SERUM DIOXIN CONCENTRATIONS AND ENDOMETRIOSIS: A COHORT STUDY IN SEVESO, ITALY

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Introduction

Concern about the reproductive toxicity of dioxin, a ubiquitous contaminant of industrial combustion processes, has been growing.[1] In the past decade, a number of animal studies have suggested that prenatal and postnatal exposure to dioxin and dioxin-like chemicals may profoundly affect the reproductive systems of both male and female animals perhaps via endocrine disruption.[1] In part, because of this concern, the United States (U.S.) Environmental Protection Agency and the World Health Organization have conducted a reassessment of dioxin, including human health consequences.[2, 3]

Experimental animal evidence supports an association of endometriosis and exposure to dioxin-like chemicals. In 1993, Rier et al.[4] reported in six to 10 year old adult rhesus monkeys a dose-response relation between TCDD levels (5 and 25 ppt) in feed and the incidence and severity of endometriosis, diagnosed a decade after dosing ceased. TCDD has also promoted the survival and growth of surgically-induced endometrial implants in non-human primates[5] and in mice[6-8], but not in rats.[7] More recent animal data suggest that endometriosis may also be associated with increased body burden of dioxin-like polychlorinated biphenyls (PCBs), in particular, PCB 77 and PCB 126, and of total serum TCDD Toxic Equivalency Quotient (TEQ).[9]

A series of hospital-based case-control studies have been conducted in humans to evaluate the association with endometriosis found in rhesus monkeys.[10-14] The results, however, have been inconsistent. In general, small sample size and/or a failure to evaluate exposure to dioxins, or other dioxin-like compounds, limited most of these studies.

We sought to determine if there is an association of dioxin exposure and endometriosis in humans. We conducted a population-based historical cohort study 20 years after the 1976 factory explosion in Seveso, Italy, which resulted in the highest known population exposure to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD). The Seveso cohort represents the largest population of enumerated TCDD-exposed women and among the highest exposure known in humans [15]. The relatively pure exposure to TCDD[16] and the ability to quantify individual level TCDD exposure from sera collected in 1976 for the Seveso cohort affords a unique opportunity to evaluate the potential dose-response relationship between TCDD exposure and endometriosis.

EPIDEMIOLOGY

Methods and Materials

SWHS Study Design

The Seveso Women's Health Study (SWHS) is the first comprehensive epidemiologic study of the reproductive health of a female population exposed to TCDD. The primary objectives of the SWHS are to investigate the relationship of TCDD and the following reproductive endpoints: (1) endometriosis; (2) menstrual cycle characteristics; (3) age at menarche; (4) birth outcomes of pregnancies conceived after 1976; (5) age at menopause; and (6) other reproductive conditions. Women eligible for the SWHS were 40 years old or younger in 1976, had resided in one of the most highly contaminated zones, A or B, and had stored sera collected soon after the explosion.

Informed consent was obtained followed by venipuncture, and a structured 1-1/2 hour personal interview. Each woman was interviewed by a highly trained nurse-interviewer who was blind to TCDD level and residence of the woman. Information collected during the interview included sociodemographic information, personal habits, work history, detailed gynecologic and other medical history, detailed pregnancy history including time to conception of first pregnancy after the accident, menstrual cycle history and exposure to TCDD. Women who were still menstruating were scheduled for a gynecologic examination and transvaginal ultrasound. They were also asked to complete three months of a daily menstrual diary. We requested permission for medical records for any previous gynecologic ultrasound, procedure, or disease diagnosis. In addition, we requested medical records for specific chronic diseases.

The women underwent a gynecologic examination and a transvaginal ultrasound by a gynecologist who was blind to exposure status and residence of the woman. The ultrasound was recorded on videotape, and photographs were taken of ovaries or any pathology noted. For each woman, the gynecologist completed a data form for the ultrasound and exam.). "Cases" had surgically-confirmed disease or an ultrasound consistent with endometriosis. "Non-diseased" had surgery with no evidence of endometriosis, or no signs or symptoms. Other women had "uncertain" status.

If any abnormality was noted on ultrasound, the woman was offered a repeat ultrasound. Laparoscopy was offered at the time of examination to women who met the criteria established based on the current standard of medical care.

SWHS Participation

Recruitment of the women began in March 1996 and was completed in July 1998. Of 1271 eligible women, 17 (1.3%) could not be located or contacted, and 33 (2.6 %) had died or were too ill to participate. Of the 1221 (96.1 %) women who could be contacted approximately twenty years after the explosion, 981 (80 %) completed the interview and blood draw. Of the 770 women who were still menstruating, 665 (86 %) underwent the gynecologic examination/ultrasound and 612 (79 %) completed the daily diary. The current analysis focuses on the 601of the 665 women who were 30 years old or less in 1976.

TCDD Exposure Assessment

For each participant, we preferentially selected the earliest serum sample collected (1976-1981) that was of adequate volume and sent the samples from Desio to the U.S. Centers for Disease Control and Prevention (CDC) for TCDD analysis by high-resolution mass spectrometry methods[18]. Values were reported on a lipid-weight basis in parts per trillion (ppt)[19]. TCDD was measured in sera collected in 1976 or 1977 for 899 women (92 %), from 1978 through 1981 for 54 women (5 %), and in 1996 or 1997 for 28 women (3 %) whose earlier samples had become concentrated by dessication. For four women whose post-1977 TCDD values were detectable but less than or equal to 10 ppt, the measured value was used. For 27 women whose post-1977 TCDD levels were greater than 10 ppt, and who were

16 years old or younger in 1976, the serum TCDD level was back-extrapolated to 1976 using the Filser Model.[20] For 42 women whose post-1977 TCDD levels were greater than 10 ppt and who were more than 16 years old in 1976, the first-order kinetic model was used for back-extrapolation.[21] For the 96 women with non-detectable values, a serum TCDD level equal to one-half the detection limit was assigned.[22]

Results and Discussion

We identified 19 "cases", 305 "uncertain", and 277 "non-diseased" women. The overall median serum TCDD level for the 601 women was 54.9 ppt with a range of 2.5 to 17, 300 ppt. The median serum TCDD levels for "cases" was 77.3 ppt; for "non-diseased" was 61.0 ppt; and for the "uncertain" group was 49.0 ppt. The TCDD levels for "cases" and "non-diseased" overlapped at the ends of the cumulative distribution, but the "cases" had higher TCDD levels in the middle of the distribution.

Although the age-adjusted percent of "cases" increased from 1.7 % for women in the lowest dose group($\leq$20.0 ppt serum TCDD) to 4.6 % for those in the highest dose group (>100 ppt), the percent of "non-diseased" also increased with exposure levels. Relative to women with levels $\leq$20 ppt, the relative risk ratios (RRR) for women with serum TCDD levels of 20.1 to 100 ppt and >100 ppt were 1.2 (90 % CI=0.3-4.5) and 2.1 (90 % CI=0.5-8.0) respectively. The test for trend for the "cases" to "non-diseased" ratio (scoring categories as 1,2, 3) was non-significant (p=0.25). The test for trend with continuous log TCDD in the polytomous model was also non-significant (p=0.84). The most notable limitation of the study was our inability to perform laparoscopy on every woman and thereby, to definitively diagnose or rule out endometriosis.

In summary, we found a doubled, non-significant, risk for endometriosis among women with serum TCDD levels of $\geq$100 ppt. To eliminate the possibility of exposure misclassification, future studies should determine whether there was substantial exposure to other PCDDs, PCDFs, and dioxin-like PCBs in this population.

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