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| RESEARCH ARTICLE

Serum Concentrations of Organochlorines in Breast Cancer Women: A Pilot Hospital Based-case Control Study in Vietnam

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| ABSTRACT
Cancer, in general, and breast cancer in particular, is one of the noncommunicable diseases (NCDs) that is increasing rapidly in the world, especially in developing countries like Vietnam. Along with other etiological factors (e.g., genetics, family history, age, etc.), there is growing scientific evidence that exposure to environmental carcinogens, especially endocrine disrupting chemicals-EDCs (e.g., organochlorine pesticides (OCPs) and some other organic compounds), is potentially associated with increased incidence of several NCDs including breast cancer in animal and human studies. People are frequently exposed to various carcinogens, such as pesticides, in their lifetime. Organochlorine pesticides (OCPs) are frequently used worldwide as insecticides, fungicides, herbicides and termiticides, and people may be exposed to these substances at different levels due to direct and/or indirect ways. Therefore, the aim of this paper is to study the accumulation level of serum organochlorines in breast cancer in a case-control study in Vietnam. A random collection of blood samples was carried out from the cases (breast cancer patients, n=146) and controls (healthy women, n=146) with informed consent in a hospital-based case and control study. Serum was separated within 2h of blood collection and then subjected to further purification before analysis by Gas chromatography–mass spectrometry (GC-MS) method. The determination of 18 organochlorines (Aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC, Heptaclor, Heptaclor epoxide, Diendrin, Endosulfan I, Endosulfan II, Endosulfan sulfat, Endrin, Endrin aldehyde, Endrin ketone, p,p′ DDD, p,p′ DDT, p,p′DDE, Methoxyclor) showed that only p,p′-DDE (as a main metabolite of p,p′-DDT) was detected in the blood samples of the cases (26.0%) and controls (10.3%). In addition, p,p′-DDT was the only pesticide detected in the disease group with low concentration (3.4%). The average concentration of p,p'-DDE in the case (3.51 ± 0.99 ppb) was higher than that in the control (1.89 ± 0.43 ppb) with a significant statistical difference ($p < 0.05$).

| KEYWORDS

Breast cancer, Pesticides, Organochlorines, Endogenous agents

| ARTICLE INFORMATION

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1. Introduction

According to a report by the World Health Organization, breast cancer is one of the most common cancers in women worldwide, accounting for about 12% of new cancer cases and 25% of all cancers in women. Breast cancer rates are the highest in developed countries, about 100 per 100,000 people in Europe and 90 per 100,000 in the US, and the lowest in developing countries in Asia and Africa. However, the rate of breast cancer in women has rapidly increased in recent years, and 50% of new cases are diagnosed in developing countries (Abolhassani et al., 2009).

Vietnam is one of the developing countries and faces an increased risk of non-communicable diseases (NCDs), including cancer. In 2018, Vietnam had 165,000 new cancer cases, including 115,000 deaths, making Vietnam a country with an alarming cancer rate. Breast cancer is the most common malignancy in women in Vietnam, with a rate of 25.8/100,000 women, a threefold increase

compared to the previous decade (from 5538 cases in 2000 to 15229 cases in 2018), becoming the most common cancer in women in recent years in Vietnam (Jenkins et al., 2018). Epidemiological studies have shown that less than 10% of cancers are due to disorders from within the body - called endogenous causes. In contrast, 75-80% of cancers arise from outside the body – called exogenous causes, and environmental carcinogens are one of the main exogenous agents (Attaullah et al., 2018).

Pesticides are chemicals widely used throughout the world. Organochlorine is a group of chlorinated compounds frequently used worldwide as insecticides, fungicides, herbicides and termiticides. These chemicals belong to a class of persistent organic pollutants (POPs) with high persistence in the environment and long half-lives (30–50 years) (Aktar et al., 2009). These organochlorines were banned decades ago in many countries around the world. However, the use of this type of pesticide still continued after they were banned due to their availability and efficacy in the agriculture sector. Therefore, these substances are still detected today in environmental samples (soil, water, food...) and biological samples such as blood and urine samples in humans due to their persistence/difficulty in degrading in the environment (FAO, 2005; Gupta, 2004; Lallas, 2001).

Organochlorines are known to be stored in adipose tissues as they have lipophilic properties, resulting in extremely high concentrations in humans and posing a public health hazard (Andreotti et al., 2010). There is speculation that they may increase cancer risk in the hematopoietic system, prostate, breast, pancreas, and liver. It is supposed that environmental factors may play a crucial role in the pathogenesis of the disease (Nabi et al., 2014). Long-term exposure to environmental pollutants, especially those having an estrogenic effect, might make a significant contribution to the process of carcinogenesis, particularly hormone-related tumors such as breast cancer (Knower, 2014). In Vietnam, environmental pollution is at an alarming rate and may be one of the reasons related to the increase in cancer rates in recent years due to the community's exposure to residues as carcinogenic chemical agents in the environment (through food sources, water sources, etc.). However, the presence of organochlorines in biological samples of breast cancer patients is still unknown in Vietnam. Therefore, the aim of this work was to comparatively determine the concentration of some organochlorine pesticides in breast cancer patients and healthyl humans for further study on the association of organochlorine exposure and breast cancer risk in Vietnam.

2. Methodology

2.1. Study design, setting, and population

The present work is a comparative cross-sectional study that was carried out at the Ha Noi Oncology Hospital in Ha Noi, Vietnam. Determination of organochlorines in the blood samples of participants was performed at Laboratory center, Hanoi University of public health. In this study, breast cancer patients served as the cases, while healthy women served as the controls.

2.2. Sample, Sampling, and Eligibility Criteria

2.2.1 Sample size

WHO software was used to calculate sample size based on previous studies. The study consisted of 292 participants (146 cases and 146 controls). Participants are people aged 18 years or older, divided into two groups; the case and control groups.

2.2.2 Inclusion criteria

Participants who agreed to participate in the study were non-smokers. The breast cancer patients did not receive either hormonal therapy, chemo-or radiotherapy before the collection of blood samples. The control group did not have cancer or non-communicable diseases, and they did not use any medicine before the collection of blood samples.

2.2.3 Exclusion criteria

Participants with a positive family history of breast cancer or who had previous surgery and came for recurrence or metastasis were excluded from the study.

2.3. Materials and methods

2.3.1 Chemicals, reagents

All solvents and reagents were analytical grades. Reference chemical standards (18 organochlorines) were purchased from PerkinElmer Inc. (Lot No: LK150619006). The purities of the standard pesticides were 97.4–99%. Pesticides analyzed in this study were Aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC, Heptaclor, Heptaclor epoxide, Diendrin, Endosulfan I, Endosulfan II, Endosulfan sulfat, Endrin, Endrin aldehyde, Endrin ketone, p,p′ DDD, p,p′ DDT, p,p′DDE, Methoxyclor. The content of each substance in the mixture is 200 ppm.

2.3.2 Analytical procedure

All samples were stored at −20°C until analysis. Then, samples were extracted and analyzed by gas chromatography for the detection of organochlorine pesticides and the estimation of their concentrations in blood samples.

The serum samples were prepared according to a previous study (Mohammad et al., 2018). The samples were cleaned up by florisil column chromatography, and the residue was subjected to analysis for determination of serum organochlorines by gas chromatography (PerkinElmer, GC clarus 680) coupled with MS clarus SQ 8T mass spectrometer and auto-sampler injection.

Gas chromatographic conditions

GC Parameters			
Injector Type	Capillary injector with capillary split/splitless ultra deactivated single taper inlet liner with wool.		
Analytical Column	Elite-5MS (30 m x 0.25 mm x 0.25 µm)		
Inlet	Temp 280 °C		
Injection Volume	1 µL		
Initial Oven Temp	50 °C		
Oven Hold	1.0 min		
Ramp	20 °C/min		
2nd Oven Temp	170 °C		
Oven Hold	0 min		
Ramp	6 °C/min		
3rd Oven Temp	300 °C		
Oven Hold	1 min		
Instrument Time Event	Time	Event	Value
	-1.00 min	Car	4 ml/min
	-0.75 min	Spl	Off
	1.50 min	Spl	50 ml/min
	1.55 min	Car	1 ml/min
MS Parameters			
Mass Range (amu)	45 to 450		
GC Inlet Line Temp	290 °C		
Ion Source Temp	260 °C		
C Function Type	Scan		
Ionization	EI		

2.3.3 Statistical analysis

Data entry and statistical analyses were performed using STATA 14.0. Statistical analysis included frequencies, percentages, means, and SD. p value < 0.05 was considered as statistically significant.

3. Results and Discussion

3.1. Characteristics of the studied participants

The average age of patients in our study was 52.2 ± 11.1 years old, and the lowest was 22 years old, while the highest was 86 years old. There was no difference in age between the group of patients and the control group ($p > 0.05$). The mean age of breast cancer patients in this study is similar to some previous studies in the country and other countries in Asia. The majority (>90%) of the participants are married, and they are mainly housewives (>80%). Regarding their residence, both the cases and controls lived in the rural area, so they could be indirectly exposed to pesticides as the number of pesticide users was low, 5% for the cases and 3% for the controls. The main characteristics of the studied participants (e.g., age, residence, occupation, pesticide use, and marital status) were illustrated in Table 1.

Table 1. Characteristics of the cases and controls

Characteristics	**Cases (N=146)**	**Controls (N=146)**
Age	Mean ± SD: 52.2 ± 11.1 Min-Max: 22-86	Mean ± SD: 48.9±10.3 Min-Max: 26-80
	N (%)	N (%)
Marital status		
Married	140 (96%)	136 (93%)
Single	6 (4%)	10 (7%)

Residence		
Urban	22 (15%)	29 (20%)
Rural	124 (85%)	117 (80%)
Occupation		
Employees	22 (15%)	30 (20%)
Housewives	124 (85%)	116 (80%)
Pesticide use		
In the past	7 (5%)	4 (3%)
Recent years	0 (0%)	0 (0%)

3.2. Concentrations of pesticides p,p'-DDE

The data on the concentration of serum organochlorines in the cases and controls are presented in Table 2. Of the studied substances (Aldrin, α-BHC, β-BHC, δ-BHC, γ-BHC, Heptaclor, Heptaclor epoxide, Diendrin, Endosulfan I, Endosulfan II, Endosulfan sulfat, Endrin, Endrin aldehyde, Endrin ketone, p,p' DDD, p,p' DDT, p,p'DDE, Methoxyclor), only p,p'-DDE, which was the metabolite of p,p'-DDT, was the major detectable substance in both the disease and control group. Meanwhile, p,p'-DDT was only detected in the disease group (3.4%). In addition, some other substances, such as Alpha-BHC, were also detected in serum samples at very low concentrations. The results showed that the concentrations of p,p'-DDE detected in the case and the control group were significantly different ($p < 0.05$). The mean concentration of p,p'-DDE in the breast cancer group (3.51 ± 0.99 ppb) was higher than that in control (1.89 ± 0.43 ppb). In addition, the detection rate of p,p'-DDE in the disease group (26.0%) was also higher than that in the control group (10.3%).

Table 2. Serum Concentrations of Organochlorines in breast cancer and healthy women

No	Organochlorines	Cases (n=146)			Controls (n=146)		
		Detection rate (>LOD, %)	Medium	Mean	Detection rate (> LOD, %)	Medium	Mean
1	Alpha-BHC	0%	-	-	0.7%	-	-
2	Beta-BHC	0%	-	-	0%	-	-
3	Gama-BHC	0%	-	-	0%	-	-
4	Delta-BHC	0%	-	-	0%	-	-
5	Heptaclor	0%	-	-	0%	-	-
6	Aldrin	0%	-	-	0%	-	-
7	Heptaclor epoxi	0%	-	-	0%	-	-
8	Alpha- Endosunfan	0%	-	-	0%	-	-
9	p,p'-DDE	26.0%	3.63	3.51 ± 0.99	10.3%	1.98	1.89 ± 0.43
10	Dieldrin	0%	-	-	0%	-	-
11	Endrin	0%	-	-	0%	-	-
12	Endosunfan II	0%	-	-	0%	-	-
13	Endo sunfan sulfat	0%	-	-	0%	-	-
14	p,p'-DDD	0%	-	-	0%	-	-
15	Endrin aldehyde	0%	-	-	0%	-	-
16	p,p'-DDT	3.4%	-	-	0%	-	-
17	Endrin keton	0%	-	-	0%	-	-
18	Methoxyclor	0%	-	-	0%	-	-

Our research results are also consistent with some following studies on determining the concentration of organochlorine

pesticides in the serum of breast cancer patients and controls (Bayat et al., 2011; Behrooz et al., 2012; Boada et al.,2012; Bratton et al., 2012; Bretveld et al., 2006; Ellsworth et al., 2018; Itoh et al., 2009; He et al., 2017; Ingber et al., 2013; Wiley et al., 1999). A recent case-control study (42 diseases and 42 controls) in China indicated that HCB, α, β, γ, δ - HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, p,p'-DDT, p,p'-DDE, p,p'-DDD, and methoxychlor were detected in both the disease and control group. The levels of these organochlorines were found to be elevated in the blood samples of breast cancer patients compared with the control group. Especially the content of p,p'-DDE was found to be different statistically ($P<0.01$) between the disease group (5.75 ppb) compared with the control group (3.67 ppb). A case-control study in Singapore also showed that organochlorine concentrations in the disease group (13700 ng/g lipid) were higher than that in the control group (9330 ng/g lipid), and this difference was statistically significant ($p < 0.01$).

Organochlorine pesticides were banned several decades ago in Vietnam, so the detection of p,p'-DDE (as a metabolite of DDT) is of interest. The presence of the metabolite and its parent DDT in breast cancer patients and healthy women suggested that people are possibly exposed to this kind of pesticide in the general public rather than occupational exposure as the number of pesticides users in our study was low, with 5% for the cases and 3% for the controls (Table 1). The detection of p,p'-DDE and p,p'-DDT in biological samples of the studied participants could be explained by the following hypothesis: 1) The cases and controls were exposed to p,p'-DDT through the environment or food intake in the past. They were mainly born between 1950-1990, and p,p'-DDT was heavily used in the period from 1960-1980 in Vietnam, so those born before 1980 may be the groups most at risk of exposure to p,p'-DDT, which is mainly converted to p,p'-DDE; 2) The cases and controls could continue to be indirectly exposed to p,p'-DDT through the environment or food intake during their lifetime as illegal pesticides, which may contain organochlorines, are still used in agriculture by farmers in Vietnam although these chemicals were banned several decades ago; and 3) p,p'-DDT has been shown to be transmitted from mother to child through the placenta or breast milk, so p,p'-DDE in the blood samples of the next generation could be detected if mothers were exposed to p,p'-DDT in the past.

The presence of higher levels of p,p'-DDT metabolite (p,p'-DDE) in breast cancer patients compared with the controls strengthens the possibility of a significant association between organochlorine exposure and the risk of breast cancer. DDT and its metabolites are capable of causing *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 p,p'-DDT metabolites (p,p'-DDE) in cancer cases of the present study also strengthen the assumption that these chemicals may be carcinogenic by damaging DNA and causing various malignancies in the body.

4. Conclusion

The determination of 18 organochlorines showed that p,p′-DDE (as a main metabolite of p,p′-DDT) was detected in the blood samples of the breast cancer patients (26.0%) and controls (10.3%). The average concentration of p,p'-DDE in the cases (3.51 ± 0.99 ppb) was higher than that in controls (1.89 ± 0.43 ppb) with a significant statistical difference ($p < 0.05$). In addition, p,p′-DDT was the only pesticide detected in the disease group with low concentration (3.4%). Organochlorine pesticides were banned several decades ago in Vietnam, so the detection of p,p'-DDE (as a metabolite of DDT) is of interest. The main findings in our study also strengthen the evidence that exposure to organochlorine pesticides may increase the risk of breast cancer. The limitations of this study included a limited number of pesticides identified in the blood samples of the studied participants. Therefore, additional studies are needed for the investigation of other pesticides in biological samples and their role in breast cancer in Vietnam.

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