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Editorial

EDITORIAL

Prenatal Diethylstilbestrol Exposure: A Harbinger for Future Testicular Cancer Incidence?

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Testicular cancer incidence has increased 65% during the course of the last 40 years. In 1975, the age-adjusted rate of testicular cancer was $3.73 \times 10^{-5} y^{-1}$ and in 2016 it rose to $6.17 \times 10^{-5} y^{-1}$ (1). Possibly this increase could be partially attributable to increased prenatal exposure to estrogen-like compounds. As early as 1979, the disease was believed to have prenatal origins (2). Numerous studies have since been conducted to support the hypothesis that prenatal exposure to hormone or hormone-like compounds, specifically diethylstilbestrol (DES), influence testicular cancer development. These studies, however, have been hampered by considerable statistical uncertainty because of two factors. Testicular cancer occurs relatively rarely with only a 0.4% likelihood of it developing over the course of a male's lifetime. Furthermore, testicular cancer accounts for only 0.5% of all incident cancers in a particular year. Contrasted with prostate cancer, which is associated with a 11.6% lifetime risk and attributable to 9.9% of all cancers developing in a year, testicular cancer is a particularly infrequent occurrence (1). This makes prospective studies of testicular cancer challenging to design as evidenced by the study by Strohsnitter et al. Only seven testicular cancer cases developed during approximately 40 years of follow-up among a cohort of 1787 men exposed to DES before birth. Although the investigators observed an increase in testicular cancer risk among this cohort when compared with the national rates, the estimate of this effect was imprecise (relative risk [RR] = 2.04, 95% confidence interval [CI] = 0.82 to 4.20). The imprecision was more pronounced when testicular cancer rates among this cohort were compared with those among a cohort of unexposed men followed for the same length of time (RR = 3.05, 95% CI = 0.65 to 21.96) (3). Also, DES was not frequently used. It was administered to between two and four million women for, among other indications, threatened miscarriage between 1940 and 1971 (4). Its use was then banned on report of women prenatally exposed to the drug having increased risk of clear cell adenoma of the cervix and vagina (5). Nonetheless, during this time period, it was not frequently used, with an exposure prevalence ranging from 1.5% (6) to 7% (7). The infrequency of DES usage also made case-control studies of the effects of DES on testicular cancer prone to imprecision.

To address this dual issue of rare outcome and rare exposure rendering both cohort and case-control studies statistically uncertain, Hom et al. conducted a much-needed meta-analysis of six studies to present a precise summary estimate of the effect of prenatal DES exposure on testicular cancer risk (8). Although the resultant estimate of RR = 2.98 (95% CI = 1.15 to 7.67) reduced the imprecision of the estimate, it was based in part on the results of three retrospective case-control studies, including one not yet published. The number of exposed cases in these studies ranged from two (9) to five in the unpublished study in the current analysis. Exposure misclassification due to erroneous recall by the mother of a case may have inflated the estimates of these studies. Shifting of one case from the exposed category to unexposed, and recalculating the summary estimate, however, did not exert much influence and the summary estimate was only slightly reduced (RR = 2.64, 95% CI = 1.05 to 6.66). For the sake of completeness, the possibility should also be considered that one mother of a selected control incorrectly recalled that she was unexposed when in actuality she could have been exposed. This is reasonable because two studies had a DES exposure control distribution of less than 1% (9,10). Cited prevalence of DES exposure during the time it was in use had a lower range of 1.5% (6). There were also, however, some regions where the drug was not used at all (6). Nonetheless, shifting one unexposed control to the exposed category in these two studies resulted in a reduced summary estimate (RR = 1.96, 95% CI = 0.83 to 4.65). It is therefore somewhat reassuring that the association withstands challenges posed by exposure misclassification scenarios that have uncertain plausibility. Furthermore, these resultant confidence interval bounds derived by Hom et al. resulted in an estimate equally consistent with a minute effect and one that is appreciable. The precision in this estimate is a vast improvement over that in the comparison of the rates

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among the exposed and unexposed cohorts followed by Strohsnitter et al. (3).

Interestingly, when studies were included that did not necessarily limit estrogen exposure to DES but also steroidal hormone exposure, the summary estimate strengthened (RR = 3.43, 95% CI = 1.93 to 6.10). This is a curious finding especially when considering that these hormone exposures included oral contraception use near the time of conception and for pregnancy determination. It is possible that oral contraceptive use at conception and hormone use for pregnancy determination may not play a role in testicular cancer development. These formulations consisted predominantly of progestins and contained low levels of estrogen analogs in the microgram range. Furthermore, hormones given for pregnancy determination were administered only over the course of 2 to 5 days to amenorrheic women at the first suspicion of pregnancy (11). If these exposures were not etiologically relevant, then the estimate would be expected to weaken. It is, in fact, believed that these incidental hormone exposures do not play an appreciable role in abnormal male genital tract development (12). Nonetheless, the positive association remained despite the challenge of considering exposures that might not have played a role in testicular cancer development.

Possibly the hormonal facet of testicular cancer etiology lies in its action and not particularly the carcinogenicity of nonsteroidal hormone-like exposures. It is suspected that exposure to estrogen acts on the in utero formation of testicular cancer, more specifically testicular germ cell tumors (TGCTs), by interfering with normal genital tract development in the male fetus, including sperm cell production. TGCTs are believed to originate from pluripotent primordial germ cells whose progression toward more differentiated germ cells has been arrested. Specifically, Sertoli cell formation is regulated by follicle-stimulating hormone, whose pituitary gland secretion is suppressed by estrogen (13). Animal studies in conjunction with epidemiologic evidence support this notion. As Hom et al. astutely indicate, the genital developmental abnormalities produced by DES administration to laboratory animals are absent in those in which the alpha estrogen receptor has been genetically removed (8).

It is widely suspected that exposure to endocrine-disrupting chemicals (EDCs), which are believed to have estrogenic activity, is playing a role in the increase in testicular cancer that has been observed over the past few decades (14). Some studies have provided evidence that this is the case (15), whereas others have not (16). Interestingly, in those studies in which an association between EDC exposure and TGCT incidence was observed, there was no association between EDC exposures and other male genital malformations such as cryptorchidism and hypospadias (12). Possibly TGCT formation may occur at lower levels of estrogen-like exposure than do those other outcomes. The strongest association between prenatal DES exposure and testicular cancer incidence was observed among the cohort members whose mothers received their prenatal care at the Mayo Clinic. The doses prescribed there were lower than those at other study centers (17).

The meta-analysis conducted by Hom et al. lends more evidence supporting the hypothesis that prenatal exposure to exogenous estrogen-like compounds may influence TGCT development (8). There are, however, prevailing threats of biases that are difficult to circumvent in most observational studies of this nature. It is possible that an indication for DES for threatened miscarriage was exhibited by symptoms that speculatively presented themselves after prenatal testicular cancer development. This would be an example of reverse causality and an intractable bias. Confounding by indication or prescribing DES exclusively for a symptom that is also highly associated with TGCT development would require the conditioning on that symptom after ascertaining that it occurred before and not after the in utero development of TGCT. Again, this would require speculation. Identifying participants whose mothers participated in a trial of DES to prevent miscarriage circumvented this issue because these women received DES simply by random assignment to a particular treatment arm. This cohort unfortunately was uninformative because there were no testicular cancer cases that developed within it.

In conclusion, the study by Hom et al. provides supportive evidence of prenatal DES exposure having a moderate effect on TGCT development (8). Also, the narrow bounds of the resultant estimate are indicative of minute effect and a large effect having equal probabilities due to statistical variation. Furthermore, the results appear to be robust to perturbations resulting from differential exposure recall based on case status. It is not likely that in the absence of any more DES exposure and TGCT data collected in the past there will be any further empirical insight into DES' role in the etiology of TGCT development. Usage of the drug ceased in 1971, and men born before this time have aged out of that period in their life span when TGCTs are normally diagnosed. Although it is implausible that the secular increase in testicular cancer incidence can be explained by prenatal DES exposure, suspicions abound regarding other xenoestrogen exposures and their influence on TGCT incidence. The design of such studies to provide supportive evidence of this occurrence will be challenging in its own right. Hopefully, such studies can be effectively designed and further insight into the underlying reason for the increase in TGCT rates observed over the past four decades can eventually be gained.

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