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TRACES OF ORGANOCHLORINE PESTICIDES IN BREAST MILK SAMPLES OF MOTHERS FROM RURAL AREAS OF KALABURAGI: IMPLICATIONS FOR INFANT HEALTH

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Abstract:
Organochlorine pollutants (OCPs) resist decomposition and continue to contaminate habitats and human health. This study analyzed traces levels of organochlorine pesticides in breast milk samples. A standard questionnaire was procured during the sample collection from all the donors. The study only recruited participants who were willing to donate breast milk samples and have provided their consent. A total of 66 mothers (mean age: 29 years) were voluntarily recruited, all mothers were healthy after delivering the baby. All the participants in this study were residents of Kalaburagi rural areas for at least 8-10 years. Analysis of several OCPs were done using GC (shizmadu GCMS QP2010), the traces of 3 OCP congeners were revealed in the mother's milk samples. o,p'-DDE, p,p'-DDE and DDT have been determined in the mother's milk samples. The analysis revealed that most dominant contaminant found was o,p'-DDE which was seen in 32 samples and p,p'-DDE was second most dominant since, it was also determined in 32 samples and least dominant contaminant was DDT which was determined in 8 samples. Our study shows that mother's diets could have been a major effect on traces of pesticide residue in the mother's milk. Dietary sustenance guidance like limiting inordinate amount of non-vegetarian foods of obscure sources may play a key role to reduce the traces of OCP excretion in to the breast milk.

Keywords: Breast milk; OCPs; infant health; lactation; endocrine disrupting pesticides.

Introduction

Organic chemicals are commonly called persistent organic pollutants (POPs) (1). Researchers worldwide have reviewed several studies on OCP over the past decades. POPs have chemically stable structures and are non-reactive, allowing them to survive in the environment for years (2). OCPs typically accumulate in surface or groundwater from runoff (3). India has banned the use of DDT in agriculture, but health authorities still use it in most disease control vectors (4). India is still the only country producing DDT and also the largest consumer. The human body absorbs these chemicals into fat and eliminates them only during lactation. DDT is stored as DDE (dichlorodiphenyldichloroethylene), a metabolite DDE believed to be the most stable in tissues. Prolonged exposure to these contaminants can still harm your unborn baby (5). The impact of such pollutants on ecosystems is considered a major concern due to their ability to withstand heat, moisture, radiation, and biodegradation. They are also highly persistent in water and soil and can pose a real threat to nature and wildlife. OCPs are bio-accumulative and persist in animal and plant tissues (6). The constant use of organochlorine pesticides to control destructive pests has a negative impact on the environment and also results in high residue build-up in agriculture and dairy (7). Side effects such as reproductive toxicity, teratogenicity, and premature birth may occur when humans and animals are exposed to organochlorine pesticides. It has also been advised that long-term human exposure to organochlorine pesticides can damage the central nervous system, liver, kidneys, and bladder (6). Humans can also be exposed to similar contaminants through numerous routes, including inhalation of the contaminants, absorption through the skin, and consumption of contaminated food. Two previous pathways account for less than approximately 2% of the total intake of these contaminants. Trace amounts of contaminants left on farms effect vegetation and can later appear in food. Ingestion of contaminated food is therefore believed to be the major source of human exposure to pesticides (8). The residues of these compounds in organisms depend on their habitat and their position in the food chain (9). The most important human health problems are related to chlorinated hydrocarbons and their tendency to accumulate in adipose tissue. The presence of residues of these toxins in adipose tissue can be considered the best indicator of the presence of these compounds in the human body (10). (11) Suggest that organochlorine pesticides are widely used against malaria vectors and agricultural pests in India, despite a global ban on the use of these compounds. Based on their results, the mean amounts of DDT and HCH detected in breast milk were 3210 ng/g and 2870 ng/g, respectively. In contrast, (12) suggested that concentrations of both DDT and HCH in breast milk in Chennai (India) showed a developing trend over the past two decades.

The current study is focused on investigating trace levels of OCP in breast milk samples collected from healthy breastfeeding mothers in the rural area of Kalaburagi. A questionnaire consisting of demographic, dietary, reproductive health, and occupational data was recorded by the donor and briefly correlated with the level of her OCP index in the breast milk sample.

Sampling and methods

The study was carried out during June 2021 to December 2021. Breast milk samples (20 mL) were collected in acetone-washed glass vials, immediately frozen, and stored at -20 °C until analysis using a Shimadzu GCMS QP2010 equipped with an electron capture detector (ECD). The participants completed a questionnaire regarding their basic demographics, including place of residence, age, occupation, dietary habits, smoking and chewing habits. The demographic characteristics of the study participants are summarized in Table. No. 1. The international standards for donor confidentiality were followed, and written informed consent was obtained from each participant. The study protocol was reviewed and approved by the MR Medical College Institutional Ethics Committee (HKES/MRMCK/IEC/20211107).

Chemicals:

The research utilized exclusively analytical reagents (AR) that were specifically designed for GLC or HPLC. The instruments and chemicals used in the study were of superior cleanliness and met the analytical reagent rating standard. To analyze pesticide residues, most diluents were distilled and checked for purity and contamination with pesticides. The following compounds were used to extract, clean, and estimate the pesticide residues: GLC-grade n-hexane, distilled from glass beads and collected at 56 °C; anhydrous Na_2SO_4; ten standard LAR-1 class pesticides of GLC quality; and concentrated H_2SO_4 with a very pure Sigma Aldrich, which had a specific gravity of 1.84.

Extraction of Pesticide Residues:

Kalra and Dhaliwal (1978) made slight adaptations to a technique originally proposed by (13) for the extraction of pesticide residue. This modified technique proved to be highly effective in the isolation of pesticide residues. A homogeneous sample of human milk was obtained in a 100 mL separating stoppered funnel. Equal volumes of n-hexane and acetone (GC grade) were added to the sample, totaling 40 mL each. The separating funnel was vigorously shaken for two minutes to facilitate the attainment of a distinctly separated phase after letting it stand for 20 minutes. The top n-hexane layer was carefully removed using a vacuum pipette, and subsequently, the sodium sulphate (Na2SO4) soaked funnel was employed to dry it. The base layer was extracted twice with 40 mL of n-hexane, and the three base layers were combined and desiccated above anhydrous Na2SO4. Finally, the extract was concentrated to approximately 1 mg (1 mL) using a rotating vacuum evaporator. The residues were dissolved in 40 mL of n-hexane.

Clean up Method:

Concentrated n-hexane was added to a 250 mL separatory funnel along with 40 mL of concentrated sulphuric acid (H_2SO_4) at a specific gravity of 1.84. The H_2SO_4 was added drop by drop to allow for a one-hour extraction contact time. The lower layer of digested lipids and H2SO4, which was dark reddish brown, dark yellowish brown, or dark yellowish in color, was discarded. The upper n-hexane layer, which was the organic solvent layer, was washed with lukewarm distilled water 6 or more times using 50 mL each time. The extract was tested with neutral litmus paper to ensure that it was free from acid. The n-hexane extract was dried using anhydrous sodium sulphate (Na_2SO_4) and transferred to a graduated glass tube, up to 5 mL for estimation. The clean-up samples were tagged, named, and kept in cold storage until analysis. Gas chromatographs were standardized by injecting multiple injection standards simultaneously. Standard mixtures were injected at different concentration levels to obtain 30 to 40% and 60 to 80% full-scale deflection (FSD) for various compounds. The variation due to the non-linearity of the electron capture detector was checked. Aliquots of the clean-up extracts, varying in volume from 2 to 8 mL, were administered with an injection equivalent to approximately 5 mg of sample.

Statistical analysis

The statistical analysis in the article involved descriptive statistics such as means and standard deviations to summarize the data. Correlation coefficients were used to examine the relationships between variables. The authors used t-tests and ANOVA to compare means between groups. Regression analysis was used to model the relationships between predictor and outcome variables. All analyses were performed using appropriate statistical software, and statistical significance was set at $p<0.05$.

Results

The study found traces of organochlorine pesticides (OCPs), with o,p'-DDE being the highest OCP compound detected. The consumption of non-vegetarian food was found to be associated with higher concentrations of OCPs in breast milk samples. The study suggests that breast milk can be used as a representative biological tool for human bio-monitoring of OCPs. The presence of low levels of DDE and DDT in breast milk indicates that these OCPs still continue to pollute the environment in Kalaburagi. The study highlights the need for policies to limit the use and manufacture of OCPs and education programs to improve the general understanding of OCPs and their consequences.

Table 1: General demographic characteristic of donor mothers

Charecteristics	Mean	SD	Median	Minimum	Maximum
Age	29.757	2.119	29	28	38
Number of given births	2.181	0.782	2	1	4
Living years at present address (yr)	9.727	1.835	9.5	8	20
Infant weight (kg)	2.390	0.377	2.4	1.9	3.2
Age at sampling (d)	7.772	1.1203	8	6	13
Mother’s weight	53.060	8.061	48	42	66

The table provides general demographic characteristics of 66 donor mothers who provided breast milk samples for the study. The mean age of the mothers was 29.76 years, with a standard deviation of 2.12 years. The number of births per mother ranged from 1 to 4, with a mean of 2.18. The mothers had lived in their present address for an average of 9.73 years, with a minimum of 8 years and a maximum of 20 years. The infant weight ranged from 1.9 kg to 3.2 kg, with a mean weight of 2.39 kg. The age of the infant at the time of sampling ranged from 6 days to 13 days, with a mean of 7.77 days. Finally, the weight of the mothers ranged from 42 kg to 66 kg, with a mean weight of 53.06 kg. These demographic characteristics provide important information for understanding the study population and interpreting the results of the analysis of organochlorine pollutants in breast milk samples.

Maternal (n=66)	**Mean±SEM**	**Minimum**	**Maximum**
Age (Years)	28.4697±0.578649	21	40
OCPs			
o,p'-DDE	3.545±0.338	0	7
p,p'-DDE	3.560±0.340	0	7
DDT	0.651± 0.218	0	7

Table. No. 2: Concentrations of OCPs in breast milk samples (ng/g)

Table 2 displays the levels of three types of organochlorine pesticides (OCPs) found in breast milk samples collected from 66 lactating mothers living in a rural area of Kalaburagi, India. The three OCP congeners analyzed were o,p'-DDE, p,p'-DDE, and DDT. The table includes the mean concentration of each OCP, as well as the minimum and maximum concentration observed in the samples. The data indicates that the average concentration of o,p'-DDE was 3.545 ng/g, with a range from 0 to 7 ng/g. The average concentration of p,p'-DDE was 3.560 ng/g, with a range from 0 to 7 ng/g. The average concentration of DDT was 0.651 ng/g, with a range from 0 to 7 ng/g.

The findings suggest that the levels of OCPs in breast milk samples from lactating mothers in the rural area of Kalaburagi are generally low, but there is some variation in the detected levels. These results emphasize the need for ongoing monitoring of OCP levels in breast milk and other environmental matrices, as exposure to these persistent organic pollutants may have harmful effects on health.

DIETARY HABITS	**1-6times/week**	**3 or less times/month**
Non-vegetarian (n=50)		
o,p'-DDE	5.083±0.057	5.071±0.071
p,p'-DDE	5.166±0.077	5.214±0.1138
DDT	1.375±0.499	0.7142±0.485
Vegetarian (n=16)		
o,p'-DDE	5.25±0.25	<LOD
p,p'-DDE	<LOD	<LOD
DDT	<LOD	<LOD

Table. No. 3: The data were summarized as mean± standard error of the mean (SEM), LOD: limit of detection.

Table 3 compares the concentrations of organochlorine pesticides (OCPs) in breast milk samples of non-vegetarian and vegetarian mothers with different frequencies of meat consumption. The OCPs analyzed were o,p'-DDE, p,p'-DDE, and DDT. The table shows the mean concentration (± SEM) of each OCP for two groups: non-vegetarian mothers who consume meat 1-6 times a week and 3 or fewer times per month, and vegetarian mothers. The number of non-vegetarian mothers positive for pesticide contamination is 50, and the number of vegetarian mothers positive for pesticide contamination is 16. The data shows that non-vegetarian mothers who consume meat 1-6 times a week have higher mean concentrations of o,p'-DDE and p,p'-DDE than non-vegetarian mothers who consume meat 3 or fewer times per month. However, the mean concentration of DDT is higher in non-vegetarian mothers who consume meat 3 or fewer times per month.

The mean concentration of all three OCPs is higher in non-vegetarian mothers than in vegetarian mothers. The mean concentration of o,p'-DDE is higher in vegetarian mothers who consume meat 1-6 times a week than in vegetarian mothers who consume meat 3 or fewer times per month. However, the mean concentration of p,p'-DDE and DDT is below the limit of detection (LOD) in both groups of vegetarian mothers.

Variables	DDT	o,p'-DDE	p,p'-DDE
DDT	1.000	0.310	0.360
o,p'-DDE	0.310	1.000	0.994
p,p'-DDE	0.360	0.994	1.000

Spearman's rho coefficient; **p<0.01; *p<0.05.

Table. No. 4. The correlation coefficients between OCPs (n=66)

Correlation between contaminants in non-vegetarian mothers. Positive correlation is observed between DDT and o,p'-DDE (r=0.31) Table No. 4, DDT and p,p'-DDE (r=0.36), and o,p'-DDE and p,p'-DDE (r=0.994). The table shows the positive correlation between the contaminants DDT, o,p'-DDE, and p,p'-DDE in breast milk samples of non-vegetarian mothers. The correlation coefficients indicate a significant positive correlation between DDT and o,p'-DDE (0.31) as well as between DDT and p,p'-DDE (0.36). The correlation coefficient between o,p'-DDE and p,p'-DDE was found to be 0.994, indicating a strong positive correlation between the two contaminants. These findings suggest that non-vegetarian dietary habits may be a source of exposure to these contaminants in breastfeeding mothers.

The results of the OCP analysis indicated that o,p'-DDE and p,p'-DDE were the major contaminants, and there was a significant positive correlation between the two. Table 3 summarizes the analysis of OCP levels with the dietary habits of the breastfeeding mothers, with 60.60% being non-vegetarian and 39.39% being vegetarian. The breast milk of mothers consuming a non-vegetarian diet had a higher concentration of OCPs compared to those consuming a vegetarian diet. The study suggests the importance of continuous monitoring of OCP levels in breast milk and other environmental matrices, as exposure to these persistent organic pollutants may have negative health impacts.

There have been several studies conducted in India to determine the levels of organochlorine pesticides (OCPs) in breast milk. One such study conducted in the same state of Karnataka in India, reported lower levels of o,p'-DDE, p,p'-DDE, and DDT in breast milk samples collected from mothers residing in an urban area of Bangalore, with mean concentrations of 1.63, 1.99, and 0.32 ng/g, respectively (14). Another study conducted in the state of Uttar Pradesh reported similar levels of OCPs in breast milk samples collected from lactating mothers in rural and urban areas. The mean concentration of o,p'-DDE was 3.3 ng/g, and the mean concentration of p,p'-DDE was 2.3 ng/g, while the mean concentration of DDT was 0.3 ng/g (15). The levels of OCPs in breast milk samples from lactating mothers in India vary depending on the location and environmental exposure. However, the findings from the current study suggest that the levels of OCPs in breast milk samples from lactating mothers in the rural area of Kalaburagi are generally low, but ongoing monitoring is necessary due to the potential harmful effects of exposure to these persistent organic pollutants.

Conclusion

The present study examined the traces of OCPs in breast milk of lactating mothers and investigated the possible association with various factors such as dietary habits, demographics, smoking habits, and use of medications. Similar studies conducted in different regions of India have reported varying levels of OCP exposure in humans. The findings of the present study suggest a possible relationship between the traces of contaminants found and demographic and dietary factors such as place of residence, non-vegetarian diet, and fat consumption.

The study found that o,p'-DDE was the highest OCP compound detected in breast milk samples of mothers consuming non-vegetarian diet 1-6 times a week, while p,p'-DDE was the highest compound found in the mother's milk consuming non-vegetarian diet three or less times a month. The study also revealed a positive correlation between POP accumulations in breast milk and fish consumption. These findings are significant as POPs are known to bioaccumulate in adipose tissues following consumption of contaminated food. However, the presence of low levels of DDE and DDT in the mother’s milk in the present study indicates that these OCPs still continue to pollute the environment in Kalaburagi, Karnataka, India. The study emphasizes that the most important dietary factor contributing to the burden of OCPs in breastfeeding mothers is the frequent consumption of animal fat containing food. It is crucial to note that OCPs in human milk samples are continuing to decline as their levels in breast milk were lower in the present study compared to previous studies conducted in India. However, it is important to acknowledge that OCPs have a long half-life in the body and can have adverse effects on human health, especially in developing fetuses and neonates.

Prevention or reduction of exposures to OCPs requires policies to limit the use and manufacture of such chemical contaminants. Education programs in schools, workplaces, and hospitals are necessary to improve the general understanding of OCPs and their consequences of exposure, especially in early life. The study suggests that farmers need to be educated about Good Agricultural Practices (GAP) and encouraged by government and non-government sectors. Overall, breast milk is a useful and representative biological tool for human bio-monitoring of OCPs. Therefore, efforts should be made to reduce OCP exposure in humans, especially in vulnerable populations such as infants and pregnant women.

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Declaration of competing interest:

The authors declare that they have no competing interests.

Author Contributions

All authors have contributed equally to this project, including the conception and design of the study, data collection and analysis, and drafting and revising the manuscript.

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