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Prenatal diethylstilbestrol (DES) exposure is associated with uterine leiomyoma development

Donna Day Baird[a,*], Retha Newbold[b]

[a] *Epidemiology Branch, A3-05, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, P.O. Box 12233, Research Triangle Park, NC 27709, USA*
[b] *Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC 27709, USA*

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Abstract

Early life exposure to DES causes uterine leiomyomata in laboratory animals. We examined the relationship between prenatal DES exposure and development of uterine leiomyomata in women. Among randomly selected study participants (819 black women, 504 white women), leiomyoma status was determined by ultrasound screening (70%) or surgical record review (7%). We relied on self-report of prior diagnosis in 13%. Leiomyoma status could not be ascertained for 10% and they were excluded from analyses. Prenatal DES exposure was assessed by interview. All five of the black women who reported DES exposure had leiomyomata. Among white women, 76% who reported prenatal DES exposure had leiomyomata compared with 52% of the unexposed (adjusted odds ratio for whites: 2.4; 95% confidence interval CI: 1.1–5.4). Exposed women tended to have larger tumors. Results were robust to sensitivity analyses. Findings support experimental animal data and indicate a role for prenatal estrogen exposure in the etiology of human uterine leiomyoma.

Keywords: Diethylstilbestrol; Prenatal exposure; Uterine leiomyoma; Uterine fibroids; Epidemiology; Environmental estrogens; Endocrine disrupters

1. Introduction

Uterine leiomyomata, commonly called fibroids, are benign tumors that can cause pain, bleeding, infertility and pregnancy complications [1]. They are the leading indication for hysterectomy [2,3]. The tumors are of smooth muscle origin and develop as clonal growths [4]. Incidence increases with premenopausal age, and they regress after menopause [5]. Both estrogen and progesterone have been implicated in their growth, though the mechanisms by which these hormones stimulate tumor development is not understood [6].

In mice, early-life exposure to the exogenous estrogen, diethylstilbestrol (DES), causes uterine leiomyoma development in adult animals [7,8]. Data obtained from our developmentally-exposed DES animal model has shown uterine leiomyomata occurring in ∼9% of treated mice, compared to <1% incidence in unexposed mice. Characterization of these DES fibroids shows similarities with human tumors [8].

The relationship between prenatal DES exposure and uterine fibroid development has not been investigated in humans, though prenatal exposure occurred in an estimated 2–10 million pregnancies in the United States and Europe from the late 1940s to the early 1970s [9]. In 1970, vaginal adenosis and adenocarcinoma were discovered in women who had been prenatally exposed to DES [10]. As a consequence, DES treatment was banned in the U.S. in 1971 and throughout Europe by 1978 [11]. Although DES was used to prevent miscarriage and other complications in high-risk pregnancies (despite a trial showing no efficacy in 1953 [12]), many women with normal pregnancies were also treated [11].

* Corresponding author. Tel.: +1 919 541 2786; fax: +1 919 541 2511.
E-mail address: baird@niehs.nih.gov (D.D. Baird).

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Research has mainly focused on DES-induced epithelial lesions, but smooth muscle cells are also DES targets [13].

We examined the relationship between self-reported prenatal DES exposure and uterine fibroids in women aged 35–49, born during years when DES was being prescribed. Because DES daughters know that they can have reproductive problems, they might initiate more gynecological investigation and thus be more likely than unexposed women to receive a clinical diagnosis of fibroids. To avoid such diagnostic bias, we examined the relationship between prenatal DES exposure and uterine fibroids among randomly selected participants who were screened for fibroids with pelvic ultrasound, regardless of any prior diagnosis of fibroids.

2. Materials and methods

The NIEHS Uterine Fibroid Study was designed to estimate uterine fibroid prevalence. Detailed methods have been described [14]. Briefly, the computerized membership records of a prepaid health plan in Washington, DC, were used to randomly select 35–49 year old women. This age group was selected because it is the age-group most likely to be clinically treated for fibroids, and these women were born during years when DES could have been prescribed. Those selected were screened for eligibility by telephone interview. Eligibility criteria were: (1) the computerized listing had correctly identified a 35–49 year old female with current membership, and (2) a telephone interview could be conducted in English. Eighty-three percent of black and white women who had been identified as eligible agreed to participate. The research was approved by the NIEHS Human Subject's Review Board, and participants gave informed consent.

Premenopausal women were asked to undergo ultrasound screening for fibroids by study-trained sonoagraphers. Medical record review was conducted for women with surgically-induced menopause. If neither ultrasound nor medical record review could be conducted, we accepted self-report of a prior diagnosis. We did not rely on self-report of "no fibroids" because undiagnosed fibroids were common (about half of the undiagnosed women in our study were found to have fibroids at ultrasound screening) [14]. We obtained sonogram data for 76% of participants and medical record data for an additional 7%. We relied on self-report in 9% of black women and 4% of white women. Fibroid status could not be ascertained for 10% of both ethnic groups, so these women were excluded from this analysis.

Prenatal DES exposure was determined by asking women whether their mothers had taken DES while pregnant with them. The data were analyzed as a three-level variable: no, maybe, and yes. Data on potential confounders were collected at clinic visit (body mass index, kg/m^2) or telephone interview (reproductive history, exercise, age of menarche, family history of fibroids).

The relationship between DES exposure and uterine fibroids was evaluated by logistic regression. We first estimated the relative odds for developing fibroids. Then, size of the largest fibroid was considered. The continuous measure of fibroid diameter was not normally distributed, nor did various transformations produce normality. Therefore, we categorized the largest tumor diameter (<4 or 4+ cm), and compared each category as a separate outcome to women without fibroids (CATMOD procedure in SAS, SAS Institute). Black women have a significantly higher risk of fibroids [14], so all analyses were carried out separately for the two ethnic groups.

3. Results

A higher proportion of both black and white women who reported prenatal DES exposure had developed fibroids (Table 1). Large fibroids tended to be more common in those reporting DES exposure. Of unexposed white women, 15% had developed large tumors compared with 26% of the DES-exposed. Among blacks, 32% of the unexposed had developed large fibroids compared with 60% for the DES-exposed. Only five black women reported prenatal exposure; all five (100%) had developed fibroids. Therefore, we proceeded with further multivariate analyses only in white women.

Adjusting for age, the odds ratio (OR) for fibroids associated with DES exposure in white women was 2.4 (95% confidence interval (CI) of 1.1–5.4), and the association was stronger for large fibroids compared with small fibroids (Fig. 1). The estimates were virtually unchanged when we controlled for age of menarche, body mass index, exercise, or participant's mother having had fibroids. Adjusting for number of full term pregnancies delivered at age 25 or older reduced the DES association slightly (OR for any fibroids: 2.1; CI: 0.9, 4.7 and OR for large fibroids: 2.5; CI: 0.9, 6.9).

Table 1
Prenatal DES exposure and fibroid development in black and white women in the NIEHS Uterine Fibroid Study

	Blacks			Whites		
	No fibroids, *n* (%)	Small fibroids, *n* (%)	Large fibroids, *n* (%)	No fibroids, *n* (%)	Small fibroids, *n* (%)	Large fibroids, *n* (%)
Prenatal DES exposure						
No	108 (22)	227 (46)	158 (32)	175 (48)	137 (38)	53 (15)
Maybe	58 (25)	116 (49)	61 (26)	27 (38)	30 (42)	14 (20)
Yes	0 (0)	2 (40)	3 (60)	5 (26)	9 (47)	5 (26)

Small fibroids are under 4 cm in greatest diameter, large fibroids are 4 cm or larger.

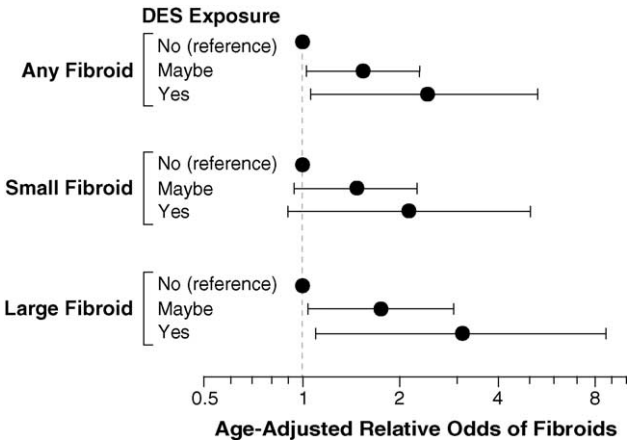

Fig. 1. The age-adjusted relative odds of developing any fibroid, small fibroids (<4 cm in largest diameter), or large fibroids (4+ cm in largest diameter) associated with prenatal exposure to DES for white participants in the NIEHS Uterine Fibroid Study. The horizontal lines show 95% confidence intervals.

We repeated the analyses of white women restricting the sample to those who were premenopausal, since women who had early menopause would have had little opportunity to develop fibroids. With exclusion of the 35 postmenopausal women, the associations were slightly stronger (age-adjusted OR for any fibroid 2.5; CI: 1.1, 5.7 and OR for large fibroids: 4.4; CI: 1.5, 13.1).

Further sensitivity analyses were conducted to evaluate the robustness of the finding. To investigate exposure misclassification, we excluded women who were in the "maybe, DES" group. The age-adjusted OR associated with "yes, DES exposure" changed very little though the confidence intervals were broader with the smaller sample size (OR: 2.1; CI: 0.7, 6.0). To investigate misclassification of fibroid status, we excluded women whose fibroid status was not confirmed by either surgery or sonogram. The relationship between DES and fibroids changed very little (age-adjusted OR for fibroids: 2.3; CI: 1.0–5.2). We also excluded participants who were born to older moms ($\geq$35 years old) because older women may have been more likely to receive DES because of problem pregnancies caused by fibroids. Again, results were very similar, though the confidence interval was larger with the reduced sample size (age-adjusted OR for fibroids: 2.3; CI: 0.9, 6.0).

4. Discussion

The adverse effects of prenatal exposure to DES have been documented in both human and animal studies [15], but, typically, research has focused on DES-induced epithelial lesions. However, alterations in smooth muscle cells following developmental DES treatment have also been reported in numerous laboratory studies [13,16,17]. Furthermore, exogenous administration of estrogens has been associated with the development of uterine leiomyoma in guinea pigs [18] as well as other rodents [7,8]. Despite these reports, there have been no studies investigating benign and malignant smooth muscle changes in prenatally DES-exposed women.

The greatest challenge in studying uterine fibroids is that undiagnosed fibroids are common [19]. Thus, there is likely to be selective over-diagnosis of fibroids among DES daughters because of their known reproductive problems. An ideal study to control for such detection bias would be to use ultrasound to screen for fibroids among DES-exposed and unexposed women from the original clinical trials of DES, such as those in Chicago [12] and Britain [20]. However, most women in these cohorts are now over 50 years of age. Because fibroids can shrink with menopause, the opportunity for conducting such a study will soon be lost.

The NIEHS Uterine Fibroids Study was designed to reduce detection bias by randomly selecting women from a health plan's membership roles and screening for fibroids with a standardized ultrasound protocol. Medical records were sought for women with prior hysterectomies. The study relied on self-report of fibroid status in only 4% of white women and 9% of black women. Our overall results showed increased risk of uterine fibroids in women prenatally exposed to DES, and DES-exposed women tended to have larger fibroids. The number of women who were certain that they were DES daughters was small, but the association with fibroids was robust. When we excluded participants whose diagnosis relied on self-report, the DES effect was essentially unchanged, indicating that our findings cannot be explained by differential detection of fibroids.

DES exposure was based on self-report. Those who said they were exposed are likely to have been exposed, since public discussion about prenatal DES exposure has been broad. There was no evidence that women who knew they had fibroids were especially likely to report DES exposure. Twenty-six percent of white women had been diagnosed with fibroids before the study began and 5.0% of them reported DES exposure compared to 5.2% of those with no prior diagnosis. The group of women in our "maybe" category is likely to include some exposed and some unexposed, but we have no prenatal records of their births to clarify exposure. When we dropped this group, the estimates for the "yes, DES" group changed very little.

Controlling for several potential confounding factors had little impact on the risk estimates associated with DES, with the exception of full term pregnancies, which did reduce the OR from 2.4 to 2.1. Whether it is appropriate to control for this factor is uncertain, given that the adverse developmental effects of DES may affect both fertility and risk of fibroids through a single mechanistic pathway. Therefore, we present both analyses.

There is another possible confounding effect that might account for our findings. Because DES was prescribed for high-risk pregnancies, it is possible that participants who had mothers with fibroids were more likely to be treated with DES, and the increased risk seen in the prenatally exposed

could be merely a reflection of the increased familial risk. We evaluated this in two ways. First, we asked participants whether their mother had ever been diagnosed with fibroids. When we adjusted for this in the multivariate models, the DES association did not substantially change. Secondly, we reasoned that older women would be those most likely to have pregnancies complicated by fibroids that could have been treated with DES. We asked women the age of their mother when she gave birth to them, and limited analysis to women who had been born to mothers who were less than 35. The increased risk of uterine fibroids among DES exposed again remained essentially unchanged. Thus, we found no evidence that our findings could be explained by increased familial risk among DES exposed.

Though members of DES cohorts are aging out of child-rearing, many DES daughters in the general population are still in their reproductive years. Our findings suggest that uterine fibroids should be added to the list of long-term health problems they may experience. In the United States the youngest are in their early 30s, and in Europe, where treatment continued into the 1970s, they are in their late 20s. Our study could not assess whether DES daughters develop fibroids earlier than unexposed, but the finding that DES daughters tended to have larger fibroids suggests that they may have earlier onset or faster growth or both.

The consistency of our findings with data from DES-exposed laboratory animals supports the relevance of animal models for studying fibroid development. Noninvasive treatments to shrink or eliminate fibroids are still limited to gonadotrophin releasing hormone agonists, a treatment that has adverse side-effects. Documentation of commonality between fibroids in experimental animals and humans enhances the prospects that mechanistic research in rodents will shed light on the human disease so that better treatments can be developed.

Though DES treatment during pregnancy has been banned, there are concerns that chemicals in the environment possessing estrogenic or endocrine-disrupting activity may contribute to increased incidence of reproductive problems and various diseases. Although it remains to be shown whether exposure to these chemicals at environmental levels is harmful, the findings support the need for research on a broad range of health outcomes, including uterine fibroids.

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