Case study

Residue levels of DDE and PCBs in the blood serum of women in the Port Said region of Egypt

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Abstract

An investigation was conducted to detect residues of 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE) and polychlorinated biphenyls (PCBs) in blood serum samples collected from a cohort of fasting females attending the health insurance outpatient clinic at Port Said between July 1999 and July 2000. Females involved in the study included 43 females diagnosed with invasive adenocarcinoma of the breast, 21 female suffering benign breast disease, and 11 normal healthy females. Serum was separated and its contents of DDE and PCBs were extracted and determined, using gas chromatography, equipped with electron capture detector. Mean residues of DDE detected in the three examined groups of females were 41 ± 5.2, 48 ± 6.2 and 31 ± 2.5 ng/g for breast cancer cases, benign breast disease cases and controls, respectively, indicating some significantly less residues in blood serum of control females. While PCBs residues detected were 54 ± 17, 59 ± 23 and 61 ± 21 ng/g, for the three groups, respectively. Residues of DDE detected in all females alike in the present study are about 15 times higher than residues detected in Canada and The Netherlands. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Organochlorine chemicals are among the most persistent environmental contaminants. Some of these chemicals, including 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (DDE) and polychlorinated biphenyls (PCBs),

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have been suggested to be significantly important in the etiology of breast cancer [1,2]. Various investigations have been performed to evaluate the involvement of these chemicals in initiating breast cancer. Moysich et al. [2] suggested that an increase in the risk of post-menopausal breast cancer is associated with environmental exposure to PCBs and Mirex, an organochlorine insecticide. Dorgan et al. [3], found that females with higher levels of hexachlorobenzene, an organochlorine insecticide, in their blood serum were at twice the risk of breast cancer compared with those with low hexachlorobenzene level. However, there was no evidence of a dose–response relationship, and the association was limited to females whose blood was collected close to time of diagnosis.

Breast cancer is a major public health concern. According to Moller et al. [4], it constitutes about 33% of all tumours affecting females, with an estimated 135,000 new cases and 58,000 recorded deaths per year in The European Union. In Egypt, Abou El Nasr and Botrous [5] mentioned that breast cancer constitutes 27% of all reported malignancy cases. In a study conducted in Alexandria, Egypt, El Sebaie and Soliman [6] revealed that breast cancer is the most frequent type of cancer affecting females population.

The present investigation was conducted to monitor residues of DDE, the primary metabolite of DDT and PCBs in blood serum of different groups of females that include breast cancer patients, benign breast disease (benign mass) cases and control females. The main objectives were to provide baseline information on the levels of DDE and PCBs in blood serum and a case-control study nested in a cohort, of females attending the health insurance outpatient clinic at Port Said between July 1999 and July 2000 was performed. Females involved in the study included 43 females diagnosed with invasive adenocarcinoma of the breast, who served as cases, 21 female suffering benign breast disease fibrocystic and 11 normal healthy females, who served as control. Females selected in this study were mainly married, with an average age of about 45 years, with comparable background and style of life. Residues of DDE and PCBs in the sample was determined, using electron capture gas liquid chromatography.

2. Materials and methods

2.1. Blood collection

Blood samples were collected from fasting females. One blood sample was collected from each female. Samples, each of 10 ml were collected in clean glass tubes, and no coagulant was added. Serum was separated within 1 h by centrifugation at $1000 \times g$ for 10 min and a 2 ml sample was used for PCBs and DDE determination.

2.2. Extraction and clean-up

Residues of PCBs and DDE were extracted from serum samples according to the method reported by Dale et al. [7], 2 ml serum was mixed with 6 ml hexane in a glass stoppered test tubes. Tubes were placed on a rotor running at 50 rpm for 2 h. Hexane was transferred into a 10 ml graduated concentrator tube and the extract was evaporated in a hot water bath at 40°C. The tube was allowed to cool down at room temperature, residues were quantitatively

transferred into a glass vial ready for separation of DDE and PCBs using adsorption column chromatography.

2.3. Adsorption column chromatography

Extracted residues were fractionated to separate PCBs and DDE using a method based on that reported by Mes et al. [8], with some modifications. Serum extract was loaded on top of a Florisil, silisilic acid column and the column was eluted with 25 ml hexane (I), and again with 30 ml of hexane-dichloromethane 1:1 mixture (II). Fraction (I) contained PCBs while fraction (II) contained DDE residues.

Each fraction was concentrated to about 1 ml using a rotary evaporator, then residues were quantitatively transferred with some hexane into a glass-graduated tube ready for injection into a gas chromatograph.

2.4. Gas chromatography

A Hewlett-Packard, model 5890 gas liquid chromatograph equipped with an ^{63}Ni electron capture detector was used to monitor residues of DDE. A capillary column PAS 5, 30 m i.d., 0.32 mm i.d. was used. Injector temperature was 270°C, and detector temperature was 300°C. The temperature program used was as follows: initial temperature 160°C, held for 1 min, with a rate of increase of 5°C/min, and the final temperature 260°C held for 20 min. Nitrogen was used as a carrier and make up gas running at a flow rate of 3 ml/min. Splitless, on column injection of 3 μl was performed throughout.

2.5. Quantification

Standard curves of DDE and PCBs were constructed by plotting the peak areas against concentrations (external standard). Good linearity was observed over 200-fold range (0.1–20 ng).

Determination of PCBs contents was based on the general pattern shown by the standards. Peak identification was performed using the relative retention time (RRT) of DDE. PCBs were quantified by measuring the peak heights of corresponding peaks in samples and standards.

2.6. Reagents and analytical standards

Organic solvents used in the present study were glass distilled or analytical grade (Merck, Dermstadt, Germany). Authentic DDE and PCBs analytical standard were kindly supplied from Central Laboratory for Pesticides (Agricultural Research Centre, Dokki, Cairo).

2.7. Validation study

Samples of serum were fortified with standard solutions of DDE in hexane (10 mg/l) at three levels. To 1 ml serum 10, 100, and 250 μl standard solutions were added. Final

concentration of DDE in serum was 0.1, 0.5, and 2.5 μg/l. At each fortification level three replicates were made. Fortified samples were extracted as described earlier and the obtained average of recovery rates was 91% (standard error of the mean (S.E.M.), 4.1).

Meanwhile available samples were not sufficient to run a recovery study for PCBs. However, previous PCBs assessment, using the same methodology gave a recovery rate of 87%. In the present study, results were not corrected according to recovery rates.

3. Results and discussion

In PCB monitoring studies, de Voogt et al. [9] suggested that quantification via pattern comparison can be done in a variety of ways. Either one can take into account all peaks in the chromatogram, or one selects individual peaks. They added that the latter method is less time consuming, but a proper selection must be made to avoid biased results. In the present study, PCBs contents were based on selecting individual peaks and PCBs exposure was examined as total number of detected PCBs peaks. A selection of 29 peaks was made, based on resolution, abundance and retention times (see Table 1). Identification of PCB peaks was based on the peaks RRT to that of DDE, allowing a difference of 0.1 RRT, with peaks in the standard PCBs injected earlier. Table 1 shows the RRTs of selected peaks in relation to the retention time of the internal standard DDE. Mean residues of DDE, PCBs, their range and frequencies of detection in the three examined groups of females are shown in Table 2. Mean DDE residues detected were 41 ± 5.2, 48 ± 6.2 and 31 ± 2.5 ng/g for breast cancer cases, benign breast disease cases and the control groups, respectively, indicating significantly lower residues of DDE ($P = 0.03$) in the control group, in comparison to the other two groups of females.

Table 1
Relative retention times of PCBs (Aroclor 1254) peaks relative to DDE retention time[a]

Peak no.	RRT	Peak no.	RRT
1	0.712	16	1.05
2	0.720	17	1.06
3	0.761	18	1.08
4	0.790	19	1.12
5	0.827	20	1.14
6	0.857	21	1.15
7	0.868	22	1.17
8	0.883	23	1.20
9	0.910	24	1.23
10	0.925	25	1.28
11	0.938	26	1.30
12	0.979	27	1.38
13	0.992	28	1.42
14	1.002	29	1.46
15	1.020		

[a] Retention time of DDE, 11.43 min. Peaks used for quantitation of PCBs, see text for chromatographic conditions.

Table 2
Mean residues of DDE and PCBs detected in blood serum collected from breast cases, benign breast disease cases and control females at Port Said, Egypt (ng/g)

Compounds	Group of females	Number (n)	Mean residue (ng/g)	Range
DDE	Breast cancer	43	41 ± 5.2	25–82
	Benign breast disease	21	48 ± 6.2	19–73
	Control	11	31 ± 2.5	12–44
PCBs	Breast cancer	43	54.9 ± 7.3	43–104
	Benign breast disease	21	59.2 ± 5.6	36–101
	Control	11	61.9 ± 8.3	45–109

Corresponding residues of PCBs detected in the three examined groups were 54 ± 17, 59 ± 23 and 61 ± 21 ng/g whole serum, there being no significant difference in the concentration levels.

Fig. 1 shows the frequencies of DDE and PCBs detection in the three examined groups. The relative number of females with DDE detected in their blood serum is rather similar in the three groups. Frequencies of DDE detection were 72, 71 and 72% in the breast cancer group, benign breast disease group and the control group, respectively. However, variation in exposure intensity conditions and other factors possibly explain, why some women developed cancerous diseases while others did not.

PCBs were less frequent than DDE in the three examined groups of females. The comparative distribution of PCBs group peaks in the breast cancer cases, benign breast disease and control groups is shown in Fig. 2, while the average concentrations of each peaks group per female is shown in Table 3. Results indicate some uneven frequencies of PCBs peaks in the three examined groups with a higher frequency and concentration of group (IV) peaks in breast cancer cases in comparison to the other cases. Unfortunately, there are no established frameworks for grouping a large number of PCB congeners into meaningful analytic
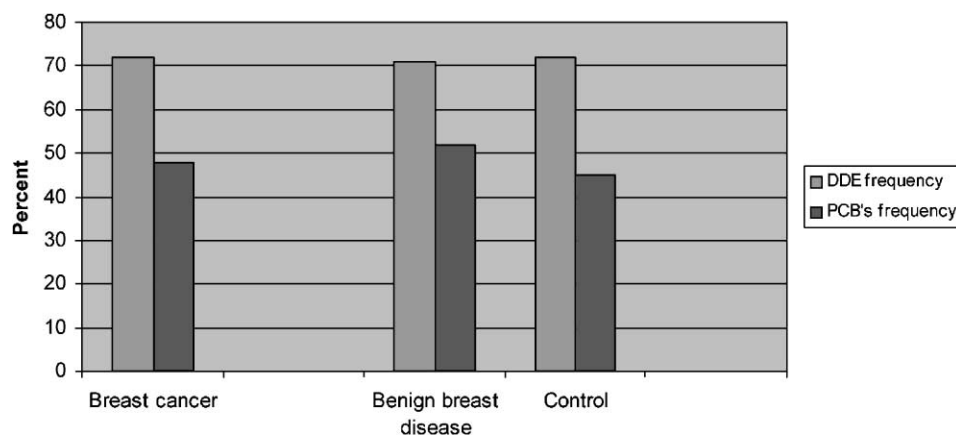

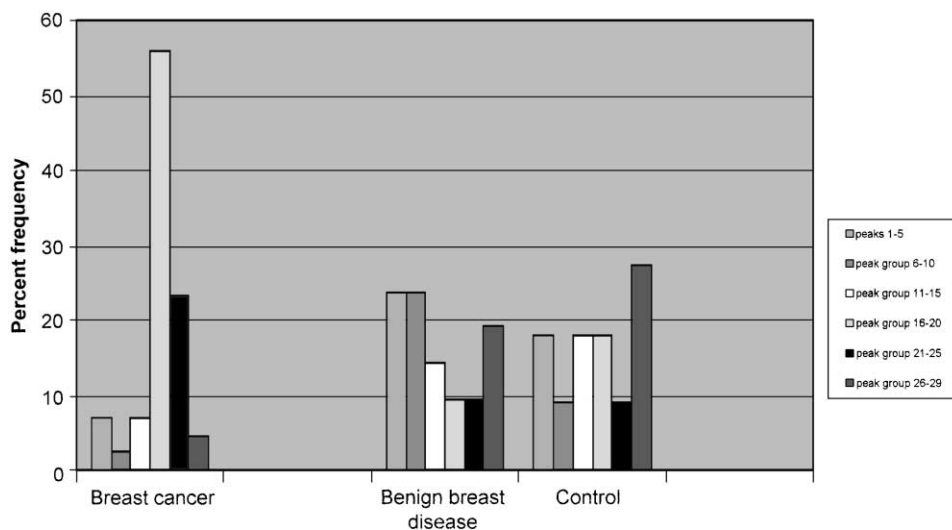Fig. 1. Frequency of DDE and PCBs detection in breast cancer cases, benign breast disease and control.

Fig. 2. Frequency of PCBs peak groups detected in blood serum in breast cancer cases, benign breast disease and control.

units. Moysich et al. [10], discussed several approaches for grouping PCBs based on chlorination, factor analysis, enzyme induction along with some other factors. They concluded that grouping with respect to degree of chlorination, enzyme induction, occurrence, were the most useful approaches to reducing a large number of PCB congeners into meaningful analytic units. Interpretation of such variations in the PCBs residue pattern in the examined groups is beyond the scope of this research. However, it may reflect some differential exposure and/or metabolism of these xenobiotics in human body and the relationship between metabolic pathways and some other factors.

Residues of DDE detected in the present study are about 15 times higher than those reported in other studies [8,11]. Such high levels of DDE detected in all females involved in this study is consistent with the extensive use of organochlorine pesticides in Egypt [12–14]. In the present study, residues of DDE detected in control females are lower than residues detected in the other two groups, though this is of a borderline ($P = 0.03$) statistical significance type. Moreover, the comparable concentrations of DDE in the breast cancer

Table 3
Average concentrations per person of group peaks of PCBs detected in blood serum of breast cancer cases, benign breast disease and control (ng/g)[a]

Serum sample	I	II	III	IV	V	VI
Breast cancer cases	3.4	5.2	4.2	19.6	9.7	0.4
Benign breast	5.1	6.1	6.9	7.6	7.2	8.3
Control	6.5	8.2	7.6	6.7	5.3	9.2

[a] I: peaks from 1–5; II: peaks from 6–10; III: peaks from 11–15; IV: peaks from 16–20; V: peaks from 21–25; VI: peaks from 26–29.

cases and the benign breast disease cases are inconsistent with the idea of DDE residue, as an elemental factor in the etiology of breast cancer. Such a conclusion is shared by a number of workers [11,15]. In Egypt, El Shiekh et al. [16] found levels of DDT residues in human milk in rural and urban communities around Ismailia are quite comparable to residues detected in breast cancer patients involved in the present study. None of the females with higher residues in their milk, however, had cancer at the time of study. This may imply that residue level is not the main contributor to breast cancer. Genetic factors may be more important. Studies involving much larger cohort of females would be required to delineate the main factors.

4. Conclusion

Studies on priority pollutants and their residue level in blood are rather meagre in Egypt. The present study is an attempt to provide baseline information on the levels of DDE and PCBs in blood serum of groups of females in the Port Said area of Egypt and to assess whether the concentrations of these chemicals varied significantly between the three groups studied. PCBs residue levels were 54 ± 17, 59 ± 23 and 61 ± 21 ng/g whole serum in the breast cancer patients, benign breast disease and the control group, respectively; the differences are not statistically significant, but the distribution of PCB peak groups for the groups of females showed differences. The corresponding DDE residues levels were 41 ± 5.2, 48 ± 6.2 and 31 ± 2.5 ng/g, respectively; levels for the control group were about 30% lower (significance level $P = 0.03$).

Although concentrations of DDE and PCBs for women in the Port Said region are about 15 times higher than residues detected in blood samples taken from Canadian and Dutch females, our findings suggest that residues of DDE or PCBs are not the main cause of breast cancer. Other factors may be equally, or more important for the incidence of cancer, but studies involving much larger groups of females would be needed to draw a definite conclusions.

Acknowledgements

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