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Serum organochlorine pesticides residues and risk of cancer: A case-control study

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ABSTRACT

Organochlorine pesticides (OCPs) are frequently used worldwide as insecticides, fungicides, herbicides and termiticides and have been associated with a variety of cancers in animal and human studies. In the present study, we examined residues of fourteen OCPs in the serum samples of diagnosed cancer patients and healthy residents of Karachi, Pakistan. A random collection of fasting blood samples was carried out from the donors with informed consent. Serum was separated within 2 h of blood collection and was then subjected to extraction with organic solvents followed by purification with florisil column. The final organic extract of each serum sample was processed with Gas Chromatograph coupled with Electron Capture Detector (GC-ECD). OCPs were detected in 97.59% of the cancer cases and 93.75% of the healthy subjects. Mean concentrations of total OCPs (ΣOCPs) was found elevated in the cancer group (0.606 mg/kg) compared with the control group (0.322 mg/kg). Endosulfan was the highest prevalent OCP with a mean concentration of 0.214 mg/kg in the cancer group and 0.166 mg/kg in the control group. The second most prevalent OCP was 4,4-DDE with a mean concentration of 0.131 mg/kg in the cancer group and 0.019 mg/kg in the control group. Highest level of ΣOCPs was detected in the breast cancer cases (20.411 mg/kg) with a mean level of (2.041 mg/kg). In light of the obtained results and available literature on the subject, it has been concluded that OCPs are positively associated with the risk of various cancers in humans.

1. Introduction

Organochlorine pesticides (OCPs) are synthetic organic compounds used worldwide as insecticides, herbicides, fungicides and termiticides for agricultural and household purposes. OCPs have been associated with a variety of cancers in humans (Arrebola et al., 2015; Koutros et al., 2015; Prada et al., 2016; Louis et al., 2017). These chemicals are highly resistant to breakdown, persist for longer times in the environment and bodies of living organisms. Half-life of DDE in soil is estimated to be higher than 20 years (ATSDR, 2002). Biological half-lives of several years have been reported for various OCPs (Morgan and Roan, 1971). OCPs are highly soluble in fats, bioaccumulate and biomagnify in the fatty tissues of animals and are converted into metabolites which are more harmful than their parent compounds.

Dieldrin, a commonly used OCP has been associated with various cancers in humans such as lung cancer (Purdue et al., 2003),

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pancreatic cancer (Clary and Ritz, 2003), breast cancer (Hoyer et al., 1998), and NHL (Quintana et al., 2004). Aldrin, DDT, Dieldrin, heptachlor, heptachlor epoxide are classified as group B2 carcinogens by EPA (EPA, 1998). Endosulfan damages human RBCs at concentrations ranging from 1 ppb to 1 ppm (Daniel et al., 1986). Endosulfan is carcinogenic in humans and causes breast cancer (Romeo and Quijano, 2000; Ibarluzea et al., 2004). A number of studies to date have reported the potential association between exposure to OCPs and risk of various cancers, e.g. breast cancer (Wolff et al., 1993; Ward et al., 2000; Clark and Snedeker, 2005), lung and skin cancers (Zahm and Ward, 1998), pancreas, liver and prostate cancer (Dharmani and Jaga, 2005) and cancers of the brain, kidney, leukemia and lymphoma (Infante-Rivard and Weichenthal, 2007). In Pakistan, prevalence of cancer is higher in urban areas compared to rural areas. One reason behind this may be the high exposure of citizens to increased levels of chemicals and environmental pollutants. Quantity of banned pesticides in Pakistan has been estimated to be approximately 3805 tons in Punjab, 2016 tons in Sindh, 179 tons in Khyer Pakhtunkhwa and 128 tons in Balochistan while the Federal Department of Plant Protection has a stock of 178 tons out of which 2.54 tons are OCPs (Ahad et al., 2010). Pesticides consumption in Pakistan increased from 665 tons in 1980 to about 130,000 tons in 2004 (Planning and Development Division of Pakistan, 2006). Residues of OCPs in various biological matrices of humans across Pakistan have been reported by Azmi et al. (2006); Soomro et al. (2008); Khawaja et al. (2012) and Attaullah et al. (2016). OCPs are reported recently in a local community from Vehari District, Pakistan with farmers being at high risk (Saeed et al., 2017). Role of OCPs in the etiology of cancer in non-occupational subjects has not yet ascertained in Pakistan. The increasing trend in the frequencies of various cancers has raised concerns in the scientific community to investigate on every possible cause of the subject. Environmental pollutants like OCPs have been associated with the risk of various cancers and their role has yet to be ascertained. The present study was aimed to comparatively evaluate the residue levels of fourteen Organochlorine Pesticides in the serum samples of diagnosed cancer patients with various malignancies and healthy human subjects. This will through light on the possible association of these chemicals with various cancers in humans.

2. Materials and methods

2.1. Study cohort

The study cohort consisted of 83 cancer cases and 32 healthy subjects with no past history of exposure to OCPs. The cancer cases were categorized based on the major cancer sites and sub sites of the body. Eight major cancer sites (Oral Cavity and Pharynx, Digestive System, Respiratory System, Breast, Skin, Female Genital System, Blood and Lymphatic System) and their respective sub sites were studied for the determination of OCPs in the cancer samples. The studied cohort was divided into five age groups (15–28, 29–42, 43–56, 57–70 and 71–84 years) for the assessment of OCPs in various age groups. Fasting blood samples (8 ml each) were collected from diagnosed cancer patients and healthy residents with informed consent at various hospitals of Karachi, Pakistan. Gel clot activator tubes were used for blood collection.

2.2. Extraction, clean up and quantification of pesticides

Extraction, cleanup and quantification of OCPs in the serum samples through Gas Chromatograph coupled with Electron Capture Detector (GC-ECD) was carried out in light of the previously described methods (Atuma and Aune, 1999; DeCaprio et al., 2000; Moreno Frias et al., 2001). Guidelines of the USEPA methods 8081A (USEPA Method, 1996a), 8081B (USEPA Method, 2007a), 3620C (USEPA Method, 2007b) and 3665A (USEPA Method, 1996b) were followed for the extraction and clean-up of the OCPs. Concentrations of 14 OCPs and their metabolites (α-HCH, β-HCH, γ-HCH, δ-HCH, Heptachlor, Hepta-exo-epoxide, Hepta-endo-epoxide, Endosulfan, Aldrin, Dieldrin + Endrin, 4,4-DDD, 4,4-DDE, 4,4-DDT and Methoxychlor) were measured through GC-ECD at HEJ Research Institute of Chemistry, University of Karachi, Pakistan. Aliquot of 2 ml serum each was extracted with organic solvents (Methanol, n-Hexane and Diethyl Ether) in light of the previously described methods. The final organic extract (1 ml each) was mixed with 1 ml of H_2SO_4, agitated for 1 min and then centrifuged for 5 min at 2500 rpm. The organic phase was collected and the aqueous phase was extracted twice with 1 ml of n-Hexane. The organic phases were evaporated to dryness in a vacuum concentrator and then dissolved into 1 ml of n-Hexane.

The final extract (1 ml each) was dissolved in n-Hexane and purified through Column Chromatograph (42 cm × 6 mm) containing Florisil (1 g) topped with anhydrous Sodium Sulfate (1 g). The extracted samples were passed 2–3 times through the column for complete recovery of the OCPs and were then dissolved in 1 ml of n-Hexane for analysis on GC-ECD. Gas Chromatograph Model Schimadzu GC-17A equipped with ^{63}Ni Electron Capture Detector attached to a CBM-102 Chromatopak recorder system with ZB-5 Column (60 m × 0.53 mm × 1.5 μm) was used for the analysis of OCPs. Temperature program of the injector port (220 °C), oven 50 °C to 295 °C at 3 °C/min and detector (290 °C) with a split ratio of (1:×):4 was used. Column pressure was kept at 87 Kpa with a flow rate of 11.8007 ml/min. Nitrogen was used as the carrier gas at flow rate of 40 cm/s. Standard chromatograms were first obtained by processing 1 μl each of the standard mixtures of OCPs (0.1%) through GC-ECD. Sample extracts (1 μl each) were then processed with the same procedure. The detected OCPs were quantified with their respective peak areas and peak heights in the chromatograms while identification was carried out through retention times of the detected OCPs and comparison with the standard chromatograms. Fasting blood samples need no further adjustment for lipid contents and the calculated results were therefore expressed as milligrams of OCP residues per Kilogram of serum (mg/kg).

3. Results

The mean age for the cancer cases was higher (39.5 years) compared with controls (33.2 years) (Table 2). Male to Female Ratios in the cancer and control groups were 1.44 and 9.66 respectively. OCPs were detected in 97.59% of the cancer cases and 93.75% of the control group (Table 5; Fig. 5). Mean sum of total OCPs (ΣOCPs) was found significantly elevated in the cancer group (0.606 mg/kg) compared with the control group (0.322 mg/kg) (Tables 1, 5 and Figs. 2, 4). Endosulfan was the highest prevalent OCP with a mean level of 0.214 mg/kg and a frequency of 77.10% in the cancer group compared with a mean level of 0.166 mg/kg and frequency of 78.12% in the control group (Table 1). Mean concentration of 4,4-DDE in the cancer group was found to be 0.131 mg/kg in 14.46% of the cancer cases (Tables 1, 5). In the categorized age groups, highest mean level of ΣOCPs in the control subjects was detected in the age group 29–42 years with a mean level of 0.506 mg/kg while lowest was detected in the age group of 57–70 years with a mean level of 0.171 mg/kg) (Table 2). In the cancer cases, the trend of OCPs deposition in various age groups was different from the control group with highest mean level of OCPs (1.359 mg/kg) detected in the age group of 43–56 years and lowest mean level of OCPs (0.071 mg/kg) detected in the age group 71–84 years. No

Table 1
Detected mean levels of the studied OCPs in the cancer cases and controls.

S. no.	Name of OCP	Cancer cases			Controls		
		ΣX (mg/kg)	Mean (mg/kg)	SD	ΣX (mg/kg)	Mean (mg/kg)	SD
1	α-HCH	1.179	0.014	0.046	0.069	0.002	0.006
2	β-HCH	1.228	0.014	0.042	0.098	0.003	0.007
3	γ-HCH	0.256	0.003	0.014	0.056	0.001	0.004
4	δ- HCH	2.505	0.03	0.206	0.086	0.002	0.008
5	Heptachlor	4.436	0.053	0.159	1.608	0.05	0.124
6	Hepta-exo-epoxide	0.86	0.01	0.026	0.255	0.007	0.014
7	Hepta-endo-epoxide	1.302	0.015	0.113	0.276	0.008	0.027
8	Endosulfan	17.797	0.214	0.901	5.32	0.166	0.21
9	Aldrin	1.311	0.016	0.071	0.052	0.001	0.003
10	Dieldrin + Endrin	2.979	0.035	0.211	0.677	0.021	0.043
11	4,4-DDD	1.741	0.02	0.102	0.075	0.002	0.009
12	4,4-DDE	10.937	0.131	1.112	0.639	0.019	0.055
13	4,4-DDT	2.377	0.028	0.2	0.801	0.025	0.135
14	Methoxychlor	1.375	0.016	0.071	0.292	0.009	0.026
Total	ΣOCPs	50.27	0.606	1.789	10.307	0.322	0.334

OCP: Organochlorine pesticides; ΣX: Sum of the detected pesticides; SD: Standard deviation.

Table 2
Comparison of total OCPs in the age groups of cancer cases and controls.

Type of samples	Age statistics		ΣOCPs		
	Age groups (Yrs)	Mean age (Yrs)	ΣX (mg/kg)	Mean (mg/kg)	SD
Cancer cases	15–28	19.8	4.988	0.226	0.369
	29–42	35.6	6.907	0.246	0.6
	43–56	47.7	25.823	1.359	3.121
	57–70	63.6	12.355	1.123	2.307
	71–84	76.3	0.215	0.071	0.024
	Mean Total	39.5	50.288	0.606	1.789
Controls	15–28	22	4.815	0.283	0.339
	29–42	33.5	4.055	0.506	0.379
	43–56	47.3	0.536	0.178	0.193
	57–70	62	0.343	0.171	0.016
	71–84	79	0.557	0.278	0.352
	Mean Total	33.2	10.306	0.322	0.334

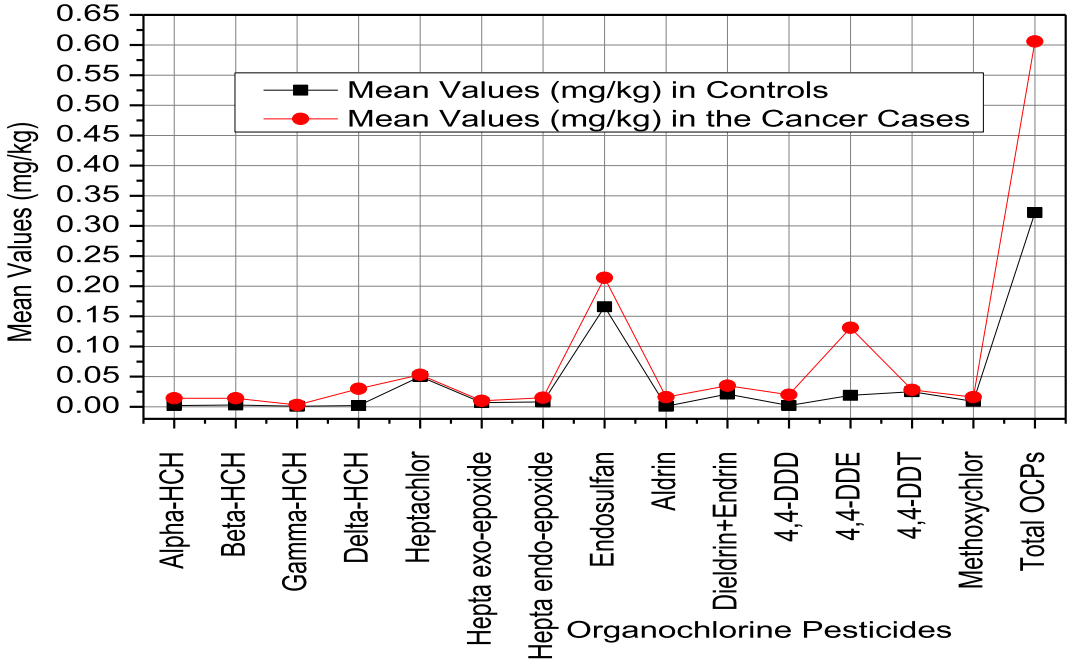

Fig. 1. Mean levels of individual OCPs in the cancer cases and controls.

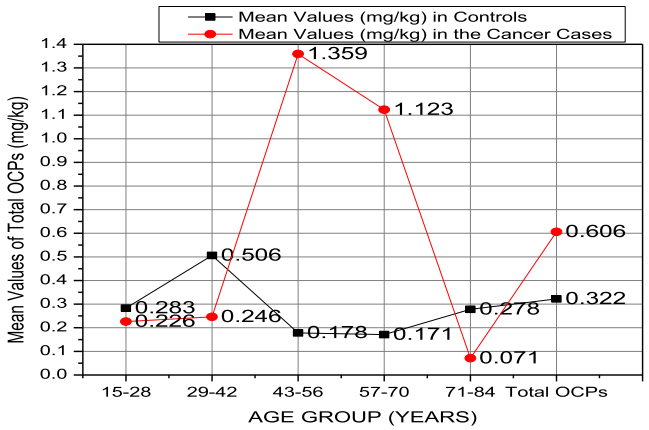

Fig. 2. Mean levels of total OCPs in the age groups of the cancer cases and controls.

linear trend of OCPs deposition was found with the increasing age in the studied cohort (Table 2 and Fig. 2).

In the major cancer sites, highest mean concentration of ΣOCPs was found in the breast cancer cases (2.041 mg/kg) while lowest was found in cases of the oral cavity and pharynx (0.092 mg/kg) (Table 3 and Fig. 3). In the sub sites of cancer cases, highest mean concentration of ΣOCPs was detected in the esophageal cancer (1.462 mg/kg) (Table 3 and Fig. 3). Amongst the individual OCPs, 4,4-DDE was the highest detected OCP with mean level of 1.017 mg/kg in the breast cancer cases followed by Endosulfan with a mean level of 0.537 mg/kg (Table 4 and Fig. 4).

4. Discussion

The studied cohort consisted of non-occupational subjects with no past history of exposure to organochlorine pesticides. The detected concentrations of OCPs in the cancer patients and healthy subjects reflect the prevalence of these chemicals in the environment. The elevated concentrations of OCPs in the cancer group compared with the control group authenticates the previous findings on the subject. Quintana et al. (2004) found higher mean residue levels in cases of NHL against controls for Dieldrin (0.24 vs. 0.20 ppm), Oxychlordane (0.20 vs. 0.14 ppm), Heptachlor epoxide (0.14 vs. 0.11 ppm) and HCB (0.05 vs. 0.04 ppm). The present study correlates with the cited study as the overall detection of individual OCPs was found higher in cases of NHL and other cancers compared with control subjects. Romieu et al. (2000) has reported a significant association between serum DDE levels and breast cancer risk with a mean serum DDE level of 3.84 *vs.* 2.51 μg/g lipids in the breast cancer cases and controls respectively. In the present study, mean serum 4,4′-DDE level of 1.017 mg/kg was detected in the breast cancer cases compared to 0.019 mg/kg in the control subjects (Tables 1, 4 and Figs. 1, 4). This correlates with the previous studies on the association of OCPs and risk of breast cancer. Kumar et al. (2010) has reported significantly higher levels of β-HCH, γ-HCH and p,p′-DDE in prostate cancer cases compared with controls ($p = .04$, 0.008, and 0.01, respectively). In the present study, the mean concentrations of HCH isomers and 4,4-DDE were found elevated in the cancer cases compared with the controls which authenticates the association of OCPs with the risk of cancer.

No linear trend of OCPs deposition with increasing age was observed in both the cancer cases and controls (Table 2 and Fig. 2). In the cancer cases, highest deposition of mean total OCPs was detected in the age group 43–56 years indicating a high risk of cancer incidence due to OCPs in this particular age group. High OCP concentrations above 50 years of age has also been reported by Ward et al. (2000) in the breast cancer cases. Stellman et al. (2000) has reported higher median concentrations of OCPs (ng/g of adipose tissue) in breast cancer cases *versus* controls, for β-HCH (19.8 *vs.* 15.8 ng/g), p,p′-DDE (419.2 *vs.* 374.1 ng/g), p,p′-DDD (16.4 *vs.* 13.3 ng/g), p,p′-DDT (12.3 *vs.* 12.1 ng/g) and total OCPs (628.6 *vs.* 546.9 ng/g). In the present study, level of 4,4-DDE in the cancer *versus* control group was found to be

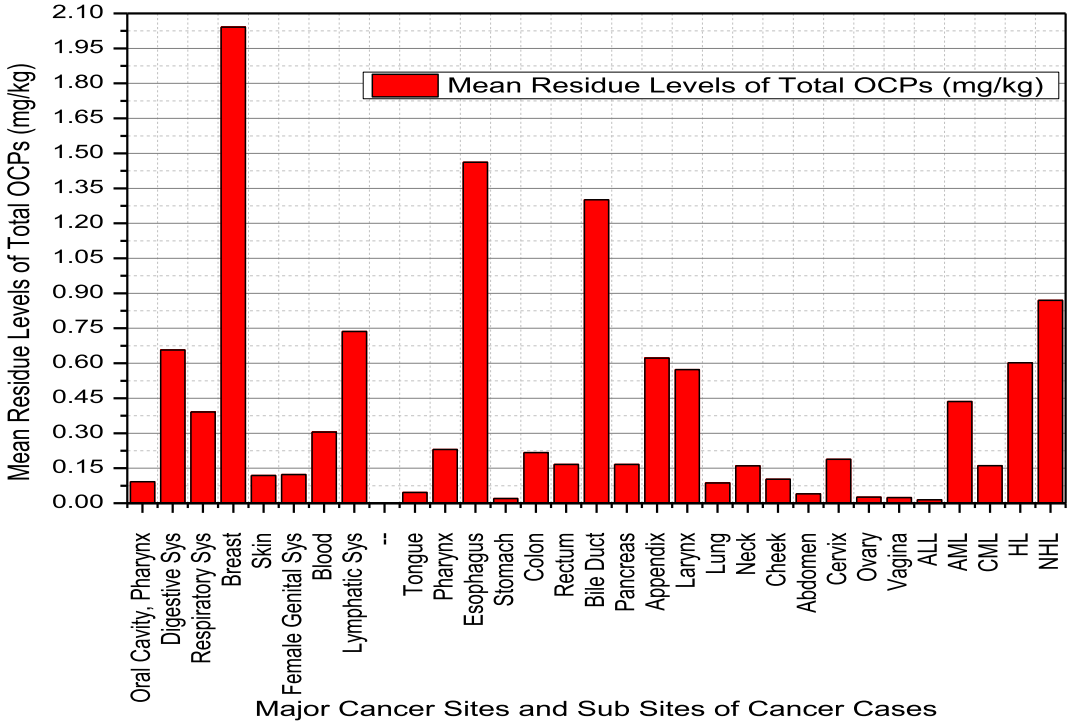

Fig. 3. Mean levels of total OCPs in the major sites and sub sites of cancer cases.

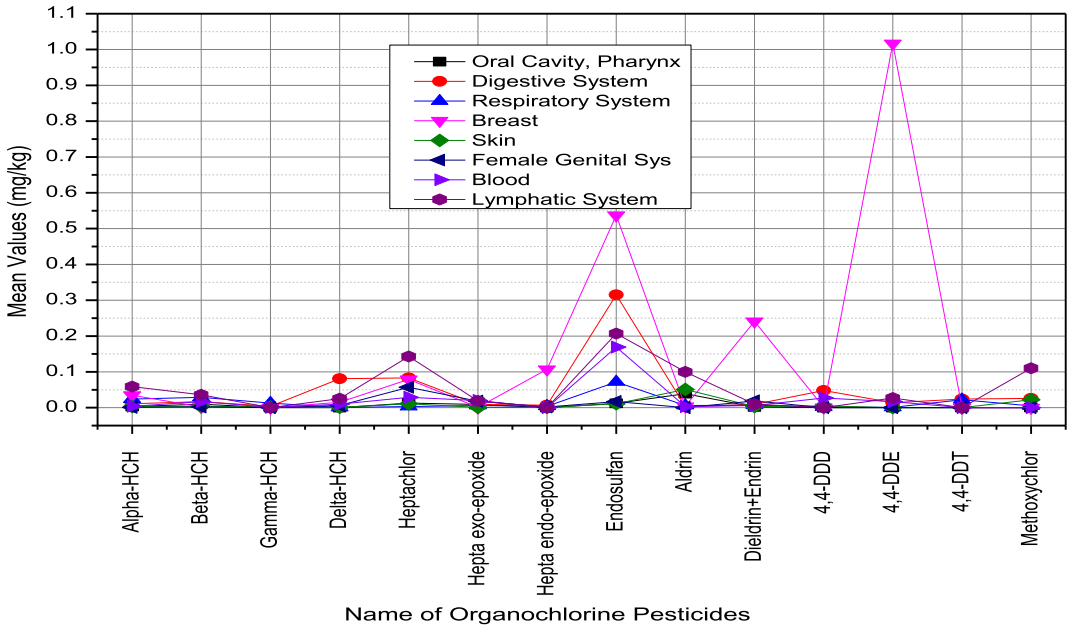

Fig. 4. Mean values of the individual OCPs in the studied major cancer sites.

Table 3
Concentrations of total OCPs (ΣOCPs) in the studied cancer sites.

Major cancer site	Sub site	ΣOCPs		
		ΣX (mg/kg)	Mean (mg/kg)	SD
Oral cavity & pharynx	Tongue	0.139	0.046	0.053
	Pharynx	0.23	0.23	0
	Mean total	0.369	0.092	0.101
Digestive system	Esophagus	10.237	1.462	2.72
	Stomach	0.04	0.02	0.021
	Colon	1.305	0.217	0.236
	Rectum	0.665	0.166	0.07
	Bile Duct	2.602	1.301	1.059
	Pancreas	0.333	0.166	0.038
	Appendix	1.245	0.622	0.808
	Mean total	16.427	0.657	1.517
Respiratory system	Larynx	2.867	0.573	0.767
	Lung	0.262	0.087	0.068
	Mean total	3.129	0.391	0.633
Breast	Breast	20.411	2.041	4.286
Skin	Neck	0.641	0.16	0.249
	Cheek	0.517	0.103	0.139
	Abdomen	0.04	0.04	0
	Mean Total	1.198	0.119	0.176
Female genital system	Cervix	0.566	0.188	0.165
	Ovary	0.026	0.026	0
	Vagina	0.024	0.024	0
	Mean Total	0.617	0.123	0.146
Blood	ALL	0.061	0.015	0.012
	AML	4.801	0.436	0.962
	CML	0.323	0.161	0.016
	Mean total	5.185	0.305	0.783
Lymphatic system	HL	1.205	0.602	0.604
	NHL	1.741	0.87	1.115
	Mean Total	2.946	0.736	0.748

1.017 mg/kg *vs.* 0.019 mg/kg indicating a significant association of 4,4-DDE and risk of breast cancer. Residues of Endosulfan were also found significantly elevated in the cancer cases but rest of the studied OCPs were mostly detected in lower concentrations in the breast cancer cases compared with controls showing somewhat different pattern from the report of Stellman et al. (2000). According to Cohn et al. (2007), high serum levels of DDT caused a 5 times increased risk of breast cancer among women who were exposed to DDT before puberty at 14 years of age. In this context, the detected DDT concentrations in the present study seem to have a significant association with the risk of breast cancer compared with other types of cancers. The presence of higher levels of DDT

Table 4
Mean levels of individual OCPs in the analyzed major cancer sites.

S. no.	Name of OCP	Major cancer sites with mean concentrations of OCPs (mg/kg)							
		Oral cavity, pharynx	Digestive system	Respiratory system	Breast	Skin	Female genital system	Blood	Lymphatic system
1	α-HCH	0.003	0.011	0.024	0.035	0.003	0.0007	0.004	0.059
2	β-HCH	0.01	0.017	0.029	0.002	0.004	0.002	0.017	0.036
3	γ-HCH	0	0.004	0.013	0.0002	0.002	0	0.001	0
4	δ- HCH	0	0.081	0.0005	0.015	0.001	0.006	0.01	0.025
5	Heptachlor	0.013	0.083	0.003	0.079	0.011	0.057	0.029	0.143
6	Heptaexoepoxide	0.009	0.009	0.006	0.001	0.001	0.018	0.02	0.019
7	Heptendoepoxide	0	0.007	0.004	0.107	0.001	0.002	0	0
8	Endosulfan	0.012	0.315	0.072	0.537	0.011	0.017	0.169	0.207
9	Aldrin	0.039	0.003	0.006	0.004	0.051	0	0.003	0.1
10	Dieldrin + Endrin	0.002	0.009	0.007	0.241	0.003	0.019	0.006	0.01
11	4,4-DDD	0	0.048	0.002	0.001	0.005	0	0.027	0
12	4,4-DDE	0	0.014	0.001	1.017	0	0	0.017	0.027
13	4,4-DDT	0	0.024	0.022	0	0	0	0	0
14	Methoxychlore	0	0.026	0.005	0	0.022	0	0	0.11

Table 5
Frequencies of the individual OCPs detected in the cancer cases and controls.

S. no	Name of OCP	Frequency (%) in the cancer cases	Frequency (%) in the controls
1	α-HCH	39.75	34.337
2	β-HCH	34.93	21.87
3	γ-HCH	18.07	31.25
4	δ- HCH	20.48	21.87
5	Heptachlor	55.42	31.25
6	Hepta exo-epoxide	31.32	46.87
7	Hepta endo-epoxide	15.66	12.5
8	Endosulfan	77.1	78.12
9	Aldrin	16.88	25
10	Dieldrin + Endrin	37.34	34.37
11	4,4-DDD	25.3	9.37
12	4,4-DDE	14.45	28.12
13	4,4-DDT	4.81	6.25
14	Methoxychlor	12.04	12.5
Total	ΣOCPs	97.59	93.75

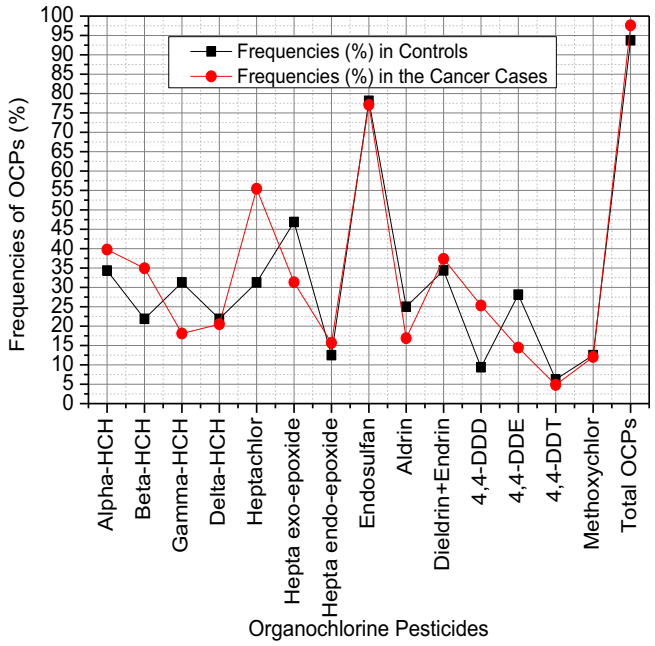

Fig. 5. Frequencies of the studied OCPs in the cancer cases and controls.

metabolites, HCH isomers, Heptachlor, Endosulfan, Aldrin and Diel drin + endrin in the cancer cases compared with the controls strengthen the possibility of a significant association of OCPs and risk of various types of cancers. Elevation in serum concentrations of OCPs may be due to bioconcentration of these chemicals as a result of weight loss in cancer patients as reported in previous studies (Chevrier et al., 2000; Imbeault et al., 2002). DDT and its metabolites are capable to cause *in vitro* DNA damage in human lymphocytes in such low concentrations which are normally found in the body fluids of human lymphocytes (Geric et al., 2012). Higher levels of DDT metabolites and other OCPs in cancer cases of the present study strengthens the assumption that these chemicals may be carcinogenic by damaging DNA and causing various malignancies in the body.

Although many of the OCPs have been banned for decades, the detected concentrations are nevertheless of interest as high body burdens indicate presence of these chemicals in various environmental compartments. Instead of occupational exposure, the present study was carried out in the general public in random with no past history of exposure to OCPs, representing the level of risk associated with these chemicals. The cohort as a whole in the present study was capable to cope with our primary research questions, however the sample sizes within cancer case groups were relatively small and high statistical significance was difficult to be achieved. The elevated concentrations of OCPs in the cancer group indicate a possible role and association of these chemicals with the etiology of various cancers in humans.

Organochlorine pesticides are positively associated with the risk of cancers particularly breast and blood cancers in humans. Endosulfan and 4,4-DDE have a more robust association with various cancers in humans compared with other studied OCPs. Deposition of OCPs in the human bodies does not increase linearly with increasing age. Serum OCP levels may increase with the severity of disease due to loss of fat contents and other confounding factors. Relation of OCPs with various cancers in humans is not statistically significant but yet important and undeniable. Further research is recommended to evaluate the role of OCPs in various cancers. The use of OCPs need to be mitigated and gradually substituted with environment friendly and biological control measures in agriculture and household uses.

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