

Risk of breast cancer in women exposed to diethylstilbestrol *in utero*: preliminary results (United States)

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Abstract

Background: A synthetic estrogen, diethylstilbestrol (DES), was widely prescribed to pregnant women during the 1950s and 1960s but was later discovered to be associated with an increased risk of clear-cell carcinoma of the vagina and cervix in female offspring. DES has not been linked to other cancers in female offspring, but studies of other prenatal factors such as twin gestation and pre-eclampsia have indicated that *in-utero* estrogen levels may influence breast cancer risk. We evaluated the relation of *in-utero* DES exposure to the risk of adult breast cancer.

Methods: A cohort of 4821 exposed women and 2095 unexposed women, most of whom were first identified in the mid-1970s, were followed by mailed questionnaires for an average of 19 years. Reported cancer outcomes were validated by medical record review. Breast cancer incidence in DES-exposed daughters was compared with cancer incidence in unexposed daughters with use of Poisson regression analysis, adjusting for year of birth, age at menarche, age at first birth, and number of births.

Findings: The rate ratio for incidence of invasive breast cancer in exposed versus unexposed women was 1.4 (95% confidence interval (CI) = 0.7–2.6). DES exposure was not associated with an increased risk of breast cancer in women under 40 years, but among women aged 40 and older the rate ratio was 2.5 (95% CI = 1.0–6.3). The rate ratio for the association of DES exposure with estrogen receptor-positive tumors was 1.9 (95% CI = 0.8–4.5).

Interpretation: While not statistically significant, the overall 40% excess risk, arising exclusively from the subset of estrogen receptor-positive cases, raises a concern calling for continued investigation.

Introduction

It has been hypothesized that *in-utero* estrogen exposure may influence later breast cancer risk [1]. Studies designed to evaluate this hypothesis have assessed

several different prenatal factors related to pregnancy estrogen levels: pre-eclampsia/eclampsia, twin pregnancy, maternal age at time of the pregnancy, preterm birth, and birthweight [2]. The most consistent findings are an increased risk associated with having been born of a dizygotic twin pregnancy [3–5] and a reduced risk from a pre-eclamptic or eclamptic pregnancy [3, 6]. These findings have biologic plausibility in that more estrogens are produced in a dizygotic twin pregnancy as compared with a singleton pregnancy [7], and pregnancy estrogen

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levels are lower than normal in pre-eclamptic and eclamptic pregnancies [8]. During the 1950s and 1960s many pregnant US women were given diethylstilbestrol (DES), a synthetic estrogen [9]. DES was initially thought to prevent spontaneous abortion and other pregnancy complications. Women to whom DES was prescribed usually began use during the first trimester and continued daily for several months or until the end of the pregnancy. Thus the offspring of these women are likely to have had *in-utero* exposure to very high levels of estrogens. In 1971 Herbst *et al.* reported a strong association between *in-utero* exposure to DES and the occurrence of vaginal clear-cell carcinoma [10]. Until recently the cohort of women exposed to DES *in-utero* was too young to assess whether their *in-utero* DES exposure also places them at increased risk of breast cancer. We assessed this question in a cohort of exposed and unexposed women who have been followed for an average of 19 years.

Materials and methods

Subjects

A collaborative prospective follow-up study of DES-exposed daughters and unexposed women of the same ages has been in progress since 1992 [11]. The cohort for this study was assembled by combining three existing cohorts: (1) women previously followed in the National Cooperative Diethylstilbestrol Adenosis Project (DESAD) [12]; (2) daughters of women who participated in a randomized clinical trial of DES in 1951–1952 (Dieckmann) [13]; and (3) daughters of women who were treated with DES by an infertility specialist in the Boston, MA area (Horne). In 1994, several hundred women who had never been studied before, but who were the offspring of women who participated in a study of DES-exposed and unexposed mothers (Women's Health Study) [14], were added to the cohort.

The methods of the original studies from which the current cohort has been assembled have been described previously [12–14]. Review of the mother's prenatal record provided documentation of exposure for all exposed participants and review of the mother's prenatal record in combination with the mother's denial of taking DES (or any other hormone) was used to classify participants as unexposed. The three cohorts that provided most of the participants were assembled in the late 1970s when the participants were in their early 20s or teens. Study enrollment was not related to breast cancer risk or to the occurrence of breast cancer. The Women's Health Study daughters were considerably

Table 1. Follow-up information on daughters exposed and unexposed to diethylstilbestrol

	No. exposed (%)	No. unexposed (%)
Total (at start of follow-up in 1978)	4821 (100)	2095 (100)
National Cooperative Diethylstilbestrol Adenosis Project	3922 (81)	1010 (48)
Dieckmann	354 (7)	319 (15)
Horne	286 (6)	221 (11)
Daughters of Women's Health Study participants ^a	259 (5)	545 (26)
Median age at start of follow-up	24	26
Median number of years followed	19	18
Lost to follow-up	859 (18)	335 (16)
Deceased	46 (1)	14 (1)
Responded to 1997 questionnaire	3916 (81)	1746 (83)

^a Followed since 1995 only.

older when invited to participate in 1994 (median age 42) and they contribute follow-up in the present analysis from 1995 forward only.

Numbers of subjects and follow-up data appear in Table 1. As shown, the great majority of subjects derived from the DESAD cohort.

Follow-up

The beginning of follow-up was taken as 1 January 1978 for all participants except for the Women's Health Study daughters, for whom 1 January 1995 was taken as the beginning of follow-up. As shown in Table 1 the median age at start of follow-up was 24 for exposed and 26 for unexposed, and the median number of years followed was 19 for exposed and 18 for unexposed. A detailed questionnaire covering reproductive factors, behavioral factors, and adverse health outcomes was mailed to all cohort members in 1994. In 1997 a shorter follow-up questionnaire that ascertained new occurrences of disease was completed by 3916 (81%) exposed and 1746 (83%) unexposed participants. Most of the non-respondents had been lost or had declined further participation a number of years prior to establishment of the combined cohort in 1994. The National Death Index was used to ascertain breast cancers in participants known to have died and in those lost to follow-up.

A total of 78 cases of breast cancer were reported and pathology reports or death certificates were obtained for 72 of them. The diagnosis was confirmed in all but one instance, and that potential case was excluded. Because of the high confirmation rate (98.6%), the six subjects whose medical records were not obtained were included as cases. Nineteen cases were classified as *in situ* from

pathology data and the rest were considered to be invasive. Laboratory reports of immunohistochemistry stains or protein assays were sought in order to classify invasive cases as estrogen receptor-positive or -negative; information on receptor status was not available for 17 cases. In addition, detailed information on histology, tumor size, and nodal status was unavailable for 11, 15, and 15 cases, respectively.

Approvals for the study were obtained from the human investigations committees at the five field centers and at the National Cancer Institute. Subjects indicated their informed consent by filling out and returning the questionnaire or by taking part in a telephone interview and, if applicable, by signing a medical record release.

Statistical analysis

Person-years at risk for each subject were computed from 1 January 1978 (or 1 January 1995 for Women's Health Study daughters) until the date of first breast cancer diagnosis, date of last known follow-up, date of death, or date of response to the 1997 questionnaire. Rate ratios and their 95% confidence intervals were calculated by means of Poisson regression analysis, adjusting for year of birth, age at menarche, age at first birth, and number of births [15]. Years of education, calendar year of entry, original cohort, duration of oral contraceptive use, family history of breast cancer, menopausal status, and use of hormone replacement therapy were examined as potential confounders but were not included in the final models because they did not materially change the estimates. Covariate information was taken from the most recent questionnaire completed by each individual, except for age at menarche for which the earliest available data were used. Nelson-Aalen cumulative incidence curves were created for the exposed and unexposed [16].

Results

Exposed and unexposed women were similar with regard to race, age at menarche, family history of breast cancer, adult height, use of oral contraceptives, use of hormone replacement therapy, frequency of mammography, and frequency of breast self-examination (Table 2). Exposed women were slightly younger, more highly educated, less likely to be parous, and had a later age at first birth.

There were 83,370 person-years of follow-up among the exposed and 29,224 among the unexposed. Forty-three cases of breast cancer occurred among the exposed and 15 among the unexposed, for an adjusted rate ratio

Table 2. Characteristics of daughters exposed or unexposed to diethylstilbestrol (figures in parentheses are percentages)

Characteristics	Daughters exposed (n = 4821)	Daughters unexposed (n = 2095)
Year of birth		
<1950	747 (15)	478 (23)
1950–1954	2068 (43)	900 (43)
1955–1959	1210 (25)	484 (23)
≥1960	796 (17)	233 (11)
Race		
White	4688 (97)	1945 (93)
Nonwhite	97 (2)	54 (3)
Missing	36 (1)	96 (4)
Education		
≤ High school	536 (11)	375 (18)
Some college	899 (19)	430 (20)
4-year college	1403 (29)	545 (26)
Graduate school	1084 (22)	394 (19)
Missing	899 (19)	351 (17)
Age at menarche (years)		
≤11	745 (15)	323 (15)
12–13	2838 (59)	1152 (55)
≥14	1106 (23)	493 (24)
Missing	132 (3)	127 (6)
Age at first live birth (years)		
≤25	768 (28)	532 (39)
25–29	987 (36)	444 (33)
≥30	993 (36)	390 (29)
Parity		
Nulliparous	1447 (30)	498 (24)
1	676 (14)	255 (12)
2	1175 (24)	635 (30)
≥3	565 (12)	360 (17)
Missing	958 (20)	347 (17)

(RR) of 1.4 (95% confidence interval (CI) = 0.7–2.6) (Table 3). (The RR adjusted for age only was 1.3 (95% CI 0.7–2.5)). The results were similar when we included 19 additional breast tumors that were *in situ* (RR 1.3; 95% CI 0.7–2.1). DES exposure was not associated with an increased incidence of breast cancer in women under age 40 (RR 0.7, 95% CI 0.3–1.7). However, there was a more than twofold increase in breast cancer incidence among women aged 40 and older (RR 2.5, 95% CI 1.0–6.3) (Table 3). As shown in Figure 1, there was no difference in cumulative incidence between exposed and unexposed before age 40, but the curves diverge from 40 to 50, with the exposed curve showing a higher incidence. There was very little person-time of experience above age 50, as indicated in Table 3.

The positive association with DES exposure was stronger for estrogen receptor-positive cancers, with a rate ratio of 1.9 (95% CI 0.8–4.5) (Table 4). Limited information was available from the pathology reports

Table 3. DES exposure in relation to risk of breast cancer

	Exposed		Unexposed		Rate ratio ^a (95% confidence interval)
	Person-years of follow-up	Cases	Person-years of follow-up	Cases	
Entire cohort	83,370	43	29,224	15	1.4 (0.7–2.6)
Age < 40	66,580	16	21,616	8	0.7 (0.3–1.7)
Age ≥ 40	16,790	27	7,254	7	2.5 (1.0–6.3)
Age 40–44	12,085	18	4,792	2	
Age 45–49	4,356	9	2,212	2	
Age ≥ 50	349	0	604	3	

^a Comparing exposed with unexposed daughters, adjusted for year of birth, age at menarche, age at first birth, and parity.

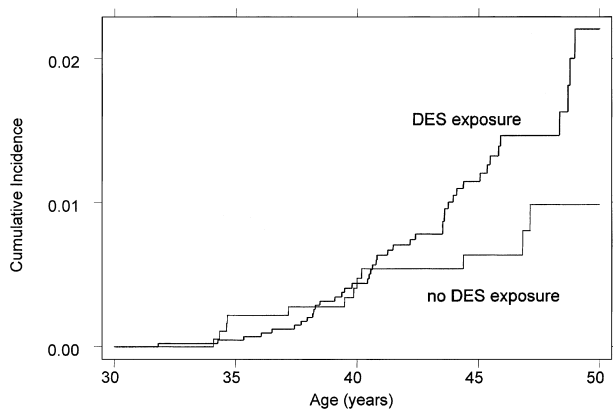


Fig. 1. Cumulative hazard plots of breast cancer incidence in DES-exposed and nonexposed women under age 50.

on other tumor characteristics such as histology, size, and nodal involvement. For tumor size the magnitude of the association was similar for the smaller tumors and for those 2 cm or larger, with rate ratios of 1.1 and 1.5, respectively. The rate ratio for DES exposure in relation to breast cancer with no nodal involvement was 3.6 (95% CI 0.9–17) as compared to a rate ratio of 0.8 (95% CI 0.3–2.1) for metastatic disease.

It was not possible to assess the risk of breast cancer according to cumulative dose of exposure to DES because of incomplete dose information on the majority of study subjects [12]. However, information on timing of first exposure was available for over two-thirds of subjects. Results were similar for exposures that began during the first trimester and for exposures that began later in pregnancy (Table 5). There was a deficit of cases among women who began use before the 9th week of gestation.

Discussion

The present results suggest that *in-utero* exposure to DES may lead to an increased risk of breast cancer, but

Table 4. DES exposure in relation to risk of breast cancer, according to estrogen receptor status, histology, tumor size, and nodal involvement

	Number of cases		Rate ratio ^a (95% confidence interval)
	Exposed	Unexposed	
Estrogen receptor			
Positive	26	8	1.9 (0.8–4.5)
Negative	4	3	0.4 (0.1–1.9)
Unknown	13	4	1.6 (0.5–5.2)
Histology			
Ductal	34	11	1.6 (0.8–3.2)
Lobular	0	1	–
Both	1	0	–
Unknown	8	3	1.4 (0.3–6.0)
Tumor size			
<2 cm	14	5	1.1 (0.4–3.0)
≥2 cm	14	6	1.5 (0.5–4.2)
Unknown	15	4	2.0 (0.6–6.3)
Positive nodes			
None	19	3	3.6 (0.9–17)
≥1	10	7	0.8 (0.3–2.1)
Unknown	14	5	1.3 (0.5–3.9)

^a Comparing exposed with unexposed daughters, adjusted for year of birth, age at menarche, age at first birth, and parity.

Table 5. Timing of first exposure to DES in relation to risk of breast cancer

Week of gestation of first exposure	Person-years of follow-up	No. of cases	Rate ratio ^a (95% confidence interval)
Unexposed	29,224	15	Reference
≤8 weeks	26,195	1	1.2 (0.5–2.5)
9–12 weeks	18,773	15	
≥13 weeks	20,814	12	
Unknown	17,586	15	1.9 (0.9–4.2)

^a Comparing exposed with unexposed daughters, adjusted for year of birth, age at menarche, age at first birth, and parity.

the data are not definitive. The overall estimate of association is 1.4 and is not statistically significant. Data on tumor size and nodal involvement provide no indication that DES exposure leads either to a higher grade or to more advanced disease. Timing of exposure appears to be unrelated to risk. On the other hand, the overall rate ratio of 1.4 was higher than the estimate of 1.2 obtained in an earlier analysis based on about 50% fewer cases [11]. In addition, there is now a statistically significant association of DES exposure with risk of breast cancer at ages 40 and older, with a rate ratio of 2.5. From ages 40 through 50 the cumulative incidence curve for exposed women increases at a faster rate than that among unexposed. There is also a suggestion that DES exposure may be associated with tumors that are estrogen receptor-positive.

Experimental studies have shown that certain proto-oncogenes associated with mitosis or mitogenic control are persistently overexpressed following neonatal exposure to DES [17]. Growth factor genes, such as transforming growth factor- α and epidermal growth factor, have also been shown to be overexpressed following exposure to DES [17]. This overexpression may result in altered tissue responsiveness to hormones, either during puberty or later in life.

Observational studies have used a number of prenatal or perinatal factors as markers for *in-utero* estrogen exposure. Several studies have linked twin pregnancy [3–5], older maternal age [3, 18–22], severe prematurity [3, 18], and high birthweight [18, 23, 24] (possible markers of relatively high estrogen levels) to an increased risk of breast cancer, and pre-eclampsia [3, 6] (a marker of lower than normal estrogen levels) with a reduced risk of breast cancer. Although some of these associations have been observed in all age groups [5, 6, 20, 22], many of the associations of prenatal factors with breast cancer risk have arisen in studies of very young women [18] or have been observed only in the subset of study subjects in the youngest age groups [23, 25, 26]. In contrast, a positive association was observed among women aged 40–50 but not among women under 40 in the present study.

Two case-control studies have reported on the relation of breast cancer risk to self-reported *in-utero* exposure to DES or other hormones [5, 23]. Neither observed an association. However, one should suspect substantial misclassification of exposure in both studies since many women do not know whether or not their mothers took medications during pregnancy.

The present study has several limitations. Because the median age of the cohort at the end of the current follow-up (1997) was only 43, the expected incidence of breast cancer is still quite low. Thus there was not

adequate power to detect relative risks lower than two, and one should view the elevated relative risks for breast cancer overall and for estrogen receptor-positive tumors as preliminary findings. Additional follow-up as the cohort ages will undoubtedly provide a sufficient number of cases for a more definitive analysis.

Follow-up was complete for approximately equal proportions of exposed and unexposed participants (about 83%) and most of the loss-to-follow-up occurred more than ten years ago and thus is probably unrelated to breast cancer risk. Due to the initial selection processes, exposed and unexposed participants were similar in most respects, except for parity, age at first birth, and education.

If exposed women undergo more rigorous screening for breast cancer, resulting in earlier detection of tumors, this could lead to a spurious positive association. Our data on frequency of mammography and breast self-exam do not support this explanation, however. Exposed and unexposed cohorts reported similar frequencies of breast self-exam and mammography in the past several years.

In conclusion, further follow-up of DES-exposed women is imperative in order to establish whether there is a causal association with breast cancer risk and to assess the hypothesis raised by the present data; namely, that *in-utero* DES exposure is related to increased risk of estrogen receptor-positive disease. The cohort of US women exposed to DES in the 1950s and 1960s has now reached the age at which breast cancer incidence is appreciable. Receptor testing currently occurs more routinely, and it should be possible to determine receptor status for almost all new cases. In the meantime it behooves DES-exposed women to follow the advice given all women aged 40 and older: undergo screening mammography every 1–2 years, and perform breast self-exam regularly [27].

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References

1. Trichopoulos D (1990) Does breast cancer originate *in utero*? *Lancet* **335**: 939–940.
2. Potischman N, Troisi R (1999) *In-utero* and early life exposures in relation to risk of breast cancer. *Cancer Causes Control* **10**: 561–573.
3. Ekblom A, Hsieh C-C, Lipworth L, Adami H-O, Trichopoulos D (1997) Intrauterine environment and breast cancer risk in women: a population-based study. *J Natl Cancer Inst* **89**: 71–76.
4. Hsieh C-C, Lan S-J, Ekblom A, Petridou E, Adami H-O, Trichopoulos D (1992) Twin membership and breast cancer risk. *Am J Epidemiol* **136**: 1321–1326.
5. Weiss HA, Potischman NA, Brinton LA, et al. (1997) Prenatal and perinatal risk factors for breast cancer in young women. *Epidemiology* **8**: 181–187.
6. Ekblom A, Trichopoulos D, Adami H-O, Hsieh C-C, Lan S-J (1992) Evidence of prenatal influences on breast cancer risk. *Lancet* **340**: 1015–1018.
7. Thomas HV, Murphy MF, Key TJ, Fentiman IS, Allen DS, Kinlen LJ (1998) Pregnancy and menstrual hormone levels in mothers of twins compared to mothers of singletons. *Ann Hum Biol* **25**: 69–75.
8. Gargoff L, Seppala M (1976) Toxemia of pregnancy: assessment of fetal distress by urinary estriol and circulating human placental lactogen and alpha-fetoprotein levels. *Am J Obstet Gynecol* **126**: 1027–1033.
9. Noller KL, Fish CR (1974) Diethylstilbestrol usage: its interesting past, important present, and questionable future. *Med Clin N Am* **58**: 739–810.
10. Herbst AL, Ulfelder H, Poskanzer DC (1971) Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* **284**: 878–881.
11. Hatch EE, Palmer JR, Titus-Ernstoff L, et al. (1998) Cancer risk in women exposed to diethylstilbestrol *in utero*. *JAMA* **280**: 630–634.
12. Labarthe D, Adam E, Noller KL, et al. (1978) Design and preliminary observations of the National Cooperative Diethylstilbestrol Adenosis (DESAD) Project. *Obstet Gynecol* **51**: 453–458.
13. Bibbo M, Gill WB, Azizi F, et al. (1977) Follow-up study of male and female offspring of DES-exposed mothers. *Obstet Gynecol* **49**: 1–8.
14. Greenberg ER, Barnes AB, Resseguie L, et al. (1984) Breast cancer in mothers given diethylstilbestrol in pregnancy. *N Engl J Med* **311**: 1393–1398.
15. Breslow NE, Day NE. *Statistical Methods in Cancer Research, II: Design and Analysis of Cohort Studies*. IARC Scientific Publication 88. Lyon: International Agency for Research on Cancer; 1987.
16. Nelson W (1972) Theory and application of hazard plotting for censored failure data. *Technometrics* **14**: 945–965.
17. McLachlan JA. The role of experimental model systems in the study of DES-associated malignancy. NIH Workshop Long-Term Effects of Exposure to Diethylstilbestrol (DES) 1992.
18. Innes K, Byers T, Schymura M (2000) Birth characteristics and risk for breast cancer in very young women. *Am J Epidemiol* **52**: 1121–1128.
19. Janerich DT, Hayden CL, Thompson WD, et al. (1989) Epidemiologic evidence of perinatal influence in the etiology of adult cancers. *J Clin Epidemiol* **42**: 151–157.
20. Zhang Y, Cupples LA, Rosenberg L, et al. (1995) Parental ages at birth in relation to a daughter's risk of breast cancer among female participants in the Framingham Study (United States). *Cancer Causes Control* **6**: 23–29.
21. Rothman KJ, MacMahon B, Lin TM, et al. (1980) Maternal age and birth rank of women with breast cancer. *J Natl Cancer Inst* **65**: 719–722.
22. Thompson WD, Janerich DT (1990) Maternal age at birth and risk of breast cancer in daughters. *Epidemiology* **1**: 101–106.
23. Sanderson M, Williams M, Malone K, et al. (1996) Perinatal factors and risk of breast cancer. *Epidemiology* **7**: 34–37.
24. Michels K, Trichopoulos D, Robins J, et al. (1996) Birthweight as a risk factor for breast cancer. *Lancet* **348**: 1542–1546.
25. Braun MM, Ahlbom A, Floderus B, Brinton LA, Hoover RN (1995) Effect of twinship on incidence of cancer of the testis, breast, and other sites (Sweden). *Cancer Causes Control* **6**: 519–524.
26. Le Marchand L, Kolonel L, Myers B, et al. (1988) Birth characteristics of premenopausal women with breast cancer. *Br J Cancer* **57**: 437–439.
27. NCI adopts New Mammography Screening Guidelines for Women (1997) *J Natl Cancer Inst* **89**: 538–539.