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Human milk is a primary and the potent source of the infant nutrition

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ABSTRACT:

Human milk is a primary and the potent source of the infant nutrition It is rich in fat and stands at the end of the food chain Since the lipid content of the milk is high and organochlorine pesticides are lipophilic in nature, therefore, they can accumulate in human milk in alarming concentrations. Therefore, human milk can be used as an evaluation index of environmental contamination by these noxious chemicals, although the main objective of its analysis is to determine the amounts ingested by children, who without a doubt, will have to face other sources of contamination during their lives. Therefore, average levels of organochlorine pesticides in the mother's milk may be counted as an index for their daily intake by the neonates, which may in turn pose various problems of management of neonatal health and nutrition. It is now a well-accepted fact that only organochlorine pesticides (OCPs) especially DDT, HCH and to some extent Aldrin / dieldrin and heptachlor play a vital role in the tissue accumulation of pesticides.It has been well established that pesticides, particularly the chlorinated hydrocarbons directly affect the fetuses and neonates as they get transferred through placenta and mother milk respectively. This has been confirmed in all the mammalian species that have been examined including humans.Taking above points into consideration, a continued surveillance on the levels of pesticide pollutants in human conception is an important task to ensure the well-being of the human pregnancy. It was, therefore, planned to conduct such as a study in Jaipur, the capital of Rajasthan and the pink city of India. Findings of this research work may provide base line data of the extent of pesticide contamination/exposure in women body, fetus and offspring. The data obtained from the above work may also provide some clues, possible reasons for abortions, premature deliveries, still births, some infant diseases andmortalitiess. The study is mainly concerned with the pesticides burden in the pregnant women and its transfer to Prenates, so it will require the analysis of pesticide residues in the blood of pregnant women before and after the lactation using gas liquid chromatography. Thirteen women are included in the present study. In this study, blood of the women was collected before parturition and the blood of the same subject was also collected at the 40th day of parturition. Concentration of organochlorine pesticides in blood of women before parturition and at 40th day of parturition was compared. A general trend of low residues was found in the blood collected at the 40th day of parturition in comparison of blood before parturition. The results revealed the presence of isomers of HCH, heptachlor, DDT and its metabolites and Aldrin in mostly all the samples analyzed. The OCPs residues present in the maternal blood indicate the pesticide burden in the human population, which in turn is a risk to human health. Secondly, this may be considered as an indication of the transfer of these chemicals

from maternal to fetal circulation across the placenta which may pose various problems of management of prenatal health.

KEY WORDS:
Mother's blood, Organochlorine, Pesticides, Contamination, Residues, Gas Chromatograph, Mothers milk, Followup study

INTRODUCTION

Pesticides occupy a rather unique position among the many chemicals that manencounters daily, in that they are deliberately added to the environment for the purposeof killing or injuring some form of life. Ideally, their injurious action would be highlyspecific for undesirable target organisms and non-injurious to desirable non-targetorganisms. In fact, however, most of the chemicals that are used as pesticides are nothighly selective but are generally toxic to many non-target species including man, andother desirable forms of life that co-inhabit the environment. Therefore, lacking highlyselective pesticidal action, the application of pesticides must often be predicted onselecting quantities and manners of usage that will minimize the possibility of exposureof non-target organisms to injurious quantities of these useful chemical.It has been known since the last century that maternal milk sometimes may contain chemical contaminants which could have adverse effects on nursing infants. Such experiences came mainly from cases of exposure to occupational chemicals or drugs. Since 1950, it has further been known that human milk may contain potentially hazardous persistent environmental chemicals in concentrations higher than in cow's milk [1].It is well known that the females significantly reduce their body burden of pesticides during lactation [2,3]. Therefore, the neonate would get exposed to them orally even if there is no immediate exposure of the mothers to these toxic compounds.

Whatever the infant's chemical exposure from maternal milk, it is superimposed on an existing neonatal body burden. Transplacental migration of toxic chemicals to the growing fetus causes a prenatal exposure and subsequently burden of maternally carried chemicals. But whereas prenatal exposure cannot at present be avoided in the course of childbearing, breast feeding has an acceptable alternative. The question that parent and their physicians must address is whether or not to nurse, and it is one that cannot be answered with any clear substantiating evidence. For most chemicals, level in milk cannot be assigned. Even more equivocal is the choice between avoiding exposure to chemicals in breast milk and obtaining the "beneficial factors" in human milk.

Passive transfer which may account for some of the beneficial components in human milk, accounts for all of the potentially detrimental chemicals found therein. It is a process involving diffusion of a compound from the blood, within capillaries surrounding the secreting mechanism of mammary glands, into the milk. Since, it relies on relative solubility in these compartments, the extent of passive transfer of a substance is determined by its chemical properties and by the chemical constitution of blood and milk. The efficiency of the process is often depicted in the milk plasma ratio, indicating the relative concentration of a chemical in maternal milk and blood. We know that residue levels of pesticides in the blood of developing child (cord blood are almost in dynamic equilibrium with those in maternal blood.In India, although percapita consumption

ofagrochemicals is much less than that in other developed countries, the tendency to accumulate them in body tissues and fluids is relatively high [4]. There are many reports which have drawn attention to the pesticide burden in the human milk from all over the world [5]. No such report is available on the accumulation of chlorinated pesticides in human milk of women from Jaipur, the capital ofRajasthan and pink city of India.

More scary studies have indicated that we have largely over looked the darker side of these chemicals as OCPs are reported to be carcinogenic [6], [7] mutagenic [7],[8] teratogenic [8],[9] immunosuppressive [10],[11] create endocrine dysfunction such as hypothyroidism or high estrogenic activity [12],[13] disturb reproductive processes [14],[15] growth depressants [16],[17] induces several psychogenic and neurogenic abnormalities in adult stages [18],[19], and are associated with abortions, premature deliveries, still births and infants with low birth weights [20]-[23]. OCPs have been in use in India nearly for a half century now. Even after having clear cut evidence suggesting that these chemicals have the ability to eliminate entire species from the planet, the annual consumption of pesticides in India is about 85,000 tons of which OCPs comprise the bulk [24]. Therefore, today OCPs are perhaps the most ubiquitous of the potentially harmful chemicals encountered in the environment and are still widely detected in humans despite the considerable decline in environmental concentrations [25-30].

Since there is no such data is available from Jaipur, pink city of India and capital of Rajasthan, therefore, it was planned to carry out systematic study which is mainly concerned with estimation of the pesticides burden in the pregnant women and its transfer to Prenates, so it will require the analysis of pesticide residues in the blood of pregnant women before and after the lactation using gas liquid chromatography. Thirteen women are included in the present study. In this study, blood of the women was collected before parturition and the blood of the same subject was also collected at the 40th day of parturition. Concentration of organochlorine pesticides in blood of women before parturition and at 40th day of parturition was compared. A general trend of low residues was found in the blood collected at the 40th day of parturition in comparison of blood before parturition. The results revealed the presence of isomers of HCH, heptachlor, DDT and its metabolites and Aldrin in mostly all the samples analyzed. The OCPs residues present in the maternal blood indicate the pesticide burden in the human population, which in turn is a risk to human health. Secondly, this may be considered as an indication of the transfer of these chemicals from maternal to fetal circulation across the placenta which may pose various problems of management of prenatal health.

MATERIALS AND METHODS

Since, organochlorine pesticides are lipophilic in nature they may accumulate in humanmilk in alarming concentrations. During the lactation mother may reduce body burdenof these pesticides Therefore, a follow up study was also conducted in which maternalblood of the mother was collected at the 40th day of the parturition, to find out whetherthere is a fall in the levels of different pesticides in maternal blood collected at the 40thday of parturition. A private clinic "Sanjeevini hospital Bani Park, Jaipur has beenidentified for the proposed work. In general, they had no history of any occupational or accidental exposure to pesticides. However, they were asked to fill up a questionnaire giving information about their health and relevant to the pesticide

residue accumulation such as age, dietary habits, area of residence, parity, social status, accidental or occupational exposure to pesticides etc. according to WHO methodology [31] by interviewing the subjects at the time of collection of samples.

Sample Collection

Maternal blood:

Five ml of maternal blood from each case was collected by venipuncture in pre-heparinzedvials, 4-8 hours before parturition andat the 40th day of the parturition while mother has been breast feeding the child for nearly forty days. to find out whether there is a fall in the levels of different pesticides in maternal blood. Mothers blood after collection was stored at -10°C in a deep freeze until analysed.

Extraction of Pesticide from Samples

Pesticides were extracted and separated from samples by liquid partition and column chromatography so that they could be analyzed by Gas Liquid Chromatography (GLC) and Thin Layer Chromatography (TLC) procedures. All reagents and chemicals used were of analytical grade and checked for any pesticide contamination. Specimens of maternal bloodblood, placenta and cord blood were extracted and then cleaned by florosil column as per the methodology given by Bush and his coworkers with little modifications according to the prevailing laboratory conditions [32].

Quantitative Estimation

Quantitative estimation of pesticide residues in all the extracts was done by HP 5890 series II gas chromatograph (GC) equipped with Ni 63 Electron capture detector (ECD) coupled to HP 3396A integrator. Glass coiled column (1.43 m x 4 mm L x I. D) was packed with Solid Support, Chromosorb 100/120 mesh size along with the Liquid phase: 1.5% OV -14±1.95% OV-210. Purified nitrogen (IOLAR-1) gas was used as the carrier gas and a known volume of sample was injected in the column with the help of the 10 µl Hamilton syringe. Different peaks of the samples were identified by comparing their retention times with those of standards. Quantitation of the samples were done by the data obtained from the integrator and were based on peak areas. Standards were obtained from Environmental Protection agency (EPA) U.S.A.

Recovery Analysis and Confirmation of Pesticide residues

Recovery analysis was done by fortification experiments and the percentage recovery was 95–98%.TLC was used for confirming the identity of the OCPs already detected by the GC. The pesticides for which the GC was standardized and were estimated were Aldrin, isomers of HCH (α, β & γ), metabolites of heptachlor (Heptachlor & Heptachlor epoxide) and DDT (DDE, DDD and DDT).

Statistical Analysis

The calculations are based on biological statistics and values are expressed as mean± standard error (S.E.). The difference in the pesticide residue levels between different groups was analyzed with the help of student t test. Significance between the residue levels of different groups was judged at 5 % and 1% levels.

OBSERVATIONS

Table.1: Concentration of organochlorine pesticides in maternal blood collected before parturition and at the 40th day of parturition. (Number of cases - 13) (ppm)

Sr. No.	Organochlorine pesticide detected	Maternal Blood Before Parturition	Maternal Blood at 40th day after delivery
		Mean + S.E.	Mean + S.E.
1.	∝-HCH	0.1197±0.0277 (n=12)	0.0574±00.0189 (n=13)
2.	γ-HCH	0.0918±0.0436 (N=10)	0.0146±0.0046 (N=8)
3.	β-HCH	0.0861±0.0221 (N=8)	0.0293±0.0162* (N=10)
4.	heptachlor	1.9538±0.5309 (n=10)	0.4025±0.1462* (n=8)
5.	Aldrin	0.1430±0.0305 (n=11)	0.0840±0.0385 (n=12)
6.	Heptachlor epoxide	0.7907±0.2252 (n=13)	0.5300±0.2385 (n=6)
7.	DDL	0.0784±0.0146 (n=12)	0.0589±0.0298 (N=13)
8.	DDD	0.0128±0.0078 (n=5)	0.0075±0.0039 (n=2)
9.	DDT	0.0213±0.0072 (n=8)	1.0776±0.9534 (n=5)
10.	Σ HCH	0.2114±0.0592 (n=13)	0.0890±0.0323 (n=13)
11.	Σ Heptachlor	2.2157±0.6810 (N=13)	0.5818±0.1979* (n=11)
12.	Σ DDT	0.0986±0.0154 (n=12)	0.4791±0.4230 (n=13)
13.	Total OCI	2.6571±0.7191 (n=13)	1.1380±0.5887 (n=13)
* Statistically Significant (P<.05)			

Σ HCH-total HCH

Σ Heptachlor-Total Heptachlor
Σ DDT-Total DDT
n-no of positive samples
ND-not detected

1. Specimen of breast milk, maternal blood and umbilical cord blood from the same 13 subjects were analysed for organochlorine pesticides. From 13 subjects, blood was also collected on 40th day of parturition, to find whether there is any fall in the level of pesticides in the women, as milk is the main excretory route of pesticides. Blood samples could be collected from 13 subjects only as it was very difficult to keep a track of follow up cases.
2. Thirteen women are included in the present study. In this study, blood of the women was collected before parturition and the blood of the same subject was also collected at the 40th day of parturition. Concentration of organochlorine pesticides in blood of women before parturition and at 40th day of parturition was compared in the Table:1.
3. A general trend of low residues was found in the blood collected at the 40th day of parturition in comparison of blood before parturition. A significant fall in the level of ß-HCH (0.293 ppm against 0.08612 ppm), heptachlor (0.4025 ppm against 1.9538 ppm and total heptachlor (0.5818 ppm against 2.2157 ppm) were found after 40 days of parturition in the blood of the women (table:1). Being lipophilic in nature, organochlorines are soluble in fat and fat rich tissues. Therefore, they are excreted in the milk. In this way women shed off the burden of these pesticides [33]. Because of this, after 40 days of lactation there is a fall in the residue level of pesticides in the blood of the women when compared with the residue level in the blood collected before parturition.

Available literature reveals, that till date no such study has been conducted in any part of world, therefore, probably this is a first report which reveals that lactation brings about a fall in the residue levels of lipophilic pesticides in the mother's blood.

Discussion

It has been reported that Indians possess highest body burden of organochlorine pesticides [34]. The medical importance ofDDT in human milk depends entirely on the dosage of the compound received by babies. The average amount ofmilk secreted daily is about 854 ml for breast feeding mothers with one child and 950 ml for a mother with twins (Adamovic et al., 1978). The present study suggests a daily intake of about 0.2555 mg of total DDT, 0.1736 mg of total HCH, 3.751 mg of total heptachlor and 0.1344 mg of Aldrin by neonates. However, this neonatal intake is lower in case of mothers with twins, but that is an exception. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have recommended as acceptable daily intake (ADI' S) 0005 mg/kg body weight for DDT, 0.01mg/kg body weight for HCH, 0.0005 mg/kg body weight for heptachlor and heptachlor epoxide and 0.0001 mg/kg body weight for Aldrin [35], therefore the calculated daily intakes for the above pesticides are far greater than the recommended values.

Further, the calculation of neonatal intake of these toxic agrochemicals per day on the basis ofaverage weights ofthe body at birth and the average amount ofmilk consumed by the baby, offers a higher safety factor as it has been reported that an infant consumes about 0.6 Liters of milk per day and the average weight of the babies at birth is 3.36 kg [36, 3]. Thus, the contamination of breast milk in the present investigation may lead to neonatal intake of 0.053 mg

of total DDT, 0.036 mg of total HCH 0.784 mg of total heptachlor and 0.028 mg of Aldrin. These values too are almost double of the recommended dose.

What is more seriously to be considered here is the synergistic action of dose effect relationship ofDDT, HCH, Aldrin and heptachlor which are simultaneously present. It is possible that dose ofeither ofthe detected pesticides which neonates carry may not be harmful but a combination of two or all of them need to be given a serious thought. The study, no doubt, throws some light on the possible consequences ofexcretion oftoxic agrochemicals through human milk, but this is not to imply that breast feeding is harmful. On the contrary, leaving out some very special cases, the breast feeding is the safest and most medically desired form of infant nutrition. As yet no chemical data are available to assess whatever danger may be associated with excretion oforganochlorine pesticides through the human milk in the quantities reported [37]. Further switching from breast to bottle feeding though herculean step, raised even greater health risk for babies [38].

Since case histories revealed no accidental or occupational exposure to any of the detected pesticides, subjects were exposed through the food chain and the environment. These findings have given rise to considerable concern among pediatrician, who must weigh possible and potential hazard against the well-known benefits to new born, with regard to nutritional status, to social relationship between mother and child, and to the prevention of infant diseases.

Most contaminants found in human milk are fat-soluble substances which will be detected mainly in the fatty phase of the milk. If the human exposure to such chemicals is high. e.g., in some occupational situations, this fat solubility, together with the degree of ionization and the molecular weight, are certainly the most important properties determining the Occurrence of chemical contaminants. However, low exposures to environmental Chemicals can also be reflected in human milk if these substances have a high degree of environmental and metabolic persistence, which together with a high fat solubility, means an ability to be bioaccumulated in organisms and biomagnified through natural food chains.

CONCLUSION

It can be concluded that the magnitude of pesticide pollution is quite high to contaminate the food and environment and as a result toxicant reach the human body through various sources mainly through the absorption from the gastrointestinal tract via contaminated food chain. From there, they are further circulated in maternal blood, cord blood stored in milk and placental tissue of the women. Since, the pesticides are reported to be carcinogenic, mutagenic, teratogenic, immunosuppressive, induces endocrine dysfunction and high estrogenic activity, disturb the reproductive processes, growth depressants, induces several psychogenic and neurogenic abnormalities in adult stages and are also reported to be associated with abortions, premature deliveries, still births, low birth weight consequences are obvious on the mother and the developing baby. It poses various problems of management of neonatal nutrition and health. It calls for suggestions like special care in nutrition and in the environment of mother throughout the life and especially during pregnancy and lactation. It would be advisable for a woman to avoid the consumption of fatty food stuffs and heavily polluted working environment. In the light of our findings stricter regulations may be discussed and such measures have to be weighed against the benefits of the use of pesticides. Present findings on obstetric toxicology of pesticides particularly in relation to distribution of pesticidal pollutants in pregnant women may finally lead to a better

understanding of the influence of chemicals on fetal development and provide grounds for further studies on placental toxicology as related to pesticide pollution in India. In the end, it must be emphasized that there is a rising protest that pesticides are destroying harmless wild life and endangering the health of man himself. The battle against the harmful insects would be much less costly and more efficient, and the problem of contamination of the environment by toxic materials would be vastly reduced, if insect activities are controlled by natural means. The use of pest-specific predators; parasites or pathogens; sterilization of insects with the help of radiations; trapping insects using insect attractants like pheromones; use of juvenile hormones or hormone inhibitors may therefore be suggested as alternate ways of pest control.

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