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Monitoring of Organochlorine Pesticides in Mother's Milk from Kalaburagi District, Karnataka, India

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Abstract Organochlorine pollutants are prevalent worldwide concern which needs urgent attention. In present study traces of organocholorine micro-pollutants (o,p'-DDE, p,p'-DDE) and DDT were determined in 22 samples of mother's milk collected from 2 different maternity hospitals from Kalaburagi district, Karnataka, India. Donors were interviewed with respect to factors that may affect accumulation of these compounds such as parity, age, place of residence, workplace, dietry habits, any previous miscarriages, and education with the help of a questionnaire. The analysis was done by GCMS. The minute traces of organochlorine pollutants (OCPs) in samples from 3 rural areas of Kalaburagi district were detected. Total DDT, p,p'-DDE and o,p'-DDE were analyzed in almost 80% of the mother's milk samples analyzed. A positive correlation was observed between traces of contaminants and consumption of non-vegetarian diet. This implies that the parameter might play an important role in influencing OCPs burdens in lactating women. The traces of contaminants in mother's milk were between 5 ng g^{-1} to 9 ng g^{-1} the results are within acceptable range and safe.

Keywords: *mother's milk, GCMS, pesticide, contamination, Kalaburagi*

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1. Introduction

Breastfeeding is recommended since, it is the only way to gain all the necessary nutrition for infants proper growth and nourishment. Infants who are breastfed are nearly six times more likely to have a higher immune system then the ones who are not breastfed and are more resistant to infectious diseases. Certain organochlorine pesticides (OCPs) are distinguished by their ability to dissolve in fats and also their tenacity in nature. Thus, the bio-accumulation in the adipose tissues of humans and animals. Thus, accumulation from diverse food chains. OCPs are the pesticide types that pose dangers which leads to unfavorable outcome towards health of human and the habitat around them. Numerous studies globally had been researched for analyzing the residue levels including breast milk and milk of buffalo, cow, goat, camel and sheep, in emergent and developed nations. From the previous studies in Kalaburagi [1] found certain organochlorine compounds such as DDT and DDE in mother's milk. Such studies synopsized briefly for some of the organochlorine pollutants analyzed were below the admissible limits provided by FAO/WHO. Such international organizations, yet in majority of emerging countries, they are even so practiced illegally [2]. In India, the authorities have forbid the use of OCPs since many years. Though, these pollutants (OCPs) released prior to the ban of these pesticides, they are still present in various environmental and biological media [3,4,5]. Breast milk bio-monitoring may also reveal specific details regarding the types and amount of pollutant traces in the nature and also in the diet we consume daily. This essential assessment is needed to evaluate the probable health risks are co-related with the intake of milk with contribution of breast milk intake into the infants throughout early stages of breastfeeding. Thus, the assessment of environmental pollutants in mother's milk is of wide research interests around the globe. Therefore, this study focused on investigating the residue traces of pollutants, as well as representing groups of such organochlorine pollutants in breast milk from mother's residing in rural regions of Kalaburagi district. The obtained results have contributed to assess the effects of organochlorine pesticide residue on human health.

2. Materials and Methods

2.1. Study Area

In Karnataka's north-eastern region, 'Kalaburagi' (Earlier Gulbarga) means stony land in Kannada. The most

prominent language used in Kalaburagi is Kannada. Kalaburagi is recognized for the ancient structures made by the Bahamani rulers. Furthermore, it is also a commercial hub for Karnataka and also Hyderabad. In addition to that an educational capability, the centre also ready as a regional marketplace and facility hub for the region. It's location in a poor area has enabled the city to become a focal point for many development initiatives, which have attracted residents from surrounding districts. Out of 30 districts in Karnataka, Kalaburagi's administrative centre is located here. Kalaburagi is situated in Deccan Plateau located at 17°-33" North and 76°-83" East and area of 10,951 Km^2. Study was conducted in Basaveshwar hospital and Sangameshwar hospital, which is one among the teaching and general hospital in Kalaburagi, which is a part of Mahadevappa Rampure Medical College, which was established in the year 1963.

2.2. Study Population and Sampling

Sample collection was done from two hospitals in Kalaburagi city. Twenty-two breast milk samples were collected during the month of February 2022. Sample were collected with the help of two staff nurses instructed for this study, only after signing informed consent form. Institutional Ethics Committee after reviewing the protocol submitted for the study was approved **(HKES/MRMCK/IEC/20211107)**, that must not be engaged for any purposes other than the study. The questionnaire prepared for the donor mothers conformed to the standards provided by the international bodies and respecting the confidentiality agreement of the donors. All the mothers who were willing to donate the breast milk samples were needed to complete a questionnaire which involved questions like occupation, type of dietary habits, parity, any habits which might increase the exposure of pesticides were recorded. The standard questionnaire modeled after the internationally recommended (UNEP, 2012). The mothers participating in the present study were all primiparous during the sampling period. All the mothers lived at the current place of residence for more than 8-10 years in rural areas. The mothers who participated in the study were between the range of 28-30 years of age. Most donors were consuming a non-vegetarian diet with 5 only consumed vegetables and eggs. The donor mothers were healthy after the parturation, had normal deliveries and nursing only one baby (no twins). Table 1 shows brief summary of the demographic characteristics.

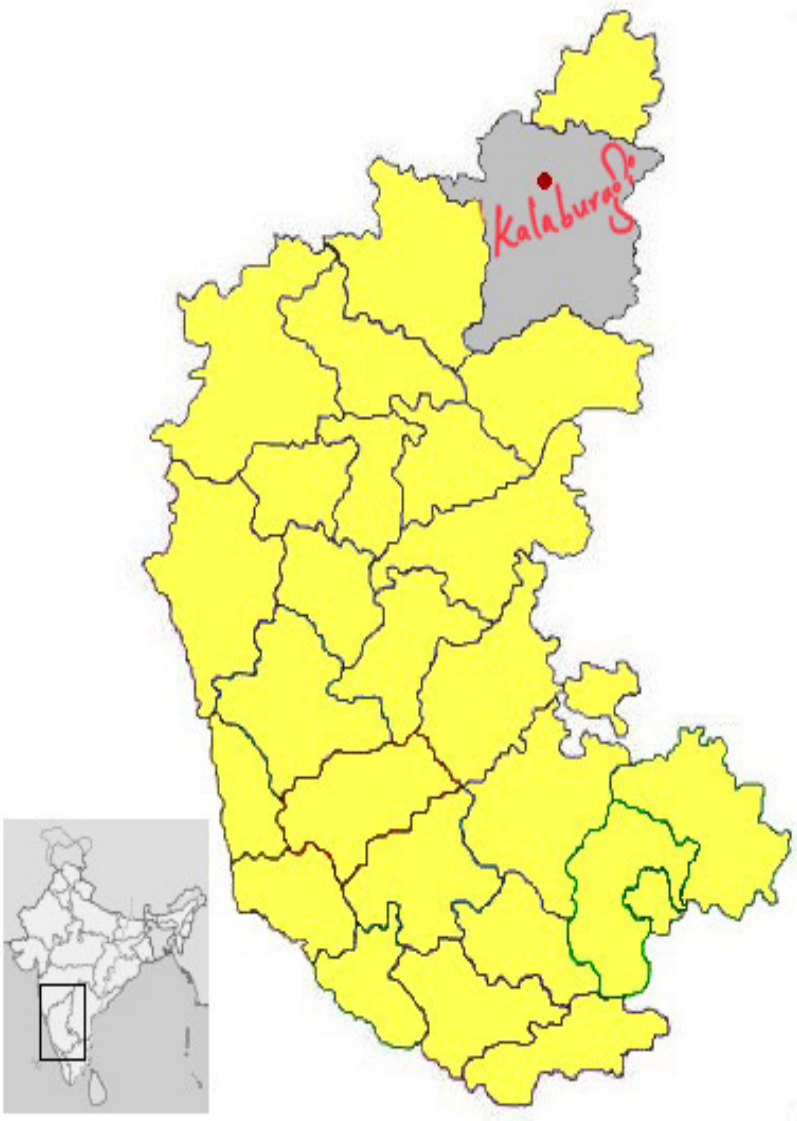

Figure 1. Study Area Kalaburagi

Table 1. General demographic characteristic of donor mothers

Charecteristics	Mean	SD	Median	Minimum	Maximum
Age	29.04545455	0.785419071	29	28	30
Number of given births	1	0	1	1	1
Living years at present address (yr)	10.27273	2.864924	10	8	20
Infant weight (kg)	2.427273	0.371845	2.45	1.9	3.2
Age at sampling (d)	8.363636	1.677454	8	6	13
Mother's weight	55	7.483315	58	42	66

2.3. Chemicals

We used only analytical reagents (ARs) designed for GLC or HPLC. All equipments and chemicals that we used in the research work were of high cleanliness and analytical reagent score. Most of the diluters were distilled and tested for pesticide purity and contamination before being used in residue analysis. In order to extract, clean, and estimate pesticide residues, the following compounds were used: n-Hexane: GLC grade, distilled over glass beads, and collected at 56°C Anhydrous Na_2SO_4: 10 LAR-1 grade standard pesticide: GLC grade Concentrated H_2SO_4: extremely pure with specific gravity 1.84 Sigma Aldrich was used to obtain the residue.

2.3.1. Extraction of Pesticide Residues

[6] Slightly modified a technique originally published by [7] for extraction of pesticide residue. This technique was considerably effective in extraction of pesticide residues. A well mixed sample of human milk was obtained in a 100 mL separating stoppered funnel. Each of which n-hexane and acetone (GC grade) were added to the sample in a quantity of 40 mL each. Thoroughly shaking the separating funnel for two minutes continued, so that distinct separation of phase is achieved after letting it stand for 20 minutes. A vacuum pipette was used to remove the top n-hexane layer, and then the sodium sulphate (Na_2SO_4) soaked funnel was used to dry it. Adding 40 mL of n-hexane, the base layer was extracted twice. The three layers from base were combined and parched above anhydrous Na_2SO_4. Lastly, the extract was concentrated over a rotating vacuum evaporator to around 1 mg (1 mL). 40 mL n-hexane was utilized to liquefy the residues.

2.3.2. Clean up Method

A 250 mL separatory funnel was filled with concentrated nhexane and 40 mL concentrated sulphuric acid (H_2SO_4), sp. gravity 1.84 was added in it drop wise slowly to allow the contact time of the extraction (1 hour). The lower dark reddish brown/ dark yellowish brown/dark yellowish layer of digested lipids and H_2SO_4 was discarded. The organic solvent layer (upper n-hexane layer) was washed with lukewarm distilled water (6 or more times using 50 mL each time) and ensured that extract was free from acid with the help of neutral litmus paper. The n-hexane extract was dried up by passage over 5-10 gm of anhydrous sodium sulphate (Na_2SO_4) in a funnel. The contents were finally transferred to graduated glass tube up to 5 mL for estimation. Clean up sample were tagged, named and kept in cold storage up until analysis. Pesticide Residues Estimation Gas chromatographs were standardised by simultaneously injecting multiple injection standards. Standard mixture were injected at all the different concentration levels of standard prepared to obtain 30 to 40% and 60 to 80% full scale deflection (FSD) for various compounds and checked variation due to non-linearity of electron capture detector. We administered aliquots of the clean-up extracts varying in volume from 2 to 8 mL, with an injection equivalent to approximately 5 mg of sample. If necessary the extracts were diluted until we get peak height within the scale, for identification of organochlorine pesticides.

3. Results and Discussion

Most of the samples analyzed were contaminated with minute traces of organochlorine pesticides. Most dominant contaminant was o,p'-DDE which was revealed in 22 samples, second most dominant contaminant was p,p'-DDE, which was analyzed in 16 samples of breast milk. Least dominant contaminant was DDT which was analyzed in 10 samples of breast milk. Allthough the traces of contaminants o,p'-DDE, p,p'-DDE and DDT were ranging from 0.006 ppm, 0.006 ppm and .005 ppm respectively shown in the Table 2, which are very low when compared to the previous studies [8]. The information about the dietary habits was obtained during the sample collection and the study shows the contamination may be due to a diet rich in meat [9]. Lactation is one of the main routes of excretion [10]. The results show that there is a need for continuous bio-monitoring of breast milk in this region for assessment of such toxic contaminants. Since, there is no data availability on pesticide contamination in this area.

Table 2. DDE p, p', DDE o, p' and DDT residues in breast milk

Residues	Whole milk (ng g^{-1})	
DDE p, p'	Mean ± SD	0.003 ± 0.0024
	Range	0.006
DDE o, p'	Mean ± SD	0.005 ± 0.0004
	Range	0.001
DDT	Mean ± SD	0.002 ± 0.0025
	Range	0.005

4. Conclusion

It is evident from the present study that rural regions of Kalaburagi has pesticide contamination. Distribution of contamination is not uniform among the organochlorine

contaminants in the rural areas; it differs from place to place. Most of the mother's milk samples are found to be contaminated with traces of organochlorine pollutants. It may be due to the unethical ways of buying the banned drugs and also in some cases the misuse of the pesticide by applying more than the recommended quantity per acre. As these pesticide pollutants has the potential to persist in the nature for an extended period of time, also can contaminate food and environment through dietary habits or by bio-accumulation in humans and also in animals. It is necessary to organize campaigns to spread awareness to the people by private agencies and government sectors. The result in present study also highlights on the for detailed investigation and monitoring of OCP levels in human and basic food items as they are the basic pathways to contamination and health risks for human health.

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