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Organochlorine pesticides and endometriosis

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ABSTRACT

Limited study of persistent organochlorine pesticides (OCPs) and endometriosis has been conducted. One hundred women aged 18–40 years who were undergoing laparoscopy provided 20 cm^3 of blood for toxicologic analysis and surgeons completed operative reports regarding the presence of endometriosis. Gas chromatography with electron capture was used to quantify (ng/g serum) six OCPs. Logistic regression was utilized to estimate the adjusted odds ratios (aOR) and 95% confidence intervals (CI) for individual pesticides and groups based on chemical structure adjusting for current cigarette smoking and lipids. The highest tertile of aromatic fungicide was associated with a fivefold risk of endometriosis (aOR = 5.3; 95% CI, 1.2–23.6) compared to the lowest tertile. Similar results were found for *t*-nonachlor and HCB. These are the first such findings in a laproscopic cohort that suggest an association between OCP exposure and endometriosis. More prospective studies are necessary to ensure temporal ordering and confirm these findings.

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1. Introduction

Endometriosis is a complex disease typically defined as an estrogen-dependent disease with an estimated incidence of 1.9/1000 person-years [1]. Prevalence of endometriosis ranges from 20% to 65% among women seeking medical care [2–6]. Confirmation of the endometriosis diagnosis requires at a minimum the visual inspection of the pelvis for overt disease. This definition impacts the choice of study cohort since not all affected women are symptomatic, seek medical care, or undergo surgery.

Despite its etiology remaining speculative, increasing evidence supports a role of environmental chemicals in the development of endometriosis, particularly dioxin [7], polychlorinated dibenzodioxins and polychlorinated dibenzo furans [8], and polychlorinated biphenyls (PCBs) [9–11]. Of note, two other studies reported an association between higher concentrations of phthalates [12,13], plasticizers frequently found in personal care products, and endometriosis. A case–control study in Atlanta aimed at measuring any potential association between serum dioxin levels as expressed by total toxic equivalence (TEQ) and serum total PCBs, as calculated by the sum of concentration of 36 congeners in women and endometriosis, found null results. The authors did not however examine the chemicals we examined in this study [14]. Limited attention has focused on persistent organochlorine pesticides (OCPs) and their association with endometriosis, despite their sharing a similar chemical structure with dioxins and PCBs and their ubiquitous presence in the environment. Organochlorine pesticides are persistent chemicals used to eliminate insects and have largely been banned in the U.S. These chemicals can bioaccumulate in fish, and diet is the main route of exposure for humans [9].

2. Materials and methods

2.1. Study population

One hundred women, ages 18–40 years undergoing incident laparoscopy in 1999–2000 at one of two participating university-affiliated hospitals were approached for recruitment for study. Participating surgeons informed women

Abbreviations: PCB, polychlorinated biphenyl; OCP, organochlorine pesticide; β-BHC, beta-benzene hexachloride; HCB, hexachlorobenzene; DDE, dichlor-diphenyl-dichloroethylene; *t*-nonachlor, *trans*-nonachlor; GC-EC, gas chromatography with electron capture; TL, total serum lipids; TC, total cholesterol; FC, free cholesterol; TG, triglycerides; PL, phospholipids; ppb, parts per billion; LOD, limits of detection; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence intervals; BMI, body mass index.

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Table 1
Characteristics of women by laparoscopic endometriosis diagnosis.

Characteristic	Endometriosis, $n = 29$		No endometriosis, $n = 51$		OR (95% CI)
	n	(%)	*n*	(%)	
Education (years)					
≤12	4	(14)	23	(45)	1.0 (referent)
13–16	17	(59)	23	(47)	4.3 (1.2–14.6)
>16	8	(27)	5	(10)	9.2 (2.0–43.0)
Household income (in dollars)					
<15,000	1	(3)	10	(20)	1.0 (referent)
15,000–59,999	13	(45)	23	(45)	5.7 (0.6–49.3)
≥60,000	15	(52)	18	(35)	8.3 (1.0–72.8)
Current cigarette smoking					
Yes	4	(14)	24	(47)	0.2 (0.1–0.6)
No	25	(86)	27	(53)	1.0 (referent)
Mean number cigarettes ± SD[a]	9	(6)	15	(6)	
BMI (kg/m^2)					
<18	1	(4)	1	(2)	1.0 (0.1–16.2)
18.5–24.9	24	(83)	23	(45)	1.0 (referent)
25.0–29.9	1	(4)	13	(26)	0.1 (0.1–6.0)
≥30	3	(10)	14	(28)	0.2 (0.1–0.8)
Mean ± SD	23	(3)	27	(6)	
Age (years)					
20–29	7	(24)	18	(35)	1.0 (referent)
30–34	11	(38)	19	(37)	1.5 (0.5–4.7)
≥35	11	(48)	14	(28)	2.0 (0.6–6.6)
Mean ± SD	32	(4)	31	(5)	
Ever pregnant					
Yes	9	(31)	32	(63)	0.3 (0.1–0.7)
No	20	(69)	19	(37)	1.0 (referent)

BMI, weight in kg/height in m^2.
OR, odds ratio; 95% CI, 95% confidence interval.
[a] Among women who reported smoking.

about the study. Following consent, each woman was contacted by research personnel. Eighty-four women (84% response) participated in the study.

2.2. Data collection

The interview conducted in the woman's home prior to surgery via standardized questionnaire elicited information on the sociodemographic characteristics, reproductive and medical history and lifestyle and potential confounders: gravidity (number of pregnancies regardless of outcome), body mass index (BMI, weight in kg divided by height in m^2), and current cigarette smoking based on recent findings [15,16]. The research assistant conducting the interview was unaware of the woman's preoperative diagnosis, and the questionnaire was designed to elicit information on potential confounders in the association between environmental chemicals and disease. Approximately 20 cm^3 of blood was obtained from all women, and 80 (95%) had sufficient sample for the OCP analysis after PCB analyses were completed. Blood was collected after the interview and before surgery using venipuncture equipment determined by the participating toxicologic laboratory to be free of the contaminants under study.

Laparoscopic surgeons visually inspected the entire pelvis and recorded all pathology and the presence of endometriosis on standardized operative reports immediately following surgery. Severity of endometriosis was staged according to the American Fertility Society's revised definition as: Stage I (minimal); Stage II (mild); Stage III (moderate); and Stage IV (severe) [17,18]. Full Institutional Review Board approval was obtained by the participating hospitals for the conduct of this study.

2.3. Laboratory methods

Gas chromatography with electron capture (GC-EC) was utilized in a blinded manner for quantifying six serum OCPs or their metabolites: aldrin, beta-benzene hexachloride (β-BHC), dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), hexachlorobenzene (HCB), mirex, and *trans*-nonachlor (*t*-nonachlor). Briefly, to each serum sample surrogate standards of PCB IPAC isomer numbers 46 and 142 were added and allowed to equilibrate overnight. Methanol was added to precipitate serum protein and the sample mixture was then extracted with hexane at 50 rpm for 20 h in a rotary extraction unit, followed by centrifugation and concentration to 2 ml under a stream of nitrogen. To separate lipids from PCBs, the mixture was passed through a deactivated Florisil column, eluted with hexane, and concentrated under a stream of nitrogen using 200 μl of isooctane as a keeper solvent. Internal standards (congeners 30 and 204) were added to the extract prior to injection into an Agilent 6890 Gas Chromatograph equipped with an electron capture detector.

Chromatographic data were collected electronically and analyzed using Turbochrome chromatographic software. All chromatographic data were burned to compact discs for permanent data archiving. Serum specimens were run in batches of 10 + 4 quality control samples (i.e., reagent blank, matrix blank, matrix blank containing a mixed standard of 15 specific congeners at known values, and one duplicate participant sample). Matrix blanks consisted of sheep serum with low background levels of PCBs. Pesticide and PCB congener concentrations were calculated from standard curves for the 15 calibration standards, and the remaining congener concentrations were calculated from response factors that were generated for each congener in our laboratory. Each congener concentration was adjusted for surrogate recovery and subtraction of reagent blanks. The limit of detection was determined as three standard deviations of the mean of at least 10 matrix blanks. We did not substitute values below the limits of detection nor did we automatically lipid-adjust concentrations to avoid biases associated with such practice [19,20]. The limits of detection for β-BHC, DDE, HCB, mirex, *t*-nonachlor, and aldrin were as follows, respectively, 0.011, 0.022, 0.002, 0.020, 0.011 and 0.017 ng/g serum.

Total serum lipids (TL) were determined using gravimetric procedures and quantified as the sum of total cholesterol (TC), free cholesterol (FC), triglycerides (TG), and phospholipids (PL) as follows: $TL = 1.677(TC - FC) + FC + TG + PL$ [21]. All lipids were expressed as mg/dl serum.

2.4. Statistical methods

Descriptive analyses were conducted to assess the missing data, the normality of OCP distributions and potential confounders for inclusion in unconditional logistic regression models. For analysis, OCP concentration was defined as the observed serum value for a particular pesticide corrected only for batch-specific recovery and blanks and expressed as nanograms per gram serum (ng/g), which translates to parts per billion (ppb).

All pesticides, except for aldrin and β-BHC, were categorized into tertiles for analyses, with the lowest tertile serving as the referent category in the logistic regression models. Since approximately 80% of women had values below the limits of detection (LOD) for aldrin and β-BHC, we undertook a series of analyses to assess the best way to correctly estimate their distributions relative to endometriosis diagnosis. Specifically, first we assumed that aldrin and β-BHC concentrations were log-normally distributed and then used the maximum likelihood ratio test for equivalence of distributions to determine if each compound discriminated women by endometriosis status. The corresponding likelihood functions were defined to