

Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States)

E. E. Hatch^{1,*}, A. L. Herbst², R. N. Hoover¹, K. L. Noller³, E. Adam⁴, R. H. Kaufman⁴, J. R. Palmer⁵, L. Titus-Ernstoff⁶, M. Hyer⁷, P. Hartge¹ & S. J. Robboy⁸

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA; ²Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, USA; ³Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, USA; ⁴Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; ⁵Slone Epidemiology Unit, Boston University School of Public Health, Boston, MA, USA; ⁶Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA; ⁷Information Management Services, Rockville, MD, USA; ⁸Departments of Pathology and Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, USA

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Abstract

Objectives: Women exposed prenatally to diethylstilbestrol (DES) have an excess risk of clear-cell adenocarcinoma of the vagina and cervix, but the effect on the incidence of squamous neoplasia is uncertain. The purpose of the current study was to evaluate the long-term risk of developing high-grade squamous neoplasia of the genital tract among women exposed prenatally to DES.

Methods: A cohort comprising 3899 DES-exposed and 1374 unexposed daughters was followed for 13 years (1982–1995) for pathology-confirmed diagnoses of high-grade squamous intraepithelial neoplasia (HSIL) of the genital tract. Poisson regression analysis was used to compute relative risks (RR) and 95% confidence intervals (95% CI), adjusting for age, calendar year, and other covariates.

Results: The RR (95% CI) among DES-exposed versus unexposed, based on 111 cases of high-grade disease, was 2.1 (1.2–3.8). Adjustment for screening history estimated by the number of years since the last Pap smear had little effect. Risk estimates were higher with earlier intrauterine exposure; the RR (95% CI) for exposure within 7 weeks of the last menstrual period was 2.8 (1.4–5.5). Only two cases of invasive squamous cervical cancer occurred in total, precluding separate analysis.

Conclusions: The findings support an association between *in-utero* DES exposure and high-grade squamous neoplasia, although a role for more intensive screening among DES-exposed women in the production of this excess could not be completely ruled out.

Introduction

The association between vaginal and cervical clear-cell adenocarcinoma in young women and their *in-utero* exposure to diethylstilbestrol (DES) was first described nearly 30 years ago [1]. Subsequently, other benign pathological findings were found in these women,

including an increased prevalence of glandular cells in the vagina (adenosis), or exocervix (ectropion) [2, 3]. The cervical transformation zone (the area of columnar epithelium that has been replaced by metaplastic squamous epithelium) was also generally wider among DES-exposed women.

A more controversial and as yet unsettled question is whether DES exposure is related to an increased risk of squamous cell neoplasia in the cervix and vagina. In the past, several investigators predicted that an epidemic of squamous cell carcinomas would occur later in DES daughters [4]. Subsequently, the National Cooperative

* Address correspondence to: Elizabeth E. Hatch, PhD, Boston University School of Public Health, Department of Epidemiology and Biostatistics, 715 Albany Street T3E, Boston, MA02118-2526, USA (current address); e-mail address: eehatch@bu.edu

Diethylstilbestrol and Adenosis (DESAD) study was initiated to determine the incidence and prevalence of cervical and vaginal neoplasia in a rigorously constructed large cohort of DES-exposed and unexposed women over a 9-year period beginning in the mid-1970s. This study did not find a higher prevalence of squamous neoplasia among exposed daughters at the first screening examination (5). However, a later follow-up study of incident cases over 7 years found a nearly 2-fold increased risk of squamous disease through 1981 [6]. A recent report from the Netherlands found a 3-fold increased risk of invasive cervical cancer among DES-exposed daughters, but the study was based on small numbers and may have been subject to selection biases [7]. The primary objective of the current study is to determine whether there is a long-term increase in risk of high-grade squamous neoplasia in DES-exposed compared to unexposed daughters.

Materials and methods

Subjects

Three previously studied cohorts are included in this combined follow-up study [8] (Table 1). The largest consists of both exposed and unexposed women who were enrolled in five medical centers during the mid-1970s as part of the DESAD study [9]. Approximately half of the exposed research participants were originally identified by review of maternal obstetric records at each of the five centers. Whenever a prescription for DES was

noted in the chart, the date and dose were recorded. The remainder of the DES-exposed group was referred by physicians or was self-referred, but was required to have written documentation of prenatal exposure. Among the 1033 unexposed women originally included in the DESAD cohort, 245 (24%) were sisters of the record review exposed women. The remainder was identified by prenatal record review from the same medical record sources as the record review exposed and was matched by year of birth (± 6 months) and age of the mother (± 5 years) to the exposed. Unexposed women were not exposed to any exogenous hormones during the prenatal period.

Between 1975 and 1983, yearly gynecological examinations, including routine Pap smears, colposcopy, and biopsies of suspicious lesions, were conducted on both the exposed and unexposed women, with identical screening protocols used for both. From 1984 to 1989, DESAD cohort members were mailed yearly questionnaires, and medical records and pathology reports were collected for cancers and gynecologic neoplasia reported during this time period.

The second group, the "Dieckmann cohort", includes 610 female offspring whose mothers participated in a clinical trial of the efficacy of DES to prevent pregnancy complications in the early 1950s [10]. During the 1970s, attempts were made to trace all women in this cohort, and 338 exposed (83% of 408 live-born) and 298 unexposed (77% of 388 live-born) responded to at least one questionnaire [11]. Clinical examinations were not regularly performed by the DES investigators in this group. Prior to the current combined cohort study, the

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

	Exposed	Unexposed	Total
Number of subjects identified	4698	1637	6335
<i>Excluded from analysis</i>	799	263	1062
Lost to follow-up or deceased before 1 January 1982	251	141	392
Diagnosis of grade 2 or greater before 1 January 1982 ^a	147	22	169
Hysterectomy before 1 January 1982	45	13	58
Treatment before 1 January 1982 ^b	356	87	443
Total subjects analyzed	3899	1374	5273
<i>Cohort</i>			
DESAD	3299	869	4168
Dieckmann	323	287	610
Horne	277	218	495
Responded to 1994 questionnaire	3222	1102	4324
Lost to follow-up (after 1 January 1982)	659	267	926
Deceased (after 1 January 1982)	18	5	23

^a Diagnoses were confirmed by pathology report.

^b Treatments included conization, hot and cold cautery, and multiple biopsies documented by medical records that were considered treatment in the original DESAD study; treatment information was available for the DESAD cohort.

last routine follow-up of the Dieckmann group consisted of a mailed questionnaire in 1990 [12].

The third group, the "Horne cohort", consists of female offspring born to women with infertility problems who were treated with DES by an infertility specialist in the Boston area between the years 1952 and 1972. The Horne cohort was reassembled for follow-up along with unexposed siblings in the mid-1970s, and mailed yearly questionnaires through the 1980s. No attempt was made to systematically examine members of this cohort.

All exposed women in these three cohorts had documented exposure to DES during gestation. The Dieckmann and Horne cohorts were exposed to high doses (total 10–12 g), following the protocol recommended by Smith and Smith [13], while the DESAD cohort, on average, was exposed to lower and more variable doses [9].

Exclusions

Because prior treatment of the cervix may lower the subsequent incidence of intraepithelial neoplasia, women with documented high-grade neoplasia (intraepithelial neoplasia grade 2 or higher) ($n = 169$) before 1982 were excluded at baseline from the main analyses (Table 1). This encompassed qualifying cases of both squamous and glandular neoplasia. In addition, 443 women who were treated with conization and/or hot or cold cautery of the cervix or excision of the vagina, and 58 women who had a hysterectomy prior to 1982, were excluded. Information from patient history and medical record review was used to ascertain prior history of diagnoses and treatments, and all women excluded due to earlier high-grade neoplasia had their diagnoses confirmed by pathology report.

Follow-up

In 1994, study subjects were sent a detailed health history questionnaire that included questions on the diagnosis of neoplasia, and the occurrence of biopsies, including dates, and names and addresses of physicians. When a participant reported biopsy-confirmed, genital-tract neoplasia of any grade (including human papilloma virus (HPV) infection), attempts were made to obtain the pathology report. Slides were also requested for pathology diagnoses of intraepithelial neoplasia grade 2 and above, and were reviewed by one pathologist (S.J.R), blinded as to DES-exposure status.

Approvals for the study were obtained from the human investigations committees at the five field centers and the National Cancer Institute. Participants indicat-

ed informed consent by filling out and returning the questionnaire and signing a medical record release form, if applicable.

A small proportion of subjects who had participated in prior studies were not mailed a questionnaire during the 1994 follow-up, either because they could not be traced or because of their unwillingness to participate during previous follow-up attempts. Among women mailed a questionnaire, response rates were 88% for both exposed and unexposed women. Approximately two-thirds of the questionnaires were returned by mail (after two mailings), and one-third was administered by trained telephone interviewers. The overall follow-up rate from 1982 through the end of the 1994 data collection for those included in this analysis was 83.1% for exposed and 80.6% for unexposed participants.

Statistical analysis

The analyses focused on the first occurrence of high-grade squamous intraepithelial lesions (HSIL). This included all instances of intraepithelial neoplasia grades 2 or 3 of the cervix, vagina, or vulva diagnosed by biopsy and confirmed by pathology report between 1 January 1982 and 30 June 1995. For purposes of this analysis the two cases of invasive cervical cancer occurring in the study population were included with the grade 3 intraepithelial cases. A separate analysis was conducted for the cases that had centralized, blinded slide review. Person-years at risk for each woman were computed from 1 January 1982 until the date of first documented diagnosis of HSIL, date of hysterectomy or surgery involving the cervix, date of last known follow-up, or date of last questionnaire response. The start of follow-up was chosen to correspond to the end of follow-up in the previous study of incident dysplasia among the DESAD cohort [6], which comprises 80% of the current study population. Poisson regression analysis was used to estimate the RR of neoplasia in DES-exposed versus unexposed women, controlling for age, calendar year, and potential confounding variables [14].

Covariates included year of birth, year of diagnosis or last follow-up, education, age at first intercourse, number of different sexual partners, smoking, and history of cervical cancer screening. Data from original study records from the DESAD and Dieckmann cohorts on first exposure to DES during gestation were available for more than 80% of the cohorts and were analyzed; data on timing of exposure for the Horne cohort were not analyzed because of the large proportion of subjects in this cohort who showed missing data on this variable. Information on total dose received during the pregnancy was not analyzed because it was often incomplete among

all three cohorts. Information on covariates was obtained from previously recorded data and from the 1994 questionnaire. Detailed history of cervical cancer screening was available only for the DESAD cohort. In 1982 and 1983 many members of the DESAD cohort still participated in yearly screening examinations. Between 1984 and 1989 the women were mailed an annual questionnaire that covered their screening history in addition to disease outcomes, and more than 90% completed these questionnaires each year. Women were asked each year whether they had a Pap smear during the previous year, in addition to questions on colposcopy, general physical examinations, and breast cancer screening. These data were used to create a time-dependent variable for the number of years since the last known Pap smear for this cohort. For the women in the Dieckmann and Horne cohorts the only information on screening history was collected during the 1994 questionnaire that asked about the frequency of Pap smears and colposcopy during the previous 5 years. Because these data in many cases would refer to post-diagnostic frequency of screening, control for cervical cancer screening was attempted only for the DESAD cohort.

The time of first exposure to DES was examined both as a categorical and a continuous variable. For the categorical variable cutpoints for analysis were chosen based on approximate quartiles of the gestational week of first exposure among exposed subjects. For the continuous variable the week of first exposure was entered directly into the model, and the significance was tested using the likelihood ratio test. Trend tests were computed both with and without the non-exposed subjects in the model [14].

All analyses were conducted among the combined cohort, and in the three cohorts separately. The primary analysis used all cases confirmed by pathology report, but the subset of cases that had central pathology review was also analyzed. The RR of HSIL associated with DES within strata of age, calendar year, sexual history, smoking, educational level, and frequency of reported Pap smears and colposcopies was also evaluated.

Additional subgroup analyses were done in the DESAD group for women originally identified by prenatal record review, and for those who were referred to the study. An additional analysis excluded women with a pathology-confirmed diagnosis of mild dysplasia, but no recorded treatment, before 1982.

Results

Questionnaire responses relating to established cervical cancer risk factors were compared for DES-exposed and unexposed women. DES-exposed women were slightly

younger (median age at last follow-up was 38 years for exposed and 39 for unexposed), had higher levels of education, and were more likely to be currently married than the unexposed participants (Table 2). DES-exposed women tended to have initial sexual intercourse at a slightly older age than unexposed women ($p = 0.06$), but reported similar numbers of male sexual partners. DES-exposed women were less likely ever to have smoked, and were more likely to report frequent Pap smears and to have had colposcopy within the past

Table 2. Distribution^a of selected covariates, DES-exposed vs. unexposed daughters

	No. exposed (%)	No. unexposed (%)
Year of birth		
<1950	489 (12.5)	212 (15.4)
1950–1954	1627 (41.7)	633 (46.1)
1955–1959	1020 (26.2)	323 (23.5)
1960+	763 (19.6)	206 (15.0)
Race		
White	3717 (97.9)	1245 (97.3)
Non-white	78 (2.1)	35 (2.7)
Marital status		
Married	2279 (71.5)	722 (66.7)
Single, divorced, widowed	908 (28.5)	361 (33.3)
Highest level of education		
≤High school	399 (12.4)	208 (18.9)
Technical School/ < 4 years college	701 (21.8)	230 (20.9)
4 Years college	1187 (36.9)	371 (33.8)
Graduate school	928 (28.9)	290 (26.4)
Ever smoked	1606 (43.2)	597 (49.2)
Age at first intercourse		
≤17	925 (27.0)	350 (30.6)
18–19	1167 (34.1)	374 (32.7)
20+	1330 (38.9)	419 (36.7)
Number of male partners		
1	833 (23.1)	253 (21.5)
2–4	903 (25.0)	289 (24.6)
5–9	746 (20.7)	271 (23.0)
10+	1130 (31.3)	363 (30.9)
Number of pap smears, 1990–1994		
≤3	963 (30.0)	427 (39.5)
4–5	1535 (47.9)	541 (50.1)
5+	709 (22.1)	113 (10.5)
Number of colposcopies, 1990–1994		
None	1605 (54.0)	740 (76.8)
One or more	1370 (46.0)	223 (23.2)
Gestational age of first exposure (no. of weeks since LMP) ^b		
≤7 weeks	782 (26.9)	
8–10 weeks	829 (28.5)	
11–14 weeks	576 (19.8)	
15+ weeks	719 (24.7)	

^a Missing values are excluded from the table.

^b Information on timing of DES exposure during gestation from DESAD and Dieckmann cohorts only.

5 years. A total of 80.2% of the DESAD and Dieckmann cohorts had information on the date of first exposure to DES during gestation. Twenty-seven percent of the women were exposed within 7 weeks of the last menstrual period, and 25% were exposed after 15 weeks.

A total of 834 women (23.1% of exposed and 8.3% of unexposed who answered the 1994 questionnaire) reported that they had ever had a diagnosis of intraepithelial neoplasia confirmed by biopsy (Table 3). Records were obtained for 665 (79.6%) of these reported diagnoses, but could not be obtained for 169 reported cases because of lack of consent to retrieve medical records from the patient, because the report could not be found, or for other reasons. A total of 124 cases of high-grade disease were confirmed among the reports that were obtained. Thirteen were downgraded to less than grade 2 after central review and were not treated as cases, leaving a total of 111 pathology-confirmed cases, including five vaginal and one vulvar case, for analysis. Two cases of invasive squamous cell cervical cancer were included among the 111 cases. Among the 665 women for whom medical records were obtained, 43% of reported intraepithelial neoplasias (including grade 1) were confirmed. Exposed women were approximately 1.6 times more likely to misreport any diagnosis of intraepithelial neoplasia than were unexposed women. The remaining 380 women (57% of those with records obtained) had "no suitable pathology" recorded on the study abstract form. Information was not collected on the exact diagnosis for all of these women, but a random sample of records for 114 women from each of the three cohorts was examined in detail. The majority (85%) had

had either cytology (29%) and/or a biopsy (64%). Multiple diagnoses often appeared on the reports, the most common being squamous metaplasia (55%), inflammation (42%), ASCUS (9%), and normal or benign changes (11%).

Slides were reviewed by one pathologist (S.J.R) for 95 (77%) of the 124 pathology-confirmed cases of high-grade disease. Agreement between pathology report diagnoses and the central review was good. Overall, 82 (86%) of the slides classified as grade 2 and above by pathology report were classified as such by central review (Table 4). A total of 13 cases (14%) which were diagnosed as grade 2 or higher by pathology report were rated as either non-dysplastic ($n=4$) or grade 1 ($n=9$) by the study pathologist. There was little difference in cases downgraded according to DES-exposure status (13.4% in exposed and 15.4% in unexposed were downgraded to be non-cases). Five of the pathology-confirmed cases originated in the vagina, and the remainder originated in the cervix.

The results were examined for known cervical cancer risk factors and were consistent with previous reports. Moderate and non-significant increased risks were found for smoking (RR for ever smoked = 1.3; 95% CI = 0.86–1.9), and younger age at first intercourse (RR for ≤ 17 vs. $20+$ = 1.3; 95% CI = 0.80–2.1), while a strong association was seen for number of male partners (RR for 10 or more vs. 1 = 6.6; 95% CI = 2.8–15.4). Risks tended to decrease with age and were lower for women whose education extended past high school.

Overall, the RR of high-grade disease, diagnosed by pathology report, was 2.1 (95% CI = 1.2–3.5) comparing

Table 3. Results of medical record collection for reported intraepithelial neoplasias among 1994 questionnaire respondents

	Exposed (n = 3222)		Unexposed (n = 1102)	
	Number	Percentage	Number	Percentage
Responded yes to IN ^a	743	23.1 ^b	91	8.3 ^b
Confirmed cases (grade 2+) ^{c,d}	91	12.1	14	15.4
Pathology-confirmed cases downgraded to < grade 2 after slide review	11	1.5	2	2.2
Confirmed cases: glandular (adeno) IN/ cancer (grade 2+)	5	0.7	1	1.1
Confirmed grade 1 (mild IN)	137	18.4	24	26.4
No IN	356	47.9	24	26.4
Unable to obtain pathology report	143	19.4	26	28.6

^a IN = Intraepithelial neoplasia.

^b Percent based on total number of exposed and unexposed who answered 1994 questionnaire; remaining percents in table based on number of respondents who responded affirmatively to question on IN.

^c Confirmed by pathology report.

^d Six additional cases of high-grade disease included in the analysis (four exposed; two unexposed) which were identified in nonrespondents from previously recorded data for a total of 124 confirmed grade 2+ cases confirmed by pathology report; after slide review 111 cases remained in the analysis.

Table 4. Agreement between pathology report diagnoses and centralized, blinded slide review

Central slide review diagnosis	Original pathology report diagnosis ^a		
	Grade 2	Grade 3+	Invasive
No intraepithelial neoplasia	3	1	
Grade 1	9		
Grade 2	23	12	
Grade 3	8	38	
Invasive			1

^a Slides were not reviewed for pathology report diagnoses of grade 1 (mild) intraepithelial neoplasia.

Table 5. Relative risk of high-grade squamous neoplasia in DES-exposed and unexposed daughters, total combined cohorts, and DESAD, Dieckmann and Horne cohorts, 1982-1995

	No. of exposed cases ^a	No. of unexposed cases ^a	Adjusted RR (95% CI) ^{b,c}
Combined Cohorts	95 (71)	16 (11)	2.1 (1.2-3.8)
Person-years	42,299	14,791	
DESAD	68 (49)	11 (6)	1.7 (0.9-3.4)
Person-years	35,856	9408	
Dieckmann	15 (10)	5 (5)	2.0 (0.6-6.7)
Person-years	3460	3087	
Horne	12 (12)	0	∞ (2.41, ∞)
Person-years	2982	2296	

^a Adjusted for age and calendar year, age at first intercourse, number of male partners, education, smoking (model in total combined cohort adjusted for original cohort also).

^b Case definition based on pathology report diagnoses.

^c Numbers in parentheses are number of cases with central review.

DES-exposed to unexposed in the combined cohorts. Adjustment for other covariates had little effect (RR = 2.1 (95% CI = 1.2-3.8)) (Table 5). When the analysis was restricted to the 82 cases of high-grade disease that were centrally reviewed, the RR was increased (RR = 2.6; 95% CI = 1.3-5.1).

The RR of high-grade disease was elevated within each of the three cohorts, but was somewhat lower in DESAD (RR = 1.7) than the other two cohorts (Table 5). When the analysis excluded women who had documented mild dysplasia (but no treatment recorded) prior to 1982, the RR in DESAD increased somewhat (RR = 2.1; 95% CI = 1.0-4.3). Also, restriction of the analysis to DESAD cases with slide review increased the estimate (RR = 2.5; 95% CI = 1.0-5.9). Twelve cases of high-grade disease, all confirmed with slide review, were found in DES-exposed Horne women, and none in their

Table 6. Relative risk of high-grade squamous neoplasia in the DESAD cohort, with adjustment for level of cervical cancer screening

	No. of exposed cases	No. of unexposed cases	Adjusted RR (95% CI) ^a
Total DESAD	68	11	1.6 (0.82-3.0)
Record review subjects only	31	11	1.6 (0.80-3.2)
Referred subjects	37	11	1.4 (0.70-2.7)

^a Adjusted for age and calendar year of diagnosis and number of years since last known Pap smear as a time-dependent covariate.

unexposed siblings. If the Horne cohort is eliminated from the analysis, the RR for high-grade disease was 1.8 (95% CI = 1.0-3.2) based on all the pathology-confirmed cases, and 2.1 (95% CI = 1.0-4.2), based on cases with central review.

Adjustment for level of cervical cancer screening in the DESAD cohort resulted in little change in the point estimates of the RR (1.6) (Table 6). The RRs for women originally identified by prenatal record review and those referred to the study were also comparable.

There was little evidence that other covariates modified the effect of DES on high-grade disease. The effect estimates for DES were somewhat higher for cases diagnosed after 1990 than before RR for cases \leq 1989 = 1.5 (95% CI = 0.8-2.7); RR for cases diagnosed after 1990 = 4.7 (95% CI = 1.4-15.3), but this was based on only three cases among the unexposed after 1990. The RRs according to age at diagnosis were not significantly different (RR = ∞ , for \leq 25; RR = 2.2 (95% CI = 0.65-7.2) for 25-29; RR = 1.1 (95% CI = 0.54-2.4) for 30-34; RR = 2.2 (95% CI = 0.66-7.6) for 35-39; and RR = 5.1 (95% CI = 0.65-39.3) for 40 and older). The effect of DES increased somewhat with the number of sexual partners, from 1.28 in women with one partner, to 1.65 in women with two to four partners, 3.44 in women with five to nine partners, and 2.17 in women with 10 or more partners, but the interaction was not statistically significant according to the likelihood ratio test.

There was a trend in risk of cervical dysplasia according to gestational age at first exposure to DES (Table 7). Fetuses exposed earlier were at greater risk for the subsequent development of high-grade disease. After adjustment for covariates the RR was 2.8 (95% CI = 1.4-5.5) among women exposed within 7 weeks of the last menstrual period, compared to 1.4 (95% CI = 0.62-2.9) in women exposed for the first time at 15 weeks or later. When gestational age at first DES exposure was modeled as a continuous variable including the non-exposed, the reduction in risk for exposure at each subsequent week of gestation was 2%

Table 7. Relative risk of high-grade squamous neoplasia according to timing of DES exposure during gestation^a, DESAD and Dieckmann cohorts

Gestational age at exposure	No. of cases ^b	No. of subjects	Adjusted RR ^{c,d} (95% CI)
≤7 weeks	24	782	2.8 (1.4–5.5)
8–10 weeks	20	829	1.7 (0.87–3.5)
11–14 weeks	13	576	1.8 (0.83–3.9)
15+ weeks	12	719	1.4 (0.62–2.9)
Unexposed	16	1156	1.0

^a Calculated as the number of weeks since last menstrual period.

^b Case definition based on pathology report.

^c Adjusted for age, calendar year of diagnosis, age at first intercourse, number of male partners, level of education, and cigarette smoking.

^d *p*-Value for trend = 0.01 for all subjects, and *p* = 0.11 for exposed subjects only.

(RR = 0.98; 95% CI = 0.968–0.998, *p* for trend = 0.02). When the trend was tested among the exposed only, the estimate was similar (RR = 0.97; 95% CI = 0.93–1.0, *p* = 0.15), but the trend was no longer significant. The gradient in risk according to timing of exposure during gestation was primarily evident in the DESAD cohort RR for ≤7 weeks was 3.1 (95% CI = 1.4–6.6). There was little variation in the Dieckmann cohort in DES dose or timing of exposure since study subjects were treated with a standard protocol and 73% of women were exposed between 8 and 14 weeks.

Discussion

This study found a two-fold increased risk of high-grade squamous neoplasia in women exposed to DES *in-utero* compared to unexposed women. The risk was highest among women who were exposed within 7 weeks of the last menstrual period, and was not significantly elevated among those exposed at 15 weeks or later. The risks tended to be higher in the analyses that were restricted to cases with centralized pathology review. The increased risk was consistent in all three groups, but was somewhat lower in the DESAD cohort than in the Dieckmann and Horne cohorts. These results are consistent with an earlier follow-up of the DESAD cohort [6] during the yearly screening program that found a two-fold increase in incidence of cervical dysplasia among the exposed after the first screening examination through the early 1980s. RRs for DES were not markedly affected by adjustment for covariates, including the time-dependent variable that measured cervical cancer screening history. In addition, stratifica-

tion by other risk factors showed little evidence of effect modification.

Because of the numerous benign histologic changes, including the wider transformation zone, which are found more frequently in DES-exposed daughters, there may be more misclassification of intraepithelial neoplasia among DES-exposed [15]. To avoid this potential bias, only cases of at least intraepithelial grade 2 lesions diagnosed by biopsy were included in this study, and one pathologist, blinded to DES exposure status but knowledgeable about typical benign changes associated with DES, reviewed 77% of the cases. Agreement between pathology report diagnoses of high-grade disease and the central review was good.

The unexposed comparison group was not entirely comparable to the exposed group (Table 2). For example, the unexposed women were less likely to have completed college or graduate school, were less likely to be married, began intercourse at an earlier age, and were slightly more likely ever to have smoked. These characteristics would tend to increase their risk of intraepithelial neoplasia relative to the exposed group, yet an increased risk of intraepithelial neoplasia among the exposed was found and adjustment for these covariates increased the effect estimates slightly among the DESAD cohort. Also, an increased risk of high-grade neoplasia was found in the Dieckmann cohort. Exposed and unexposed women in this cohort should be highly comparable because their mothers were not prescribed DES based on pregnancy problems but rather were participants in a clinical trial to assess the effectiveness of DES.

In addition to DES, elevated risks were found for other known cervical cancer risk factors [16], the most significant factor being number of sexual partners. Other factors with lower RRs included age at first intercourse and smoking status. Information on HPV infection was not available.

There was some loss-to-follow-up in this study and there is always a possibility of differential loss according to both disease and exposure status. However, loss was approximately equal between exposed and unexposed women, and was less than 20%, making the chance of substantial bias from this source less likely.

A limitation of the present study is the lack of precise information on level of cervical cancer screening among study subjects because this analysis is based primarily on diagnoses that occurred after the end of the DESAD screening program when exposed and unexposed were subjected to identical screening protocols. Therefore, it is possible that the excess risk of intraepithelial neoplasia could be due to more frequent and intense screening among the exposed during the time period of this study.

Adjusting for screening behaviors within the DESAD cohort, where reasonably complete information on screening was available, did not materially affect the risk estimates.

Two observations diminish the likelihood that the observed increased risk among the DES-exposed is simply an artifact of screening. First, the initial finding of an increased incidence of squamous neoplasia in the DESAD cohort was based upon systematic, yearly screening examinations conducted simultaneously and identically for DES-exposed and unexposed participants [6]. Secondly, in the present study, higher risks were found among women who were exposed earlier in gestation, with no significant increase in risk among women exposed after the 15th week. It is unlikely that women would participate more frequently in cervical cancer screening based on their timing of first exposure to DES during gestation.

Women exposed to DES *in-utero* have a much wider cervical transformation zone than non-exposed women. The increased incidence of high-grade squamous neoplasia observed in the present study may be the result of the increased area of the transformation zone resulting from DES exposure. It is also possible that the area undergoing metaplastic transformation to squamous epithelium may be uniquely susceptible to carcinogenic factors such as HPV infection. In a previous study of the DESAD cohort, the risk of intraepithelial neoplasia was correlated with the extent of vaginal epithelial changes (VEC) [6]. However, there was also marked decline in the prevalence of VEC with age, leading to the speculation that, over time, most of the DES-associated changes would disappear [17]. It is interesting that the present analysis found an increased risk of intraepithelial neoplasia in all age groups, including those over age 40.

Another hypothesis for the association between DES and high-grade disease is that DES exposure may have caused permanent alterations in the immune system leading to lesser ability to fight off a genital infection such as HPV. Treatment of neonatal mice with DES caused profound alterations in the immune system, especially on natural killer cells [18]. Very few studies of immune effects have been conducted in humans, and those that have are inconclusive [19–21].

In summary, the present study found a small but significant increase in the incidence of high-grade cervical and vaginal neoplasia among DES-exposed women followed between 1982 and 1995. The highest risks were found among women who were exposed earlier in gestation, and the risk declined steadily with exposure later during gestation. These results are consistent with an earlier study that found a two-fold risk of intraepithelial neoplasia following DES exposure, although it is impossible to completely rule out a screening

effect among the DES-exposed women in the current study. The findings, in conjunction with the previous study of the DESAD cohort [6] and the suggestion of a higher risk of cervical cancer among DES-exposed in the Netherlands [7], underscore the importance of continuing to educate both physicians and the exposed population about the necessity of regular screening among women with a history of *in-utero* DES exposure.

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