ORGANOCHLORINE: EXPOSURE AND RISK OF BREAST CARCINOMA

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Abstract

Organochlorines (OCs) are known to have antiestogenic and estrogenic characteristic in vitro and in vivo. The most significant and considerable influence of estrogen in the development of breast cancer suggests that the exposure to these contaminants could have an impact on the promotion or initiation of breast carcinogens. This study was conducted in order to evaluate the influence of organochlorine on the breast cancer patients. A random collection of blood samples from diagnosed breast cancer patients (N=130) and healthy women (N=50) was carried out with informed consent of the donors at various hospitals of Karachi. The Gas chromatography with electron capture detector (GC-ECD) was used to analyze the OC (alpha-HCH, beta- HCH, gamma-HCH, Aldrin, Dieldrin, DDT and DDE) compounds. The total mean concentration of organochlorine compounds was found significantly higher (ΣOCs= 0.4713 µg/mL) in experimental group while aldrin is absent in case study. It was observed that highest concentration of ΣOCs 0.9434µg/mL was found in last stages of cancer. Present study shows the aggressiveness in stages of breast cancer. Although observed elevated concentration of OC compounds in cases and stages of breast carcinoma but mean concentration were not statistically difference with control from those of population control. In light of the obtained results it has concluded that Organoclorine compounds are may be associated with breast cancer. Further research is recommended to know the precise role and mechanism of OCs in the breast oncology.

Introduction

More than one million new cases of breast cancer are diagnosed every year in all over the world. Mostly these cases are detected at the 3rd or 4th stage with the passage of time when the symptoms of breast cancer start to appear. There are many reasons of breast cancer in which organochlorine is one of the most common reasons noticed by the researchers previously. Among synthetic chemicals, there are other different chemicals which are called as organochlorines, in the past few decades such chemicals were released into the environment due to their use as the industrial products. Although the relationship among the organochlorine chemicals and breast cancer is not easy to understand, the relationship among them is quite complex (Cohn, 2015). Furthermore, this study is conducted in order to evaluate the influence of organochlorine on the breast cancer patients. It has been researched that there are several organochlorine chemicals which can cause breast cancer through their estrogenic actions. On the other hand, many harmful herbicides and as well as pesticides are strictly banned in the developing countries due to their risky effects, as these chemicals have been found as the major cause of breast cancer.

The exposure to chemicals existing in environment and more specifically the organochlorines has been considered as the most probable source of breast carcinoma. This said chemicals includes the industrial compounds like agricultural pesticides and PCBs, such as DDT, mirex, and chlordane which were excessively used in past and may be present everywhere over the word (Coughlin, 2015). In humans, the biological half-lives of many years have been recognized for the majority of persistent organocholrines, leads to the accumulation of body fat, which is comprised of milk fat, blood lipids, and adipose tissue (Demers *et al.,* 2000). The results from the early conducted studies over the area supported that there is a relationship between the risk to breast cancer and exposure to organochlorine, particularly with *p,p′*-DDE which is the main DDT metabolite. But, contrasting with the recent studies which involved the large sample size has come up with the negative results. Specifically, the study conducted by Hoyer *et al.*(2000) and Hunter *et al.* (1997) is based on *nested case control study design,* got unsuccessful in observing the association among breast malignancy and polychlorinated biphenyls plasma concentrations or p,p′-DDE. However, it was reported by Hoyer *et al.* (2000)*,* that dieldrin high plasma concentration were related with the risk of breast cancer (Demers *et al.,* 2000). Organochlorines are known to have antiestogenic and estrogenic characteristic *in vitro* and *in vivo.* The most significant and considerable influence of estrogen in the development of breast cancer suggests that the exposure to these contaminants could have an impact on the promotion or initiation of breast carcinogens. The exposure

to organochlorines and its relation to increased risk of breast cancer were observed in numbers of studies however the most epidemiological studies haven't been supportive strongly for the association among PCBs or DDT and the incidence to breast cancer (Del *et al.,* 2015).The main purpose of this study was to evaluate the influence of organochlorine on the breast cancer patients.

Materials and Methods

A favourable ethical opinion was obtained from the members of the international review board along with the agreement with assurance filed at the beginning of study.

Study Subjects: The study was conducted in the year 2012 till 2015 when a sum of a total of 130 participants were recruited in study those were diagnosed with breast cancer. Another group of 50 healthy participants were recruited without having any cancer history. The cases were then matched with the people's demographics. The study participants were aged between 30 to 60 years.

Interviews: The study participantswere interviewed by two research assistants who were trained to get it filled by participants. The questionnaire was designed for the collection of details of the participants regarding their age, pregnancy, smoking habits, past medical records, family cancer history, menopausal and lactation history. The reposts regarding the tumour size and the stages of cancer of the breast cancer woman were collected from the laboratory and from the medical examinations.

Blood Collection: Blood samples were also collected in the flasks containing EDTA at the time of examination. On the other hand, the blood samples of the breast cancer women were collected before surgery. The samples were immediately centrifuged as the serum was shifted in the flasks that have been previously washed with hexane. To perform further analysis, the samples were stored in the cooled area having temperature of -20 °C.

Chemical reagents: Solvents and reagents used were pesticide-scan grade. The chemicals used for the sample extraction were methanol, diethyl ether and hexane. Silica Sep-Pak was used as the cleanup purpose. The chemical Florisil was heated at a temperature of 130°C for 24 hours to be used as a cleaning agent. The pesticide standards were purchased from a laboratory in Germany named after Dr. Ehresnstorfer's laboratory.

Glassware: Glassware used for the sample collection and further process were washed properly with detergents, kept in acid for next 24 hours and washed with the distilled water in order to have them cleaned. Moreover, it was also kept in the oven for 4 hours at a temperature of 200^0C. The glassware apparatus was finally rinsed with hexane before use.

Extraction of Serum: An Aliquot of serum of about 5ml was kept at room temperature and 2mL of methanol was added in it. It was then agitated for 1 min. an addition of 5mL n-hexane and diethyl ether (1:1 V/V) was made to it and again agitated for 2 min. this sample of serum was centrifuged at 3000 rpm for 5 minutes. The organic phase of the sample was collected after centrifugation and the extractions of aqueous phase were made twice with n-hexane, diethyl ether (1:1 V/V). 1mL of organic phase was kept in the vacuum concentrator for evaporation and concentration. $H_2SO_4$was further added to the 1mL concentrated sample of organic phase. Furthermore, it was shaken for a minute and centrifuged at 2500 rpm for 5 minutes. In addition to that the organic phase was collected and the aqueous phase was taken out along with 1mLof n-hexane. The organic phases were then dried completely in a vacuum. The residues of the dried organic phases were dissolved in 1mL of n-hexane for cleaned-up.

Column Chromatography: The Florisil column (22 mm id) was made and activated at 600°C having 10 cm after being topped with 1 cm anhydrous $Na_2SO_4.$ The column was previously washed with 200mL of hexane. The retained compounds of the column were eluted with hexane until the collection of eluates with a total volume of 25 ml. this elute was dried and analyzed after getting diluted to 1 mL by the GC-ECD.

Gas Liquid Chromatography (GLC): A Gas Chromatograph Perkin Elmer Clarus-500 was to analyze the organochlorines with [63]Niselective electron-capture detector. Nitrogen was used as a carrier gas at 30 mL min^{-1}. The analysis is further performed on cross bond DB-35 5% biphenyl, 65% dimethyl polysilicone capillary column having 30-meter length, 0.35 mm ID and 0.50 μm df stream of nitrogen. In order to maintain the results accuracy Pesticide standards were run daily on GLC–ECD before the analysis of the extracted sample (Koutros *et al.,* 2015). The samples were calculated with the peak areas of isomers compared with the isomers standards. Peak was identified by GC software (Total Chrome) with absolute preservation period (Niehoff *et al*., 2016).

The final extract was kept at a temperature of 250^0C. The temperature was gradually increased up to 300^oC. The temperature of injector was 250^oC and 270^oC for the detector respectively.

Recovery studies: The study of recovery was taken in with the standards and the applications of the procedures for extraction. These samples were subjected to chromatography. The reagent blanks and matrix spike recovery were used for quality assurance. Verification of calibration was checked from the beginning till end for all batches of samples. The batches contain laboratory control sample, procedural blank and duplicate sample. All the samples were spiked efficiency was determined. The calculation of results was on the basis of mean value of specimens. % recovery, %RSD, LOD and LOR calculated during the analysis and values are given in Table 1.

Data tracking: Information about the participants was feed in the database with patient's medical records. The results of chemical examination were gathered in the spreadsheet. The SPSS software was used for the analysis of the combined data and analysis.

Data analysis: Statistical analysis was performed using SPSS (IBM SPSS Statistics 20.0).Data were expressed in in the form of mean and SD. The results of organochlorine compounds were less than limit. The concentration of total DDT and HCH were matched with the help of Mann-Whitney test. The assessment of relations between pesticide serum, concentration, and age and tumor size was made by Spearman's correlation coefficient. In order to analyze the link between the organochlorine residues and breast cancer the odd ratios were calculated. The patients of breast cancer were divided into the categories according to their stages and were compared with control group.

Results

Case and control group did not vary with regard to some feature showed in Table 1 in relation to family record of breast carcinoma and benign breast disorder were frequently reported in cases than control (P = 0.001). Cases were slightly older (P = 0.001), had less fertile year (P= 0.0002), showed a higher BMI (P = 0.002) and conceive first pregnancy at older age (P = 0.002) than controls. Most of the cases have been pregnant but breast fed time shorter than control group (Xu *et al.*, 2016).

In the breast cancer patients' most detectable organochlorine compound was beta-HCH (84%) while DDE and γ-HCH were found at 48%. Mean concentrations of α-HCH in women who were reported with carcinoma of breast were not statistically significant from control group (Table 2). Mean value of dieldrin and DDT were higher in case as compared to control group. Aldrin was not detected in any samples of case group. The mean concentration of β-HCH and DDT level in cases were associated to carcinoma of breast, and normal individual data also used in odd ratio calculation. Level of total DDE were related to tumor size (3). Breast cancer risk associated with dieldrin but no other organochlorine compounds in females differ by lactation (2). Breast cancer risk is slightly higher in those women who had lactated less than two years. In table 3 divided breast cancer patients according to cancer stages. Surprising, observed significantly high concentration of organochlorine compounds in those patients who are suffering with later stage disease (3rd and 4th) than those patients with early stage (1st and 2nd).

Discussion

Organochlorine can have severe effects on breast cancer patients. It was obvious that the risk of breast carcinoma is associated to the serum concentrations of some organochlorine compounds including β-HCH and DDT as well. In present study higher concentration of the analyzed organochlorine compounds was detected in the breast cancer patients. Mean level of total organochlorine compounds in the experimental versus control group was 0.4713µg/mL vs. 0.1509 µg/mL. Wolf *et al.*, (1993) observed higher DDE levels in women who were suffering from breast cancer. Early prospective study showed highest concentration of serum level in case than control group while no statistical significance was observed (Demers *et al.,* 2000). In previous prospective study mean value of DDE is higher in control group. Data did not support the theory that exposure to DDE elevate the hazard of breast cancer (Hunter *et al.,* 1997). In the present study observed same trend of accumulation of DDE in case and control group. The previous data suggested mean level of p,p'-DDE is higher (3.84µg/g, standard deviation = 5.98) as compared to control (2.5µg/g) group. The data proposed that high levels revelation of DDE in women may increase risk of breast cancer (Romieu *et al.*, 2000). In early study researcher were observed elevated mean concentration of organochlorine (beta-HHCH, DDT) compounds in case study. The authors did not report statistically difference from control group (Demers *et al.*, 2000). Early studies on organochlorine compounds do not show any relation with risk of breast carcinoma (Hoyer *et al.*, 2000; Aronson *et al.*, 2000; Dorgan *et al.*, 1999). Moreover, the previous conducted research has proven the most possible correlation among the fat or blood concentration of organochlorines and breast cancer as well, but

arguments regarding these results are still there just due to the difference in the results found after experiments, more than ever as all the epidemiological studies varies through the identified compounds including the blood sampling, the selected population and the methods used for analysis purpose.

The relationship between breast cancer patient and organochlorine compound is complex, or in other words both are in direct relation with each other, as the concentration of organochlorine will be increased in the body, the risk of breast cancer may be increased simultaneously.

Table 1. Demographic Features of Studied Subjects

	Cases (130)	Controls (50)
Age ± SE*	51±11	49±10
Age at menarche± SE	12±7	12±2
Age at first pregnancy± SE	22±5	24±7
No. of children± SE	3.0±2	3.2±3
No. of fertile years± SE	29±6	27±7
BMI± SE	26±5	23±6
Lactation period >2yrs	15%	9%
Family history of breast diseases	31%	17%

Table 2. Serum Organochlorine Concentrations (µg/mL)

Variables	ControlGr (n-50)		Experimental Gr (n-130)	
	Mean	SD	Mean	SD
α-HCH	0.0123	0.024	0.0694	0.0199
β-HCH	0.0086	0.016	0.1217	0.272
γ-HCH	0.0134	0.043	0.0606	0.181
DDE	0.0171	0.039	0.0876	0.212
DDT	0.0347	0.099	0.0699	0.188
Dieldrin	0.0103	0.226	0.0621	0.199
Aldrin	0.0545	0.0134	0	0

Table 3. Level of Organochlorine Compound(µg/mL) in Breast Cancer Stages

Variables	Stage 1 (n-40)		Stage 2 (n-50)		Stage 3-4 (n-40)	
	Mean	SD	Mean	SD	Mean	SD
α-HCH	0.0708	0.222	0.0817	0.209	0.526	0.164
β-HCH	0.1202	0.276	0.1117	0.296	0.1358	0.239
γ-HCH	0.0676	0.186	0.0515	0.169	0.0649	0.194
DDE	0.0807	0.212	0.0804	0.218	0.1037	0.209
DDT	0.1013	0.209	0.0638	0.181	0.0492	0.179
Dieldrin	0.0813	0.227	0.0455	0.169	0.0638	0.206
Aldrin	0	0	0	0	0	0

The breast cancer was categorized according to the stages of cancer (Table 3). It is observed in this study that, significantly high concentration of organochlorine (α-HCH, β-HCH and DDE) compounds in those patients who were suffering with later stage disease (3rd and 4th), as compared to those patients with early stage (1st and 2nd). Women who had the higher concentration of DDE were likely to have more probability of both axillary-lymph-node and large tumor (having diameter more than or equal to 2cm) (Anglin, 2016). The serum concentration of p,p'DDE, β-Hexachlorocyclohexane, oxychlordane or trans-nanochor analyzed at higher level in women with breast cancer who had large size tumor (diameter≥ 2cm) and axillary-lymph node involvement (Demers *et al*., 2000). These findings further recommended that organochlorine revelation can effect the growth of this disease in the specific range of organochlorine concentration which is present in the female belongs to the Karachi city, as the study is conducted in Pakistan. The processes with the help of which organochlorines can affect the growth of breast cancer is un-cleared, but perhaps involve the ability of few of organochlorine compounds to antagonize the consequences of endogenous sex hormones(Rivero *et al*, 2015).The analysis performed here is showing the direct relations between the organochlorine chemicals and the breast cancer patients, as the concentration of organochlorine will get increase inside the body, it may increase the risk of tumor inside the different organs of the body. In this specific study the selected population consists of women, whose age was between 30- 90, the finding of this research indicates the risk of breast cancer specifically after the exposure of DDE as according to the results. Although did not observed statistically relation between OC compounds and breast cancer. (Roy and Nath 2016) The strong relation between the increased cases of breast cancer and a synchronous duration of extensive pesticides requires a proper clarification. The studies in the past decades shows that the relation between the fat or blood concentration of organochlorine and breast cancer, but on the other hand debate on this topic is still same due to the variations in the results, especially all the epidemiological researches varies through the investigated compounds, the duration on blood sampling, the selected population and as well as the difference in the analytical methods, which were selected previously (Leng *et al*., 2016). Furthermore, cancer is identified in order to induce alterations in body weight and metabolism and these parameters are not encountered in the existing study, anyhow this misclassification of exposure cannot be excluded as well. However, breast cancer was always considered as a primary tumor and mostly in the cases where sampling occurred before treatments and surgery, for instance radiotherapy and chemotherapy (Ellsworth *et al*, 2015).

This study results concluded that the exposure to chemicals existing in environment and more specifically the organochlorine compounds has been considered as the most possible cause of breast cancer, and this high risk can be reduced by doing the efforts to secure the environment, as it is also true that the cases of breast cancer cannot entirely be revealed in such a way.

A higher standard deviation indicates our study has more variability in the observed data than expected. This mean the data is genuinely more variable than we assumed. We have squared to check if we have an outlier in the data as it is one of the courses. However, we have recorded multiple "0" values in our included study variables and these results were included in all statistical analyses. Second, may be this is due to small sample size as small samples can be notoriously unrepresentative. Further research is needed to determine the influence of organochlorine in patients with breast cancer in larger and multicenter trial.

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