

Dioxin concentrations in women with endometriosis

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The concentrations of the environmental pollutant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were measured in the blood of 44 infertile women with endometriosis (study group), and in 35 age-matched women with tubal infertility (control group). Eight women with endometriosis (18%) were dioxin positive as compared to one woman (3%) in the controls ($P = 0.04$). Although the concentrations of dioxin did not seem to be directly correlated with the severity of endometriosis, these observations contribute to the accumulating data linking dioxin to endometriosis in humans.

Key words: dioxin/endometriosis

Introduction

The environmental pollutant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin) is a potent teratogen and carcinogen in rodents (Chahoud *et al.*, 1989). Dioxin has also been incriminated as a carcinogen in humans (Fingerhut *et al.*, 1991; Huff *et al.*, 1994).

In rhesus monkeys, chronic exposure to dioxin was directly correlated with an increased incidence of the development of endometriosis (Rier *et al.*, 1993). A positive correlation was observed between the severity of the disease and the daily and cumulative dose of dioxin administered (Rier *et al.*, 1993).

It has been recently reported that in Belgium the incidence and severity of endometriosis in women, as well as the degree of dioxin pollution, is amongst the highest in the world (Koninckx *et al.*, 1994). These observations raise the concern that dioxin may be involved in the pathogenesis of endometriosis in humans.

In view of the accumulating data, we carried out a preliminary study aimed at evaluating the concentrations of dioxin in the blood of women suffering from endometriosis as compared to women free from the disease.

Materials and methods

Patients

The study included 79 women who were evaluated for infertility in Bikur Cholim Hospital IVF unit during 1991–1995. All women

underwent laparoscopy and in 44 patients (endometriosis group) endometriosis was diagnosed. The severity of the disease was classified according to the scalar scoring system of the American Fertility Society (American Fertility Society, 1985). The control group comprised 35 women with mechanical infertility. In these women no evidence of endometriosis was found at laparoscopy. They had had ectopic pregnancy, salpingectomy and pelvic inflammatory disease.

All women in the study had resided in the Jerusalem area during the previous 10 years, and had a similar socioeconomic status. An informed consent was obtained from all patients who participated in the study.

For dioxin and triglyceride analyses, approximately 25 ml of blood was obtained from each woman.

Dioxin determination

Blood specimens for dioxin analysis were frozen at -20°C and transported to the residue control laboratory. Dioxin was extracted and quantitated by gas chromatography (GC) and mass spectrometry (MS) essentially as previously described (Lamparski *et al.*, 1979; Firestone, 1991) with certain modifications: 20 ml of blood was extracted three times with 15 ml *n*-hexane:acetone (9:1 v/v). The combined extracts were concentrated by evaporation and applied to solid phase extraction columns. Dioxin was eluted with methylene chloride which was evaporated to dryness; the residue was dissolved in 0.05 ml of toluene immediately prior to GC analysis. Primary identification and determination of dioxin by GC was performed using a Hewlett-Packard 5890 unit (Hewlett-Packard Co., Atlanta, GA, USA) equipped with DB-5 capillary column and with a ^{63}Ni electron capture detector. Dioxin identity was confirmed by a Hewlett-Packard 5989A GC-MS instrument equipped with a computerized data analysis system, in negative ion chemical ionization mode, using methane as the reagent gas. The operating conditions were as follows: the source temperature was maintained at 200°C and a quadropole at 100°C , injector temperature was 270°C , detector temperature 280°C , programmed oven temperature increase from 100 to 200°C at a rate of $30^{\circ}\text{C}/\text{min}$ and from 200 to 240°C at a rate of $15^{\circ}\text{C}/\text{min}$. Nitrogen and helium were used as carrier gases. Dioxin identification was accomplished using ion chromatograms for mass to charge (m/z) ratios 319, 321, 323. Mass units and their relative abundance were calibrated with perfluorotributylamine.

Dioxin standard solution (10 ppm) was purchased from Eastman Chemical Co. (Rochester, NY, USA).

Statistical analysis

The statistical analysis was performed using SPSS 6.1 for Windows on a 486DX-50 PC. The association between endometriosis and presence of dioxin was analysed by the Fisher's exact test. χ^2 test was applied to test all other associations among categorical variables. In tables with an expected cell frequency <5 , exact P -values were obtained using StatXact 2.04. Differences in age and in triglycerides were evaluated by the two-sample t -test. These were preceded by Levene's test for equality of variances in order to determine the appropriateness of use of pooled variance estimate. Logistic regression

Table I. Patients' characteristics in study groups and amongst women found positive for dioxin

	No. of women	Age (years)	Ethnic origin (%)			Triglycerides (nmol/l)
			Ashkenazi	Sefardi	Muslim	
Endometriosis	44	33.6 ± 6.7	21 (48)	20 (45)	3 (7)	1.3 ± 0.7
Control	35	34.4 ± 5.8	7 (20)	27 (77) ^a	1 (3)	1.7 ± 1.2
Endometriosis dioxin positive	8 ^b	33.4 ± 6.4	2 (25)	6 (75)		1.3 ± 0.6
Control dioxin positive	1	37			1	1.1

Values are mean (SD).

^aStatistically significant difference in distribution of Sefardic women between control and endometriosis groups ($P = 0.02$).

^bStatistically significant difference in ratio of women positive for dioxin between control and endometriosis groups ($P = 0.04$).

Table II. Dioxin levels in blood of dioxin-positive women

Patient	Disease stage	Dioxin concentrations (ppt) ^a
Endometriosis dioxin-positive		
1	I–II	0.7
2	I–II	0.8
3	I–II	1.2
4	III–IV	0.6
5	III–IV	0.8
6	III–IV	1.0
7	III–IV	1.1
8	III–IV	1.2
Control dioxin-positive		
1		0.4

^appt = parts per trillion.

analysis was performed to control for potential confounding effects of ethnicity.

Results

Eight out of 44 women with endometriosis (18%) had blood samples positive for dioxin as compared to one woman (3%) in the control group (Table I, $P = 0.04$, odds ratio = 7.6, 95% confidence interval: 0.87–169.7). The concentrations of dioxin in blood are represented in Table II.

In both groups, the women's average age and socioeconomic status were matched. However, the ethnic distribution among controls differed from that of patients with endometriosis, as 77% of the controls and 44% of the endometriosis group were Sefardic, i.e. of Middle Eastern origin (Table I, $P = 0.02$).

Since dioxin is lipid soluble, it was obligatory to measure the concentrations of blood triglycerides in the women who participated in the study. As shown in Table I, in the endometriosis and control groups no significant differences in blood lipids were observed. Body-mass indexes were also similar (23.9 ± 4.7 , 24.0 ± 4.5 respectively).

In the endometriosis group, 20 women (40%) had disease stage III or IV and 24 (60%) had stage I or II. Among the severe cases (stages III and IV), five women were found positive for dioxin. Among patients with disease stage I or II, three were dioxin positive (Table II).

Discussion

The aim of this preliminary work was to investigate the presence of dioxin in the blood of women suffering from endometriosis. Although the study groups were relatively small, among the endometriosis patients 18% were found positive for dioxin as compared to 3% in the controls. This difference is statistically significant. It cannot be attributed to a higher solubility of dioxin in the blood of the endometriosis patients because triglyceride concentrations in both groups were comparable.

As all of our patients had lived in the same vicinity over a long period of time, we assume that they were exposed to similar amounts of environmental dioxin.

Although our study groups were not fully matched in the aspect of ethnicity, it did not interfere with the interpretation of the results. In the endometriosis group more Sefardic women were dioxin positive but in the control group, which consisted of 77% Sefardic women, none was found positive.

The concentrations of dioxin measured in our study are in agreement with those reported by Ott *et al.* (1993) for 102 individuals without known occupational exposure to dioxin. Although in Ott's report the mean values of dioxin concentrations were somewhat higher, this may be explained by the different characteristics of the study groups.

Since dioxin bioaccumulates in liver and adipose tissue (Firestone 1991), we cannot rule out the possibility that blood dioxin concentrations may not necessarily correlate with its distribution in body tissues. As body-mass indexes were similar and within the normal range in both groups, it seems that obesity did not interfere with the results. However, in our study, obtaining tissue biopsies was not justified. The amounts of dioxin in tissues ought to be investigated in the further search for a possible causal relationship between dioxin and endometriosis.

Among our patients we did not observe a correlation between dioxin concentrations in the blood and the severity of endometriosis (Table II). Nevertheless, one may speculate that the degree of sensitivity to dioxin might vary among different individuals. Whether the dioxin-positive patients exhibit increased rate of accumulation, decreased degradation capacity, or both, remains unanswered. Furthermore, since other environmental pollutants or toxic substances were not

analysed, it is not clear whether the presence of dioxin reflects a specific or rather a general impairment.

Our observations contribute to the accumulating data linking dioxin to endometriosis. Assuming that the prevalence of endometriosis in the general population is 10% (Olive, 1993), in order to detect a two-fold increase with a power of 90% and significance level of 0.05, 286 dioxin positive women and 286 controls need to be evaluated.

The diversity of biological effects resulting from exposure to dioxin is thought to reflect its ability to alter gene expression through binding to the aryl hydrocarbon receptor (Whitlock, 1990). In humans dioxin modulates gene expression of cytochrome P450, plasminogen activator inhibitor-2 and interleukin 1 (Whitlock, 1990). It affects the reproductive system through altering oestrogen, progesterone and prolactin receptor activities (Safe *et al.*, 1991). In addition, dioxin is a potent inhibitor of T lymphocyte function (Neubert *et al.*, 1991; Tomar *et al.*, 1991). The combination of sex steroids, growth factors and impaired immune response has been implicated in the initiation of endometriosis, although the exact mechanism by which ectopic endometrium attaches to the peritoneum has not yet been identified (Olive 1993). Because the aetiology of endometriosis seems to be multifactorial, it is suggested that the presence of dioxin in a certain proportion of endometriosis patients may be regarded as a marker of an affected immune system, or imbalance in the activities of sex hormones and growth factors.

Since dioxin is considered metabolically stable (Chahoud *et al.*, 1989), it seems reasonable to speculate that even low concentrations of continuously activated aryl hydrocarbon receptors suffice to maintain the long-term biological effects of dioxin.

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