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Distribution of Organochlorine Pesticides in Nigerian Human Serum and Risk of Cancer: A case-control study

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Abstract

This study monitored the distribution of organochlorine pesticides (OCPs) in the blood serum of breast cancer patients and control subjects. Blood samples from 50 breast cancer patients and 50 controls were analyzed using Gas Chromatography-Electron Capture Detection. Thirteen OCPs, including DDT, DDE, methoxychlor, aldrin, dieldrin, endrin, endosulfan (I, II, and sulfate), heptachlor, heptachlor epoxide, γ-BHC, and δ-BHC, were detected. Endosulfan II had the highest mean concentrations (16.505 ppb in breast cancer patients and 10.625 ppb in controls), while DDE had the lowest (0.039 ppb and 0.014 ppb, respectively). A DDE/DDT ratio below 10 indicated recent DDT exposure. The significantly higher presence of DDT, aldrin, γ-BHC, and δ-BHC in breast cancer patients suggests a potential link between OCP exposure and breast cancer risk. The clustering of OCPs further indicates possible synergistic effects that may enhance cancer risk. Environmentally safer alternatives to OCPs should be adopted in agriculture and industry to reduce exposure risks.

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Keywords: Breast cancer; Case-control study; DDT; Organochlorine pesticides; Serum

1. Introduction

Organochlorine pesticides (OCPs) are a class of chlorinated compounds, commonly categorized as persistent organic pollutants due to their environmental persistence and high lipid solubility (Baratzhanova et al., 2024). Their extensive use in agriculture and industry has led to widespread environmental contamination (Litynska, 2024) and bioaccumulation throughout the food chain (Ansari et al., 2024). OCPs are broadly classified into three groups: dichlorodiphenylethanes, chlorinated cyclodienes, and hexachlorocyclohexanes (Kartalović et al., 2020; Zuo et al., 2023). Exposure to these chemicals has been linked to various adverse health effects, including oxidative stress, metabolic disorders, immunosuppression, and neurotoxicity (Taiwo, 2019; Chen et al., 2022; Pathak et al., 2022).

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Furthermore, several studies have identified an association between OCP exposure and different types of cancer, including breast cancer (Awe et al., 2018; Abolhassani et al., 2019; Mortazavi et al., 2019; Dang et al., 2023; Ugalde-Resano., 2024). Due to their chemical stability, OCPs persist in the environment for long periods and resist degradation, leading to their accumulation in human fatty tissues, where they can be metabolized into more toxic derivatives than their parent compounds (Castro López & Castillo Rodriguez, 2024; Colosio & Rubino, 2024).

Breast cancer is the second leading cause of cancer-related mortality worldwide (Afifi *et al.*, 2020; Bray *et al.*, 2024). While its exact etiology remains unclear, both hereditary and non-hereditary factors contribute to its development. Risk factors include environmental exposures, biological and lifestyle factors such as sex, age, race, obesity, late childbearing, late menopause, physical inactivity, and alcohol consumption (Kaminska et al., 2015; Rodgers et al., 2018). Among these, environmental contaminants like OCPs have been identified as endocrine disruptors that mimic estrogen, interfere with hormone synthesis, and potentially increase breast cancer risk (Ugalde-Resano et al., 2023; Aouichat-Bouguerra et al., 2024).

Several studies have reported a positive correlation between OCP exposure and breast cancer incidence (Awe et al., 2018; Rocha et al., 2021; Garg et al., 2021; Ugalde-Resano et al., 2023; Ugalde-Resano et al., 2024). Although OCPs have been banned in many countries (USEPA, 2016), they remain in use in several regions, including Nigeria (Taiwo et al., 2020; Oyinloye et al., 2021; Iwegbue et al., 2024). Due to their long half-lives, these chemicals persist in human blood, making serum OCP levels a useful biomarker of exposure (Jia et al., 2023; Ugalde-Resano et al., 2024). However, there is limited data on OCP serum concentrations and their association with breast cancer risk in Nigeria. Given the potential health hazards linked to OCP exposure, addressing this research gap is essential. Therefore, this study aims to investigate the distribution of OCPs in the blood serum of breast cancer patients and control subjects to better understand their potential role in breast cancer risk.

2. Methodology

2.1. Samples

Samples were collected from patients in the Department of Surgery, Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. The study included 100 women, comprising 50 breast cancer patients (cases) and 50 controls without breast cancer or other OCP-related diseases. Participants, primarily from southwestern Nigeria, provided written consent, with non-consenting individuals excluded. Blood samples (5 mL) were drawn from the inner forearm using butterfly needles after skin cleansing with methylated spirit to minimize contamination. Samples were collected in labeled, decontaminated plain bottles, transported to the laboratory, centrifuged to separate serum, and stored at -20°C in labeled plastic containers until analysis.

2.2. Extraction of Organochloride pesticide

A 1–2 mL aliquot of each serum sample was treated with 1% sodium tungstate and 1N sulfuric acid (H_2SO_4) to denature serum proteins and facilitate precipitation. The precipitate was subsequently washed three times with 0.1N H_2SO_4 to remove impurities. The resulting filtrate, containing OCP residues, was extracted using dichloromethane (DCM) as the solvent. The filtrate-DCM mixture was vigorously agitated for approximately 10 minutes to enhance partitioning. The obtained extract was then subjected to a clean-up regime to remove potential contaminants and improve analytical accuracy.

2.3. Clean-up Procedure

A chromatographic column (15 cm × 2 cm) was prepared by sequentially packing it with glass wool, followed by approximately 5 g of activated silica gel. A 2 g layer of anhydrous Na_2SO_4 was added on top to absorb residual moisture in the extract. Pre-elution was performed using 15 mL of dichloromethane (DCM), ensuring that the Na_2SO_4 layer remained covered to prevent desiccation of the silica gel and maintain column integrity. The extract was then passed through the column using approximately 30 mL of DCM as the elution solvent. The eluate was collected and evaporated to dryness under a stream of high-purity nitrogen gas (99.99%). The dried residue was reconstituted in 1 mL of n-hexane, and OCP levels were analyzed using gas chromatography coupled with an electron capture detector (GC-ECD).

2.4. Recovery Experiment

Due to the unavailability of certified pesticide reference materials, a recovery analysis was conducted to assess the precision and efficiency of the analytical procedures using the standard addition method. A 2 mL serum sample was divided into two: one portion was spiked with a 10 ppm standard mixture of selected organochlorine pesticides (OCPs), including HCH, γ-BHC, aldrin, p,p'-DDE, α-chlordane, p,p'-DDD, dieldrin, heptachlor, endrin, and p,p'-DDT, while the other served as an unspiked control. Both samples underwent the same extraction and clean-up procedures.

Additionally, 10 mL of a 1000 mg/L OCP standard mixture in spectra-grade n-hexane was evaporated under a nitrogen stream at ambient temperature and reconstituted with 2,2,4-trimethylpentane. A 1.0 µL aliquot of the spiked, unspiked, and standard solutions was injected into the GC column for GC-ECD analysis. Recovery percentages were determined by comparing the peak areas of spiked OCPs with those from the standard residues, using the equation:

$$\%R = \frac{A' - A}{B} \quad (1)$$

where A' is the OCP concentration in the spiked sample, A is the concentration in the unspiked sample, and B is the spiked OCP amount.

2.5. Qualitative identification and Quantitative Estimation of Organochloride Pesticide

Organochlorine pesticide (OCP) analysis was conducted using an Agilent GC-7890A equipped with an Electron Capture Detector (ECD). A 1 µg/L reconstituted eluate was injected in splitless mode at 250°C using a microsyringe. Separation was achieved on a DB-17 column (30 m × 250 µm × 0.25 µm) with helium as the carrier gas (2 mL/min) and nitrogen as the make-up gas. The oven temperature was programmed from 150°C to 280°C at 6°C/min, with a total run time of 23 minutes. OCP residues were quantified by comparing sample peak areas with calibration standards. Analysis was done at the Nigerian Institute of Oceanography and Marine Research (NIOMR) Central Laboratory, Victoria Island, Lagos, Nigeria.

2.6. Data Analysis

Data analysis was conducted using SPSS (version 26). ANOVA was performed to assess significant differences in serum OCP levels between breast cancer and control subjects. Correlation matrix and principal component analysis (PCA) were used to evaluate associations among the detected OCPs.

3. Results

3.1. Validation of Anaytical Procedures

The recovery analysis results, presented in Table 1, confirm the reliability of the analytical procedures employed in this study. Key performance parameters—sensitivity, recovery, precision, and accuracy—were evaluated. The percentage recoveries ranged from 84.68 ± 2.78% for p,p'-DDD to 99.63 ± 4.39% for Endrin, and were within the European Union's acceptable recovery range of 70–110%.

Table 1: Percentage Recovery for Organochlorine Pesticides (OCPs)

Organochlorine Pesticides	Amount (ppm) Used For Spiking	Mean Amount (ppm)of OCPs Recovered	Percentage Recovery
HCH	10	9.116	91.16 ± 5.81
γ-BHC	10	9.320	93.20 ± 3.63
Aldrin	10	8.552	85.52 ± 3.76
P, p'-DDE	10	9.269	92.69 ± 5.33
p, p' – DDD	10	8.468	84.68 ± 2.78
Dieldrin	10	9.719	97.19 ± 5.56
Heptachlor	10	9.794	87.94 ± 3.61
Endrin	10	9.963	99.63 ± 4.39
p, p' – DDT	10	9.921	99.21 3.88

3.2. Levels of Organochlorine Pesticides in Sera

The summary statistics of the detected organochlorine pesticides (OCPs) in the sera of breast cancer and control subjects are presented in Table 2. Representative chromatograms of the serum OCP levels of breast cancer and control subjects are presented in Figures 1 and 2 respectively. Thirteen (13) OCPs were detected in the blood sera. However, DDT, Aldrin, Endosulfan I, γ-BHC, and δ-BHC were the frequently detected OCPs in the samples. In the breast cancer subjects, Endosulfan II had the highest mean concentration (16.505 ppb) while DDE had the least mean concentration (0.039 ppb). A similar trend was observed in the control subjects, with Endosulfan II and DDE having mean concentrations of 10.625 ppb and 0.014 ppb respectively. Generally, the mean levels of the OCPs detected in the sera of breast cancer subjects were higher than those of the control subjects except for Endrin, Endosulfan I, Endosulfan sulphate, Heptachlor, and Heptachlor epoxide. The levels of DDT in the sera were relatively higher than DDE. One way analysis of variance (Table 2) was carried out to determine whether there is a significant difference in the OCP levels of the sera of breast cancer and control subjects. The result indicated that significant statistical difference existed in the levels of DDT, Aldrin, γ-BHC, and δ-BHC among the samples. The mean levels of DDT, Aldrin, γ-BHC, and δ-BHC in serum of breast cancer subjects is more than twice their mean levels in the sera of control subjects.

Table 2: Descriptive Statistics of OCP levels in Blood serum of Subjects

Organochlorine Pesticides	Breast Cancer subjects			Control subjects			F-value	p-value
	Freq. (%)	Mean±SD	$\sum OCP$	Freq. (%)	Mean±SD	$\sum OCP$		
DDT	100	1.70±1.248	85.021	100	0.821±0.622	41.059	19.852	**<0.0001***
DDE	4	0.039±0.198	1.959	2	0.014±0.099	0.706	0.638	0.426
Methoxychlor	20	9.277±59.392	463.893	16	0.905±2.896	45.278	0.991	0.322
Aldrin	98	10.857±16.37	532.041	100	5.557±6.575	277.855	4.502	**0.036***
Endrin	8	0.26±1.001	13.033	2	0.267±1.892	13.38	0.001	0.982
Dieldrin	18	1.806±9.874	90.319	28	1.409±4.25	70.498	0.068	0.795
Endosulfan I	48	1.903±2.298	93.279	70	2.08±2.115	104.043	0.159	0.691
Endosulfan II	8	16.505±83.717	825.283	4	10.625±68.514	531.281	0.148	0.702
EndosulfanSulphate	8	5.435±21.009	271.782	12	10.187±58.075	509.391	0.296	0.588
Heptachlor	8	0.212±0.748	10.628	16	0.448±1.152	22.422	1.473	0.228
Heptachlor Epoxide	28	0.636±1.242	31.178	20	0.686±2.252	34.335	0.03	0.862
γ-BHC	60	1.157±1.106	56.734	34	0.507±0.799	25.389	10.534	**0.002***
δ-BHC	70	0.644±0.566	32.248	46	0.277±0.399	13.897	14.03	**<0.0001***

*significant difference at $p<0.05$

3.3. Correlation Matrix and Principal Component Analysis of OCPs in Breast Cancer Serum

Correlation matrix and principal component analysis were employed to determine the relationship and interactive effects of the studied OCPs in sera of breast cancer subjects. The correlation matrix result (Table 3) indicated that strong and significant positive correlation existed between DDE/DDT, γ-BHC/DDT, δ-BHC/DDT, γ-BHC/DDE, δ-BHC/DDE, Endrin/Aldrin, Endosulfan I/Aldrin, Heptachlor epoxide/Aldrin, γ-BHC/Aldrin, Endosulfan I/Endrin, Heptachlor epoxide/Endrin, Endosulfan II/Dieldrin, Endosulfan sulphate/Dieldrin, Heptachlor/Endosulfan I, Heptachlor epoxide/Endosulfan I, Endosulfan sulphate/Endosulfan II, γ-BHC/Endosulfan sulphate, δ-BHC/Endosulfan sulphate, Heptachlor epoxide/Heptachlor, and δ-BHC/γ-BHC.

Table 3: Correlation matrix of OCPs in Sera of Breast Cancer subjects

	OCPs	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	DDT	1												
2	DDE	**0.29***	1											
3	Methoxychlor	-0.12	-0.03	1										
4	Aldrin	-0.05	-0.08	-0.03	1									
5	Endrin	-0.06	-0.05	-0.02	**0.24***	1								
6	Dieldrin	-0.14	-0.03	-0.02	-0.03	-0.04	1							
7	Endosulfan I	-0.26	-0.06	-0.10	**0.53***	**0.46***	-0.08	1						
8	Endosulfan II	-0.05	-0.03	-0.03	-0.06	-0.05	**0.96***	-0.14	1					
9	Endosulfan Sulp	0.18	0.08	-0.03	-0.03	-0.06	**0.23***	0.02	**0.35***	1				
10	Heptachlor	-0.07	-0.05	-0.02	0.16	0.01	-0.01	**0.29***	-0.05	-0.07	1			
11	Heptachlor Epoxide	-0.15	-0.10	-0.06	**0.59***	**0.43***	0.22	**0.58***	0.22	0.07	**0.36***	1		
12	γ-BHC	**0.72***	**0.25***	-0.14	**0.23***	-0.008	-0.14	0.08	-0.10	**0.28***	0.21	0.02	1	
13	δ-BHC	**0.75***	**0.28***	-0.16	-0.18	-0.11	-0.15	-0.20	-0.10	**0.25***	-0.06	-0.33	**0.74***	1

- n = 50, * means significance at $p<0.05$

Principal component analysis (Table 4) showed that there are four components accounting for a cumulative 67.881% of total data variance.

Table 4: Principal Component Analysis of the studied OCPs in sera of breast cancer subjects

Organochlorine Pesticides	**Component**			
	1	**2**	**3**	**4**
γ-BHC	0.897	0.123	-0.033	0.260
δ-BHC	0.887	-0.236	-0.057	-0.091
DDT	0.876	-0.135	-0.034	0.006
DDE	0.437	-0.044	-0.018	-0.128
Methoxychlor	-0.258	-0.214	-0.077	0.158
Endosulfan I	-0.135	0.879	-0.093	0.080
Heptachlor Epoxide	-0.134	0.786	0.262	0.342
Endrin	-0.033	0.742	-0.083	-0.295
Aldrin	0.029	0.694	-0.036	0.338
Endosulfan II	-0.081	-0.045	0.976	-0.020
Dieldrin	-0.163	-0.023	0.943	-0.014
Endosulfan Sulp	0.394	0.062	0.511	-0.034
Heptachlor	-0.061	0.160	-0.046	0.862
Eigen values	3.205	2.447	2.160	1.013
% data variance	24.654	18.820	16.618	7.790
Cumulative %	24.654	43.474	60.091	67.881

As shown in the component plot (Figure 3), close proximity existed among γ-BHC, δ-BHC, and DDT.

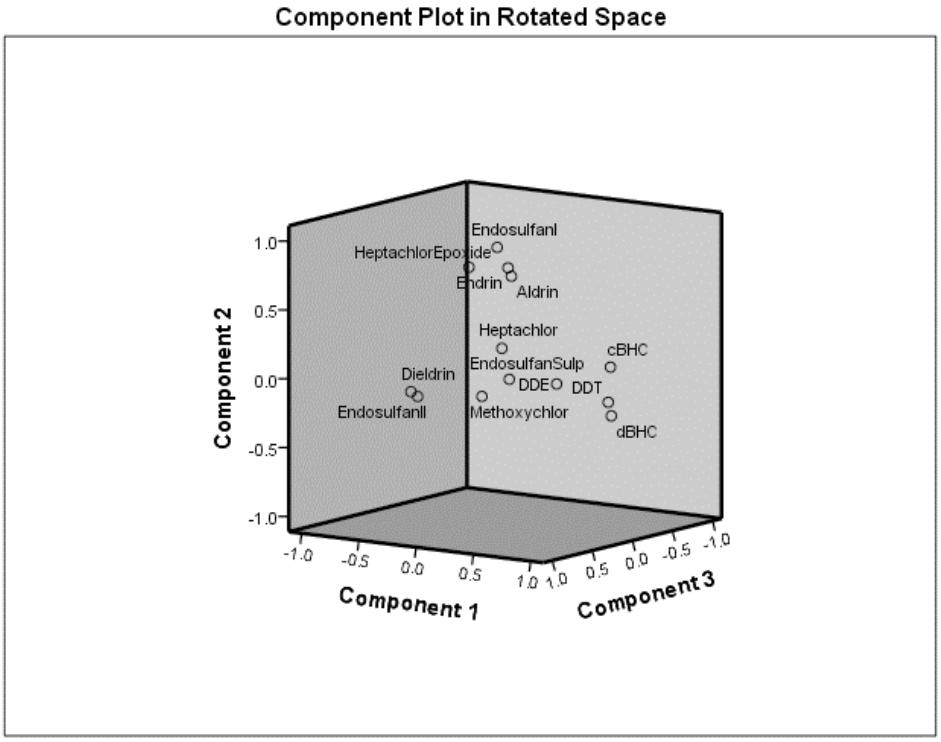


Figure 3: Component plot of OCPs in sera of breast cancer subjects

4.0. Discussion

The percentage recoveries in this study were all within the European Union's acceptable recovery range. This indicates minimal analyte loss as well as the efficiency of the extraction and quantification methods. The consistency of recoveries further confirmed the reproducibility of the procedure, accuracy and reliability in the detection of OCP residues in serum samples (Awe *et al.*, 2018).

The summary statistics of organochlorine pesticides (OCPs) detected in the sera of breast cancer and control subjects provide valuable insights into exposure levels and distribution patterns of these persistent pollutants which are essential for assessing potential health risks associated with prolonged OCP accumulation in human biological systems. The OCPs mostly detected in the serum samples in this study were compounds known for environmental persistence, long biological half-lives leading to bioaccumulation in human tissues; their widespread presence suggests ongoing exposure through environmental contamination, dietary intake, or occupational contact (Li *et al.*, 2022; Qiu *et al.*, 2023; Ansari *et al.*, 2024). The consistently high levels of Endosulfan II in both groups indicate continued exposure, possibly due to its agricultural use despite regulatory restrictions in some regions. The mean concentration detected among both groups in this study are higher than 2.22 ppb recorded by Sharma *et al.* (2019) among women in the Talwandi Sabo Block of Southern Punjab, India; this discrepancy may be attributed to differences in environmental exposure, dietary habits, regulatory measures, or geographical variations in pesticide use. The persistence of endosulfan in the environment has been attributed to slower photochemical degradation compared to biodegradation, such that even after its ban, it continues to cycle and may still be present in soil and sediment, leading to continued exposure (Lammel *et al.*, 2024).

DDE, a metabolite of DDT, is often used as an indicator of historical DDT exposure due to its prolonged stability in biological systems; the relatively low DDE levels in both groups compared to DDT suggest either recent exposure or differences in metabolic and excretion rates (Costopoulou *et al.*, 2024). The DDE levels in both groups in this study were lower than 3.51 ppb and 1.89 ppb recorded by Dang *et al.* (2023) in breast cancer patients and controls in Vietnam by Dang et al., (2023); this may be influenced by differences in exposure sources, environmental persistence, metabolic factors, or regional pesticide use patterns.

Overall, the mean serum concentrations of most detected OCPs were higher in breast cancer subjects than in

controls, except for Endrin, Endosulfan I, Endosulfan sulfate, Heptachlor, and Heptachlor epoxide (Figure 3). This disparity suggests a possible link between prolonged exposure to certain OCPs and an increased risk of breast cancer. The elevated levels in breast cancer patients may indicate bioaccumulation over time due to continuous environmental exposure, dietary intake, or occupational contact.

OCPs are known endocrine disruptors, capable of interfering with hormone regulation and contributing to hormone-related cancers, including breast cancer. Studies have shown that compounds such as DDT and its metabolites mimic estrogenic activity, potentially promoting carcinogenesis by altering hormonal balance and cell proliferation (Darbre, 2019; Kumar *et al.,* 2022; Wang *et al.,* 2022; Laxmiprasad, 2023). The higher OCP concentration in breast cancer group in this study has reinforced this hypothesis. In contrast, the lower concentrations of Endrin, Endosulfan I, Endosulfan sulfate, Heptachlor, and Heptachlor epoxide in breast cancer patients compared to controls may be attributed to differences in metabolic pathways, elimination rates, or exposure sources (Dang *et al.,* 2023). These compounds may also degrade more rapidly in the human body or exhibit different toxicokinetic behaviours.

While these findings suggest a potential association between OCP exposure and breast cancer risk, further epidemiological and mechanistic studies are necessary to establish a causal relationship. Factors such as genetic predisposition, lifestyle, and cumulative exposure must also be considered. Nonetheless, the presence of these OCPs in human serum indicates the need for ongoing environmental monitoring, stricter regulatory enforcement, and public health interventions to minimize exposure risks (Liu et al., 2023; Ugalde-Resano et al., 2024).

The frequent detection of DDT and its metabolites is particularly significant, given their persistence and ability to bioaccumulate in human tissues. DDT undergoes environmental and biological transformation to its primary metabolite, DDE, a process influenced by metabolic efficiency, environmental degradation, and physiological differences. Due to its lipophilic nature, DDE is stored in adipose tissue and released into the bloodstream over time, resulting in a prolonged half-life of approximately nine years in human serum (Martínez-Valenzuela *et al.,* 2017; Jugan *et al.,* 2020; Rubo et al., 2024). This persistence raises concerns about its long-term health effects, particularly in relation to endocrine disruption and carcinogenicity.

In this study, DDT levels in serum were higher than those of DDE in both groups, suggesting recent exposure. This is at variance with the findings of Haug *et al.* (2018) who reported elevated levels of DDE for mothers in the RHEA cohort in Crete. The DDE/DDT ratio is a useful biomarker for distinguishing between historical and recent exposure, with a ratio below 10 indicating ongoing exposure (Zhang *et al.,* 2018; Costopoulou *et al.,* 2024). The observed ratio in this study contrasts with Waliszewski *et al.* (2012), who reported a mean DDE/DDT ratio of 18.9, suggesting predominant DDE accumulation due to past exposure. Differences in DDE/DDT ratios between studies may be influenced by factors such as exposure sources, environmental persistence, dietary intake, and metabolic efficiency (Haug *et al.,* 2018; Zhang *et al.,* 2018; Costopoulou *et al.,* 2024). In regions where DDT use was banned decades ago, its presence in human serum likely results from legacy contamination, leading to higher DDE/DDT ratios. Conversely, areas where DDT is still used—either legally for vector control or illegally for agriculture—may exhibit lower ratios, reflecting recent exposure. Genetic and physiological factors also influence DDT metabolism and elimination, with some individuals metabolizing it more slowly due to genetic polymorphisms affecting enzyme activity. Additionally, dietary habits and lipid metabolism play roles in differential accumulation and breakdown rates.

Conclusion

The findings of this study indicate significantly higher levels of organochlorine pesticides (OCPs) in the blood serum of breast cancer patients compared to control subjects. Specifically, DDT, Aldrin, γ-BHC, and δ-BHC were found in elevated concentrations among breast cancer patients, suggesting a potential association between prolonged OCP exposure and breast cancer risk. The persistence of these chemicals in human tissue, despite regulatory bans, highlights the ongoing environmental contamination and the potential role of bioaccumulation in disease development. These results align with previous studies that have linked OCPs to endocrine disruption and carcinogenicity, reinforcing concerns about their impact on human health.

Furthermore, correlation and principal component analyses revealed strong associations between certain OCPs, suggesting common exposure sources or metabolic interactions. The clustering of these compounds in breast cancer patients points to the likelihood of cumulative or synergistic effects that may contribute to disease progression. This emphasizes the need for further research to explore the mechanisms through which OCPs influence cancer development, including their role in hormonal imbalance, oxidative stress, and genetic mutations to provide deeper insights into the pathways linking environmental pollutants to cancer risk.

Given these findings, it is crucial to strengthen regulatory enforcement and public health interventions to minimize OCP exposure, particularly in high-risk populations. Continuous environmental monitoring, public awareness campaigns, and stricter controls on pesticide residues in food and water sources are essential steps toward reducing human exposure. Additionally, healthcare practitioners should consider environmental risk factors in breast cancer management and encourage lifestyle modifications that can help mitigate exposure.

Conflict of Interest

The authors declare that there is no competing conflict of interests.

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