ORIGINAL ARTICLE

Analysis of Organochlorine Pollutants in Mother's Milk from Kalaburagi district, Karnataka, India

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ABSTRACT Aim of the present study was to analyse the organic pollutant residues in mother's milk (n=14) from Kalaburagi, Karnataka, India, revealed p,p?-DDE and o,p?-DDE as the primary pollutants. The study aimed to examine the present exposure levels of organochlorine pollutants (OCPs) in mother's milk of general population from Kalaburagi district. The study was carried out to explore the effects of (o,p?-DDE) 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene, and (p,p?-DDE) 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene, This was a monitoring study where milk samples from Kalaburagi district were collected and analysed for OCPs levels using GC-MS. The increased levels of DDE in mother's milk observed in rural areas with levels were 9 ng g-1 and the lowest was found in urban areas with 5 ng g-1. In India, DDT is still practiced for various control programs in health departments, which may be the credible reason for the organochlorine pollutants to reflect in breast milk samples. The amount of organochlorine residues recorded in mother's milk indicates higher traces in rural study areas of Kalaburagi. The study shows the contamination in mother's milk is below the permitted limits and harmless.

Keywords: Human breast milk, GCMS, DDE, Kalaburagi

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INTRODUCTION

Breast milk is the first food that humans consume, from birth to a recommended minimum of six months, and it provides all of the necessary nutrients [6], Breastfeeding has numerous benefits to mother and child including enhanced health after birth, child bonding, and the development of baby's nervous system and immune system. Environmental contaminants may cause concerns to infants health, when they are found in mother's milk. Like a slow-degrading, highly toxic manmade chemical, organochlorine pesticides are dangerous. The high lipid content of human milk makes it an ideal partitioning matrix for OCPs [3], which tends to be lipophilic in nature [24]. Pesticides such as DDT, DDE, and related chemicals accumulate in the environment and in living organisms, yet they are illegal in agriculture in India [11]. Via dietary habits, these pollutants accumulate in human body, which results in severe health issues [23]. The residues of OCPs are observed in food and water [10], as well as mother's milk [21,20] also reported these OCP residues in mother's milk, [22,2]. There have been previous reports of it being present in dairy milk [23,18] and skin. DDE traces reported in previous studies stated health problems [13,12]. International rules such as the Stockholm Convention restrict or ban the use and emission of such Persistent organic pollutants (POPs), which are organic compounds containing organochlorines. The fact that they are toxic, mobile and bio-accumulate in environment are reasons why they are banned in many countries. Because of their perpetual quality, they can ecotoxicity via dietary habits.

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MATERIAL AND METHODS

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, 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.

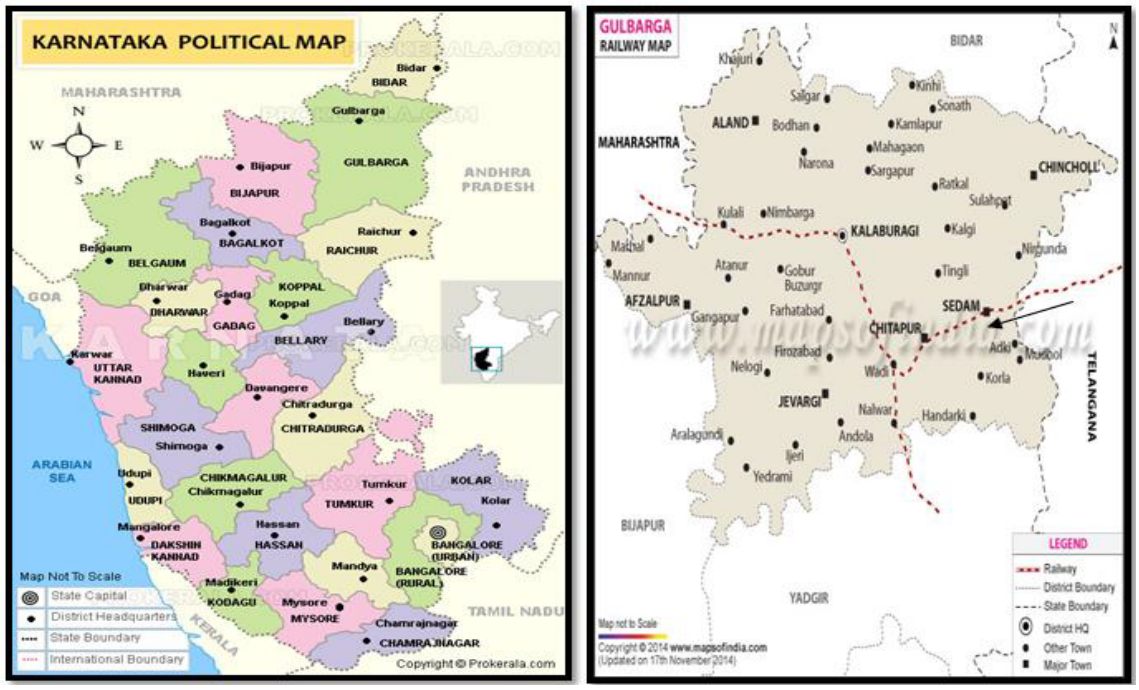
Sampling

Breast milk samples were collected from donors who were under medication in Basaveshwar Teaching and General Hospital. In the course of study period, June to November of 2021, the samples were collected. Diet frequency questionnaires were completed during the interview. There were also questions about personal information (such as age, residence, employment, chewing tobacco, beetle leaves, smoking habits) and events that could impact pesticide exposure. In the first week of breastfeeding, we collected breast milk samples only after we have obtained signatures on informed consent form. Donors were chosen on the random selected workdays, were suitable to participate in the trial. The present work was submitted and approved by the Integrities forming Committee of Mahadevappa Rampure Medical College, Kalaburagi (approval no. HKES/MRMCK/IEC/20211107), around 100 subjects were approached during the study period, from which only 19 agreed to donate us with breast milk samples. 19 samples were collected in which only 14 were eligible for analysis, since the remaining 5 samples were below 10 mL they were returned to the mothers. The amount of sample needed for analysis was a minimal of 18-20 mL. The samples were collected in amber vials, to protect the milk from UV-light and to keep the sample safe and stable during transport from hospital to laboratory. The samples were placed in ice chest packed with icepacks while in transit. Later the collected samples were transferred from amber vials to normal glass vials, special care was given to avoid cross contamination of the samples. The samples were stored under -20°C until analysis and later sent to laboratory in cold conditions for analysis by GC-MS.

Chemicals

We used only analytical reagents (ARs) designed for GLC or HPLC. All equipments and chemicals that we used in the

Figure 1: Study Area Kalaburagi



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.

Extraction of Pesticide Residues

[5] Slightly modified a technique originally published by [16] 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 stoppered separating funnel. Each of 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 (Na2SO4) 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 Na2SO4. 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.

Clean up Method

A 250 mL separatory funnel was filled with concentrated n-hexane and 40 mL concentrated sulphuric acid (H2SO4), 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 H2SO4 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 (Na2SO4) 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.

RESULTS AND DISCUSSION

In the present study, (OCP) pollutants were detected in breast milk samples as part of the investigation (Fig 2). These results suggest that there is a high level of exposure to these hazardous pollutants among the general population. There are several explanations for the high amount of OCPs observed in this research work, including pesticide exposure and malaria control measures. These reports favour the pattern of OCP residues in mother's milk samples. This study suggests that population in the Kalaburagi urban area is exposed to relatively low levels of OCPs and related compounds which implies some of the residents in the Kalaburagi rural areas are exposed to specific pollutant sources such as DDT, DDE, and related compounds. [17] Too reported that p,p2 -DDE was linked to some negative outcomes including premature births, low infant weights, and baby size at birth. Since contamination is in acceptable limits. Researchers from industrialized countries have reported higher levels of lipophilic OCPs in primipara than multipara breast milk [7,19] since lipophilic OCPs like DDE reduce body burden throughout gestation and breastfeeding. As no significant difference between primapara and multipara mothers was observed, the current findings may suggest that Kalaburagi mothers are still exposed to these pollutants. It is unclear what factors determine the pesticides in milk, but they include factors like parity and age, food habits, habitation, and extra external issues [8]. There are four factors that are most important: place of residence, consumption of tobacco, age and extremely contaminated dietary intake [26]. When these residues are mobilized during breast feeding, the result is that these residues are excreted into breast milk [27]. Present research work reveals that breast milk samples collected in rural Kalaburagi area contained minute levels of DDE. The findings of present study show that breast milk involves unusual traces of DDE residues. The DDE levels were harmless and within range as per the study. However, the study reveals traces of DDE were much higher than in human breast milk from other states, like in Punjab, where DDE levels are 0.3 ng g-1 [1]. In study area, we found negligible levels of DDE contamination that could be explained by the vectors for the purpose of malaria control, moreover the earlier utilization of DDT practiced in this area. However, we found no link between maternal age and DDE accumulation in breast milk [29]. In the study area, contamination may be linked to residues of DDT from previous decades and groundwater pollution.

Table 1: General demographic characteristic of donor mothers

Characteristics	Mean	SD	Median	Minimum	Maximum
Age	29.35	2.97	29	25	35
Number of given births	1.42	0.64	1	1	3
Living years at present address	7.85	3.18	7	4	14
Infant weight (kg)	2.34	0.32	2.35	1.9	2.9
Age at sampling (days)	6.64	2.30	6.5	3	10
Mother's weight (kg)	53.21	5.02	52.5	45	62

Figure 2: Graph Showing Contamination in Rural Subjects

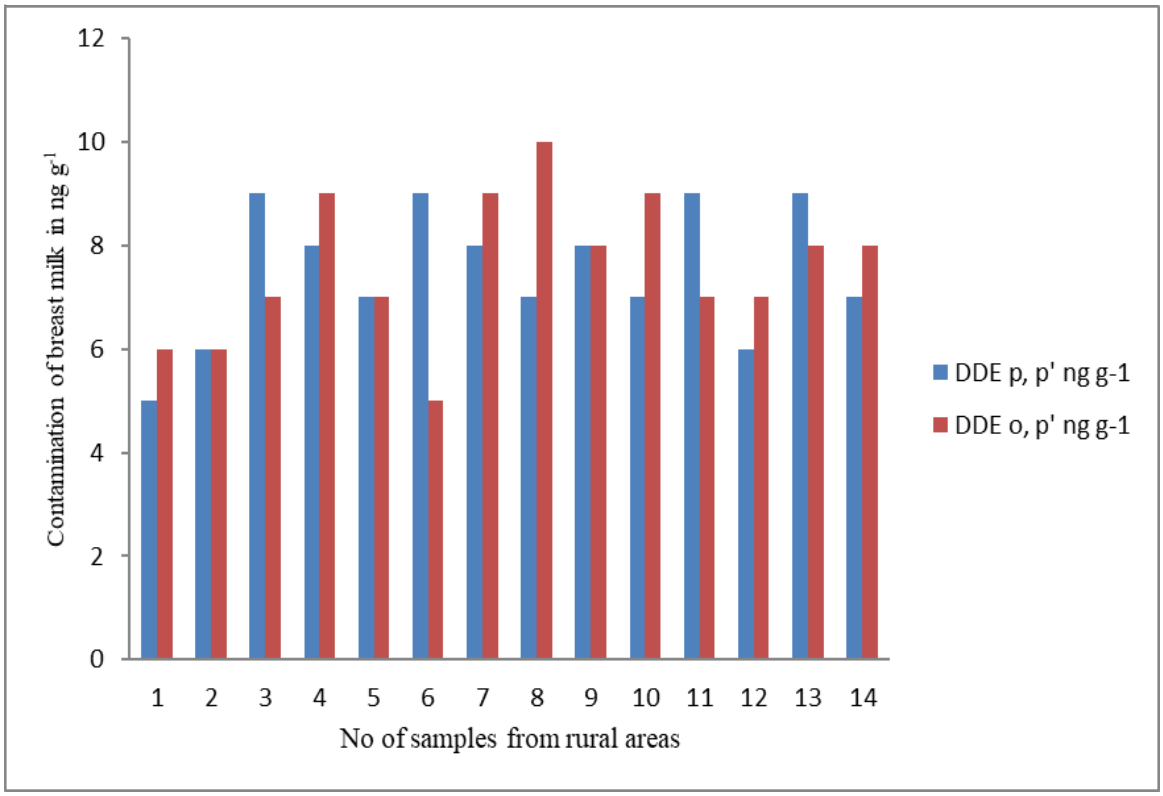


Table 2: p, p'-DDE and o, p'-DDE Residues in Breast Milk

Residues		Whole milk (ng g^{-1})
DDE p, p'	Mean ± SD	0.0072 ± 0.0013
	Range	0.004
DDE o, p'	Mean ± SD	0.0075 ± 0.0013
	Range	0.004

CONCLUSION

This study presents one of the first data of organochlorine residues in mother's milk in Kalaburagi region. However the samples examined in this study were less, it shows that the concentrations of pollutants in mother's milk samples from Kalaburagi area are slightly higher level of concentration range when compared to other parts in India. This study has the potential to represent a keystone in assessing the feasibility and the need for a continuous monitoring of Kalaburagi population.

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