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Increased Risk of Relapse After Breast Cancer With Exposure to Organochlorine Pollutants

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Human exposure to endocrine-disrupting chemicals (EDCs) is considered a possible cause for hormone-dependent cancers. Pesticide exposure is recognized as an important environmental risk factor. Insecticides, herbicides, and fungicides are associated with hematopoetic cancers and cancers of the prostate, pancreas, liver, and other body systems (Jaga and Dharmani, 2005).

A series of clinical studies have been conducted in several countries to determine the association between polychlorinated biphenyls (PCBs) or organochlorine pesticides exposure and the increased incidence of breast cancer. These studies frequently have found a lack of association between exposure to these compounds and the development of breast cancer (reviewed in Lopez-Cervantes et al., 2004). However, a limited number of studies have reported higher levels of PCBs or organochlorine pesticides in patients with breast cancer than in control subjects (Cassidy et al., 2005; Charlier et al., 2004; Falck et al., 1992; Wolff et al., 1993).

Finally, even if most environmental factors have not been strongly associated with breast cancer risk, pesticide exposure in combination with genetic predisposition and age at exposure may have a cumulative effect on breast cancer risk (Coyle, 2004). It is well known that the mitogenic and genotoxic effects of estrogens stimulate the malignant transformation of mammary gland cells (Colditz, 1998; MacMahon et al., 1973). An increased risk of breast cancer can be mediated through a prolonged or increased exposure to estrogens or estrogenic compounds (Colditz et al., 1995; Jaga, 2000). Even after breast cancer surgery, potentially disseminated breast cells can be stimulated by EDCs (Bradlow et al., 1995). These endocrine-disrupting compounds are highly lipophilic and can persist in biologic tissues many years after the last exposure has occurred (Jaga and Dharmani, 2003).

Therapy for breast cancer often is associated with weight loss, and previous studies have demonstrated that significant weight loss can result in mobilization of EDCs and a subsequent increase in blood concentration (Charlier et al., 2002; Chevrier et al., 2000). This redistribution can represent an additional risk factor for breast cancer relapse.

This study enrolled 125 women, age 60.7 ± 12.2 years with a diagnosis of breast cancer who undewent curative surgery followed by cytotoxic chemotherapy or hormonal therapy. Pollutants were quantified simultaneously using a gas chromatographic analyzer coupled with an ion-trap mass spectrometer detector. The potential association between chlorinated pesticides and various characteristics of the breast neoplasms was tested.

Material and Methods

This retrospective study investigated 125 women with breast cancer who underwent surgery followed by a cytotoxic chemotherapy treatment or, when respon-

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sive, hormonal therapy immediately before starting radiotherapy treatment. The patients were not to have undergone previous irradiation treatment. All the patients gave their informed consent for participation in the study.

Blood sampling was organized on the first day of radiotherapy treatment. Samples (10 ml) drawn in the early morning after overnight fasting were immediately centrifuged, with serum specimens kept at –18°C until analysis.

Information about the breast cancer case was collected from the patient's record by the physician at the time of blood sampling. The characteristics of the study population are summarized in Table 1.

Chlorinated pesticides in serum were identified and quantified using a gas chromatographic analyzer coupled with a mass spectrometer detector. The analytical method is described elsewhere (Charlier and Plomteux 2002). Briefly, sample preparation included a liquid–liquid extraction (petroleum ether:diethylether, 98:2) followed by a solid phase extraction (Bond Elut Certify, Varian). The eluate was evaporated to dryness, reconstituted in n-hexane, and then injected into a gas chromatograph (Saturn 2000, Varian). The column was an HP-5 trace from Agilent (30 m × 0.25 mm internal diameter). Ionization by electronic impact occurred at 70 eV. All solvents were of pesticide grade quality.

Table 1 Characteristics of the study population (n = 125)[a]

Parameter	
Age (years)	60.7 ± 12.2
BMI (kg/m^2)	25.4 ± 4.5
Smoking (yes): n (%)	28 (24.8)
Histology: n (%)	
Canalar carcinoma	108 (86.4)
Lobular carcinoma	17 (13.6)
Bloom stage: n (%)	
I	23 (25.8)
II	43 (48.3)
III	23 (25.8)
TNM classification: n (%)	
T1	65 (60.8)
T2	33 (30.8)
T3	4 (3.7)
T4	5 (4.7)
N0	77 (70.0)
N1	32 (29.1)
N2	1 (0.9)
M0	102 (100.0)
ER status (yes): n (%)	70 (57.4)
Chemotherapy treatment (yes): n (%)	55 (44.0)
Relapse (yes): n (%)	14 (11.2)

BMI, body mass index; TNM, tumor node metastasis; ER, estrogen receptor

[a] Percentages were calculated on the basis of the total of available information

Reference standards for all the compounds were obtained from Cambridge Isotope Laboratories (Andover, MA, USA) or Dr. Ehrenstorfer (Ausburg, Germany). The calibration curve was constructed from 0 to 30 parts per billion (ppb), and linearity was applied for this concentration range. Endosulfan-d4 (0.5 ppb) was used as the internal standard. Samples were analyzed using a blind procedure with controls comprising samples spiked with 0.5 or 2 ppb of each organochlorine pesticide tested. The analytical method was validated for p,p'-DDT, p,p'-DDE, HCB, lindane, and aldrine. An interlaboratory comparison program (AMAP Ring Test, Institut National Santé Publique, Québec) was performed as an external quality control.

Serum levels of organochlorine residues were expressed as mean ± standard deviation. Correction for lipid content was tested, but because the results were not affected, only crude data are presented. When organochlorine results were lower than the limit of quantification (0.5 ppb), a null value was recorded, and these results were included in all subsequent calculations. Quantitative continuous results were log transformed to normalize their distributions. The Spearman correlation coefficient was calculated to assess the relationship between pesticide serum concentration and age or body mass index (BMI).

Serum concentrations of pesticide residues were compared between women according to smoking status, tumor node metastasis (TNM) grade, histologic status, estrogen receptor positivity, and Bloom stage by analysis of variance (ANOVA). For each breast cancer characteristic, total pesticide concentrations were compared between categories and adjusted for age because it is well known that concentrations increase with age. All results were considered to be significant at the 5% critical level ($p < 0.05$). The calculations were performed using the SAS (SAS Institute, version 8.2 for Windows, SAS Campus, Cary, NC, USA) and S-Plus (version 9.0) statistical packages.

Results and Discussion

Most study patients (59.2%) had positive results for one pesticide residue only. Pesticide residue could not be detected in 24.8% of the tested women, whereas two residues (p,p'-DDE + HCB) were identified in 16% of the patients. Dichlorodiphenyltrichloroethane (DDT) was identified and quantified in only one sample, at a level of 2.9 ppb. Dichlorodiphenyldichloroethylene (DDE) was present in 76% and hexachlorobenzene (HCB) in 16.8% of the study population. Organochlorine concentrations are pre-

Table 2 Prevalences and means of p,p'-dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB) serum levels in the study population

	Frequency (%)	Mean ± SD (ppb)	Median (ppb)	Maximum (ppb)
p,p'-DDE	76.0	3.83 ± 47.2	2.28	28.63
HCB	16.8	0.26 ± 0.64	0	2.92
Total pesticides		4.05 ± 4.95	2.35	28.63

sented in Table 2. Contamination increased with age. In fact, the number of detected residues increased significantly with age ($p = 0.029$), and total pesticide concentration was highly correlated with age ($r = 0.35$; $p = 0.0004$). Our study results failed to establish a positive association between smoking and organochlorine positivity. Surprisingly, total pesticide concentration was independent of BMI ($p = 0.235$). Concentrations were similar in normal and overweight (BMI > 25 kg/m^2) patients.

Table 3 displays total pesticide concentrations according to breast tumor characteristics. No significant association with estrogen receptor presence, histologic status, Bloom grade, or TNM classification could be shown. Positive concentrations of residue serum were similar in patients who received chemotherapy and those who did not. In contrast, the concentrations were significantly higher in relapsing patients than in nonrelapsing patients (7.8 ± 6.2 vs 3.6 ± 4.7 ppb; $p < 0.005$). When tested after adjustment for age, organochlorine residue concentrations remained significantly higher in relapsing patients ($p = 0.037$).

During the past 12 years, a series of prospective nested case-control, retrospective population-based case-control, and retrospective hospital-based case-control studies have been conducted to determine the association between EDC exposure and the increased incidence of breast cancer. Most of the results from these studies indicate a lack of association between exposure to these compounds and the development of breast cancer. However, *in vitro* experiences and laboratory animal testing have shown the potential impact of EDCs on the regulation of hormone-induced responses, especially when mediated by estrogen receptor (ER) or androgen receptor (Atanassova et al., 2000; Sharpe et al., 1995; Welch et al., 1978). Studies have indeed suggested that breast cancer risk factor profiles may vary according to combined ER and progesterone receptor status, but most of the published literature has reported weak or null associations (Rusiecki et al., 2004).

Such conflicting results are questionable and probably can be explained by the bias in selection of populations or measurement of parameters. Because breast cancer incidence still is increasing (Wolff et al., 1996), and because known etiologic factors can explain only less than 20% of breast tumors, it is estimated that more than 80% of the risk for breast cancer, as for most cancers, can be associated with environmental factors, which include exposure to contaminants, lifestyle, and diet (Safe, 2005). Clearly, further research is necessary for clarification of the potential associations.

It is suspected that prenatal exposure to EDCs is implicated in the development of endocrine-related cancers, and probably is a potential explanation for some of the observed cancers (Birnbaum and Fenton, 2003). Alternative pathways, regardless of ER status, have been described to explain potent intracellular effects of EDCs (Walsh et al., 2005). Genetic polymorphism in the cytochrome P450 1A1 gene (CYP 1A1) also may affect the relation between EDC exposure and breast cancer risk (Zhang et al., 2004).

In this study, we compared serum concentrations of organochlorine residues in women with breast cancer using different tumor characteristics as potential

Table 3 Comparison of p,p'-dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB) serum levels based on breast tumor characteristics

	n	Total pesticide concentration (ppb)	
		Mean ± SD	Range
Histologic status			
Canalar carcinoma	108	4.35 ± 5.57	0–28.63
Lobular carcinoma	17	3.49 ± 4.94	0–14.40
Bloom stage			
I	23	3.97 ± 3.85	0–12.66
II	43	2.00 ± 2.43	0–9.30
III	23	6.15 ± 6.54	0–22.00
TNM classification			
T1	65	4.00 ± 5.67	0–28.63
T2	33	3.57 ± 3.41	0–16.10
T3	4	2.61 ± 2.51	0–4.90
T4	5	5.41 ± 6.67	0–14.40
N0	77	4.29 ± 5.60	0–28.63
N1	32	3.51 ± 3.40	0–12.66
N2	1	0	0
M0	102	3.97 ± 5.11	0–28.63
ER status			
Positive	70	3.92 ± 5.03	0–28.63
Negative	52	4.39 ± 4.99	0–22.00
Chemotherapy			
Yes	55	4.15 ± 4.74	0–21.94
No	70	4.00 ± 5.37	0–28.63
Relapse[a]			
Yes	14	7.76 ± 6.15	0–21.94
No	109	3.64 ± 4.66	0–28.63

TNM, tumor node metastasis; ER, estrogen receptor

[a] p = 0.037 after adjusting for age

explanation factors. If contamination increases with age, we failed to establish a positive correlation with smoking or elevated BMI. Our results do not support any association between organochlorine residues and histologic status, TNM, or Bloom grades, suggesting that the histologic profile of breast cancer may not be related to the etiologic factor. The findings show no association between ER-positive breast cancers and a higher level of organochlorines in serum.

The only positive finding in the current study was a strong association between the incidence of relapse and high serum levels of organochlorine residues, even after adjustment for age. Because this association has never been reported in previous studies, larger series of patients will be needed for its confirmation.

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