULTS

Fibroid incidence

Of the 1,208 women without fibroids at baseline, 577 (48%) were HSV-2–seropositive. Compared with women who were HSV-2–seronegative, those who were seropositive tended to be older (median age, 30 years vs. 28 years), to be current smokers (25% vs. 14%), to have ever used depot medroxyprogesterone acetate (55% vs. 38%), to have given birth (73% vs. 55%), and to have an income less than $20,000/year (54% vs. 40%). A similar proportion of those who were seropositive (24%) developed fibroids as those who were seronegative (25%). In the multivariable model, seropositive women did not have an increased risk of fibroid incidence (adjusted hazard ratio (aHR) = 0.88, 95% confidence interval (CI): 0.69, 1.12) (Table 1). In the sensitivity analysis restricted to the first follow-up visit, the adjusted hazard ratio was suggestive of a protective effect rather than the hypothesized increase in risk (aHR = 0.63, 95% CI: 0.42, 0.93). After restricting the analysis to participants aged ≥30 years, the hazard ratio was closer to the null (aHR = 0.96, 95% CI: 0.70, 1.33). The secondary analysis did not show differences by symptomatology. Compared with the seronegative, seropositive women without symptoms had a hazard ratio of 0.87 (95% CI: 0.68, 1.13) and seropositive women with symptoms had a hazard ratio of 0.93 (95% CI: 0.60, 1.46).

Fibroid growth

Of the 1,323 growth measurements, 601 (45%) were among women who were HSV-2–seropositive. The adjusted estimated percent growth difference was 3.1% (95% CI: −5.8, 13.0) greater for fibroids in HSV-2–seropositive women than for fibroids in seronegative women (Table 2). In the sensitivity analysis that excluded outliers ($n = 16$), there was no change in the estimate (3.2%, 95% CI: −5.2, 12.4). When we restricted the analysis to the first follow-up visit or participants aged ≥30 years at baseline, the adjusted estimated percent growth differences were imprecise but higher than those for the full data set (13.2% (95% CI: −4.5, 34.3) and 5.8% (95% CI: −5.0, 17.8), respectively). In the secondary analysis comparing seropositive women with and without symptoms to seronegative women, the estimated percent growth differences were 9.0% (95% CI: −9.0, 30.5) and 1.7% (95% CI: −7.6, 11.9), respectively.

DISCUSSION

In this prospective study conducted in a community-based sample of young African-American women, we did not find an increase in incidence or growth of fibroids for HSV-2–seropositive women, even among seropositive women who reported a clinical diagnosis. These findings are consistent with our previous cross-sectional analysis of prevalent fibroids in this same population (6), where we found no association between HSV-2 and fibroid presence, size, number, or total volume at study baseline. The sensitivity analysis in the current prospective study, which utilized data from only the first follow-up visit (thus limiting possible exposure misclassification due to postbaseline seroconversion), indicated a possible protective association for HSV-2 seropositivity. However, for women aged ≥30 years, another group expected to have little seroconversion, the hazard ratio

Table 1. Association Between Herpes Simplex Virus Type 2 Serostatus and Incidence of Uterine Fibroids Among 23- to 35-Year-Old African-American Women ($n = 1,208$) With 2,998 Eligible Follow-up Visits Across 3 Follow-up Intervals, Study of Environment, Lifestyle and Fibroids, Detroit, Michigan, 2010–2018

HSV-2–Seropositive	No. of Women	No. of Visits	Incident Fibroids		Unadjusted HR	95% CI	Multivariable-Adjusted HR[a]	95% CI
			No. of Women	%				
No	631	1,553	155	25	1.00	Referent	1.00	Referent
Yes	577	1,445	138	24	0.84	0.67, 1.06	0.88	0.69, 1.12

Abbreviations: CI, confidence interval; HR, hazard ratio; HSV-2, herpes simplex virus type 2.
[a] Adjusted for income, time since last use of depot medroxyprogesterone acetate, and time since last birth anchored at the end of the interval. Age was the time scale.

was null (aHR = 0.96, 95% CI: 0.70, 1.33). The first follow-up data set had small numbers, which can result in higher susceptibility to chance deviations. However, examination in other study populations is needed.

To our knowledge, our study is the first to have investigated the relationship between HSV-2 and fibroids prospectively using clinically valid measures of both the exposure and outcomes. Though we used an enzyme-linked immunosorbent assay (13) to measure HSV-2 and not the Western blot method (the gold standard for serological analysis (9, 21)), this assay has been approved by the Food and Drug Administration, with sensitivity of 96%–100% and specificity of 97%–98% (22, 23) in comparison with Western blot. However, because it can take weeks to months after infection for immunoglobulin G antibodies to be detected, we did not capture women who had very recent infections, which could have led to some misclassification of the exposure. In addition, we only captured HSV-2 seroprevalence, not HSV-1 seroprevalence, thus potentially missing genital infections caused by HSV-1 alone. However, because HSV-1 also causes orolabial infections, which are very prevalent (7), we would not have been able to distinguish those from genital HSV-1 infection (24). Lastly, our sample was a volunteer sample of women. However, they were recruited through numerous community outreach methods, and seroprevalence of HSV-2 in our cohort (47%) was very similar to the seroprevalence of 50% for African-American women aged 14–49 years during a similar time period (2007–2010) (10).

Previous studies have used only cross-sectional data, and the majority relied on self-reported diagnosis of genital herpes as the exposure measurement, which is problematic because of the high prevalence of undiagnosed infection (85% of HSV-2 infections are undiagnosed for African-American women) (10). Our study also had extensive data for addressing confounding. Finally, we were able to investigate fibroid growth, which also showed no association with HSV-2 status.

Overall, based on our prospective findings in a large cohort of young African-American women, HSV-2 seropositivity is unlikely to be an important risk factor for uterine fibroid incidence or growth. This finding is reassuring, given the high seroprevalence of HSV-2 among African-American

Table 2. Association Between Herpes Simplex Virus Type 2 Serostatus and Growth of Uterine Fibroids Among 421 African-American Women With 1,323 Growth Measurements, Study of Environment, Lifestyle and Fibroids, Detroit, Michigan, 2010–2018

HSV-2–Seropositive	No. of Growth Measurements[a]	Estimated Growth Difference Over 18 Months	
		%[b,c]	95% CI
No	722	0	Referent
Yes	601	3.1	−5.8, 13.0

Abbreviations: CI, confidence interval; HSV-2, herpes simplex virus type 2.
[a] For modeling, the growth outcome is the difference in the natural log volume from one visit to the next, scaled to 18 months, but results are converted to the estimated percent growth difference between exposed and unexposed women.
[b] Adjusted for fibroid volume, number of fibroids, age at the beginning of the interval, time since last use of depot medroxyprogesterone acetate, time since last birth anchored at the end of the interval, and income.
[c] An estimated percent growth difference of 3.1% indicates that the average growth (volume change over the course of 18 months) of fibroids among HSV-2–seropositive women was approximately 3.1% greater than that of fibroids among HSV-2–seronegative women.

women and the fact that there is currently no available medication that cures HSV-2 infection.

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Conflict of interest: none declared.

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