Title: The Heritable Legacy of Diethylstilbestrol: A Bellwether for Endocrine Disruption in Humans

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Running title: Heritable Legacy of Diethylstilbestrol

Summary sentence: Research has shown that diethylstilbestrol (DES) can exert adverse heritable consequences. The session at Beyond Genes drew attention to the latest findings and research efforts.

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Abstract:

Millions of women and their fetuses were exposed to the toxic pregnancy drug diethylstilbestrol (DES) from the 1940s into the 1970s, a time when the medical profession had little knowledge about potential developmental consequences of fetal drug exposures. Pathological

consequences of DES exposure to the pregnant mothers and their offspring are well documented, but now generational research is finding that the grandchildren of women given DES in pregnancy are also at risk. This commentary summarizes presentations on this subject from the Beyond Genes panel “Heritable Impacts of Diethylstilbestrol (DES).”

Introduction

As the executive director of the advocacy group DES Action USA, I was pleased to serve as the chair of a Beyond Genes session on heritable impacts of the drug diethylstilbestrol (DES). DES was a potent non-steroidal estrogenic drug prescribed to between five and 10 million pregnant women from the 1940s until 1971 [1]. The U.S. Food and Drug Administration (FDA) had approved DES for a variety of uses in 1941 and in 1947 approved the use of DES for pregnant women who had suffered a miscarriage. This meant that millions of embryos and fetuses were exposed *in utero* to unprecedented quantities of synthetic hormones, creating in essence a tragic human experiment, the full repercussions of which have yet to be fully elucidated [2]. I do know from my many years as an advocate in close contact with families affected by DES toxicity that heritable impacts are a preeminent and serious concern.

Reports of toxicity in humans were first published on April 22,1971, when Arthur Herbst, M.D. et al. published a landmark study in the *New England Journal of Medicine* that linked DES exposure *in utero* to a rare vaginal adenocarcinoma, clear cell adenocarcinoma (CCA), in girls and young women [3]. Previously, CCA had been diagnosed only in postmenopausal women, so a cluster of young women with it gained Herbst’s attention and was the basis of the paper. Soon after the article was published, the FDA removed the label indication for pregnant women. The FDA did not ban DES, but in 1971 only urged doctors to stop prescribing it to their pregnant patients. Most, but not all, stopped, meaning that DES was prescribed to pregnant women in America into the early 1980s [4], and later internationally.

A very powerful endocrine disrupting chemical (EDC), DES was often prescribed in escalating doses resulting in the ingestion of massive amounts of DES by each woman who followed the protocol. The husband and wife team of Olive Smith, PhD, and George Smith, MD, developed this DES prescribing protocol that bears their names [5]:

The recommended regimen started at 5mg per day in the 7th and 8th weeks of pregnancy (from first day of last menstrual period), and was increased every other week by 5mg per day through the 14th week. Then the amount was increased weekly by 5mg per day, from 25mg in the 15th week to 125mg per day in the 35th week.

In other words, many women and their fetuses were exposed to massive quantities of this drug at a time when the medical profession had little knowledge about potential developmental consequences. But we now now that the mothers who had been given DES have a higher risk of breast cancer, and both the DES-exposed daughters and DES-exposed sons had elevated instances of structural abnormalities in their reproductive organs and high rates of infertility, cancer, and other pathologies. Now generational research is finding that the grandchildren of women given DES in pregnancy are also at risk of structural abnormalities and other related problems. The December 4, 2020 Beyond Genes panel “Heritable Impacts of Diethylstilbestrol (DES)” highlighted some of these findings. This article provides a summary.

Intergenerational DES Studies in Humans

Linda Titus, PhD, Geisel School of Medicine at Dartmouth, a primary researcher on the National Cancer Institute (NCI) DES Combined Cohort Follow-up Study, has examined some endpoints in F2 offspring of F1 daughters who were prenatally DES-exposed or -unexposed. Unfortunately, the study did not ascertain data from F1 exposed sons or their offspring. Findings

to date for the F2 DES-exposed women, compared to the unexposed, suggest an increased likelihood of menstrual irregularities and a possible excess of ovarian cancer, a finding based, however, on only three cases. Further work is underway to assess these outcomes as well as the possibility of adverse reproductive outcomes, which affected their prenatally exposed mothers.

Marianthi-Anna Kioumourtzoglou ScD, Columbia University, spoke on her study looking at DES exposure during pregnancy and F2 neurodevelopmental deficits. The study explored the hypothesized link between DES in pregnancy and adverse neurodevelopmental outcomes in the grandchildren, who were exposed as germ cells in the developing parent *in utero*. EDCs potentially create molecular alterations to the germline, mediated through epigenetic mechanisms to promote outcomes in subsequent generations. While there are several studies on multi-generational EDC-caused changes in mice, epidemiological evidence on the multi-generational EDC leading to abnormal neurodevelopment in humans is lacking.

Her study, published in *JAMA Pediatrics* in 2018 reviewed 47,540 records of women from the Nurses Health Study II [67]. Of those women, 2,032 reported being “certain or somewhat certain” that their mothers (F0 generation) used DES while pregnant, creating the F1 generation, commonly called DES daughters (just as in the NCI cohort, in the Nurse’s Health Study the DES sons were not investigated). The daughters of the F0 generation, those who were exposed *in utero* to DES (F1 generation) produced 1,784 children (F2 generation). The year 1983 was the median birth year for F2.

ADHD was diagnosed in 7.7% of all the grandchildren (F2) of women given DES in pregnancy (F0), (adjusted OR, 1.36; 95% CI, 1.10-1.67), as compared to 5.2% of those not exposed, a 33% increased risk of ADHD for the F2 generation. However, looking only at the F0 women given DES in the first trimester of pregnancy reveals their grandchildren, F2, were 66% more

likely to have an ADHD diagnosis, indicating that timing of DES exposure greatly affected the likelihood of an ADHD diagnosis.

The study provides evidence of an association between grandmateral DES use and third-generation ADHD, with evidence regarding first trimester use seeming particularly strong. Dr. Kioumourtzoglou noted that there is biological plausibility as to why this window of exposure could be critical. Early gestation is a time of sensitive maternal influences resulting in embryonic and germ-cell reprogramming. During this period, a wave of genome demethylation followed by *de novo* remethylation occurs together with the establishment of genomic imprints and determination of sex. Perturbations of reprogramming could have detrimental impacts on germ cell DNA methylation, subsequent gene regulation, and offspring neurodevelopment. There could be other potential mechanisms, however, with one suggestion being assortative mating, as when an F1 generation had ADHD and had a child with someone also with ADHD, which could lead to an increased ADHD result. The F1 generation in the Nurses II study was not asked if they had received an ADHD diagnosis themselves. A comment by Jill Escher, chair of the Beyond Genes conference and a research philanthropist, raised the question of whether F1 DES daughters' potential increased rates of surgical interventions could have impacted their oocytes, thereby increasing ADHD rates in the F2. Escher noted that mammalian experimental models have demonstrated adverse neurodevelopmental consequences of general anesthesia on offspring of exposed gametes. This potential mediating factor was not studied, however.

Dr. Kioumourtzoglou concluded that the finding from this study, the first to examine links to F2 neurodevelopment, may have important implications for exposures to other environmental EDCs (e.g., ubiquitous chemicals such as bisphenol-A, phthalates, etc.) during pregnancy and grandchild adverse health effects.

Other Presentations

Subhrangsu S. Mandal, PhD of University of Texas at Arlington presented on “Unfolding the DES Epigenome: Impacts on Chromatin, Genes and Non-coding RNAs.”

Dr. Mandal spoke about his work to understand the epigenetic mechanism of human gene expression and regulation and how they malfunction in disease. In particular relevance to DES, Dr. Mandal is studying the molecular signaling mechanisms by estrogenic endocrine disrupting chemicals such as BPA or DES exert their actions and induce a variety of gene expression linked to metabolism, reproduction, development, and cancer.

Their studies demonstrated that BPA/DES exposure triggers the activation of estrogen signaling, alters the chromatin modifications in the genome, and turns on a variety of gene expression including cancer causing genes and developmental genes. Along with protein coding genes, BPA and DES also turn on oncogenic long-noncoding RNAs, such as HOTAIR, suggesting potential roles of estrogenic EDCs in increased risk of cancer. Additionally, chromatin modifying enzymes such as Mixed Lineage Leukemia (MLL) family of histone methyltransferases, which are major players in leukemia, other types of cancer and in development, coordinate with estrogen receptors and influences BPA and DES induced alterations in gene expression.

Dr. Mandal predicts that exposure to EDCs and other environmental chemicals, affect the chromatin and DNA modifications across the genomes and reprogram the epigenetic landscape in the genomes, altering pattern of expression of proteins and noncoding RNAs. These alterations in the epigenome may be transmitted to the next generations through germline cells, causing developmental defects, immune defects, and increasing the risk of cancer. Dr. Mandal plans to analyze the histone modifications and noncoding RNA profiles in DES exposed populations in multiple generations (DES-son/daughters, their children, and grandchildren) and hopes to understand the epigenetic changes that are caused by DES exposure and discover the DES epigenomes that are transmitted into next generations. This research will enhance our

understanding of the DES impacts and may guide us in developing a novel biomarker for DES impacts and provide novel therapeutic avenues.

Ms. Kari Christianson, MedShadow Foundation board member and former research director, DES Action USA.

Ms Christianson spoke as both a patient advocate and a DES daughter. Ms. Christianson explained that in the early years of DES Action and the DES research, the questions raised by DES-exposed people were mostly focused on reproductive tract abnormalities, endometriosis, infertility and cancers among the directly exposed daughters.

Because DES had been prescribed to pregnant women as early as 1945, some grandchildren were already born by 1971, the year that DES was linked to CCA in the Herbst et al. paper. Therefore, DES Action fielded questions about DES grandchildren from the start of the organization in 1978, alongside questions about DES daughters and sons.

DES Action received anecdotal reports of DES granddaughters' miscarriages, ectopic pregnancies, cervical and vaginal epithelial changes, premature ovarian failure and ovarian cancer, endometriosis, bicornuate uterus, irregular menstrual cycles, polycystic ovary syndrome (PCOS) and some cases of CCA in young girls. Many of these questions and fears were the same ones that DES Action received about DES daughters during the same period.

The health questions about DES grandsons included those about hypospadias, infertility or sterility, kidney cysts, autism and gynecomastia. Again, they were some of the same questions asked by and about DES sons.

All of these questions were shared by DES Action with the NCI as well as the National Institute of Environmental Health Sciences (NIEHS). In conclusion, Christianon noted that the unfortunate opportunity of having a DES-exposed population is that we may be helpful in understanding some of the generational effects of all endocrine disruptors.

Scott Kerlin, PhD, independent researcher and founder of DES Sons International Research Network (IRN).

Kerlin reported on his 25-year investigation into DES. As a DES son, he has focused on qualitative research about DES sons' reproductive health issues. He conducted much of his research through DES Sons IRN, which included nearly 1,000 participants in 14 countries. Four-fifths of the members or IRN had proven or "strongly confirmed/strongly suspected" exposure; because many DES-exposed people have had great difficulty obtaining their mothers' pregnancy records, confirmation through them was not always possible. Over the years, several topics emerged among his network members, including disorder of sexual development, intersex conditions, gender dysphoria, and testicular dysgenesis syndrome (TDS). TDS was identified in the early 2000s with its constellation of effects, including testicular cancer, genital urinary penile testicular effects, hypospadias and infertility.

The present area of Dr. Kerlin's inquiries are the history of psychiatric disorders, anxiety and depression among the F1 sons and F2 grandsons, and monitoring of gender dysphoria, with a connection to autism and ADHD. Although Dr. Kerlin has limited ability to conduct direct research his work is important in developing and framing new hypotheses worthy of follow-up by researchers.

Conclusion

DES exposure has had profound effects physiologically and sociologically, not only on the pregnant women prescribed the drug, but also the children exposed *in utero* and now, their children as well. Ongoing research into the grandchild generation is minimal and the primary research, the NCI's DES Follow-up Study, is being closed.

More study is needed on DES's generational effects, not only for those exposed to this toxic drug, but also for the endocrine exposure pathways the research reveals. No other cohort has the power, in human studies, to reveal the enduring legacy of endocrine disruption. Unlike environmental chemicals that are found ubiquitously in the environment, DES exposure is often quantifiable and intensive. With the disbanding of the DES Follow-up Study, we are threatened with the loss of vitally important information about EDC human impacts across generations.

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Conflicts of Interest

The author serves as the executive director of DES Action USA and as president of MedShadow Foundation. Both organizations accept donations from the public and refuse donations, grants or other support from pharmaceutical companies. The author receives no compensation for either role. She has no other conflicts of interest.

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