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Presence of organochlorine pesticides in Breast Milk

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ABSTRACT:

Pesticides occupy a rather unique position among the many chemicals that man encounters daily, in that they are deliberately added to the environment for the purpose of killing or injuring some form of life. Ideally, their injurious action would be highly specific for undesirable target organisms and non-injurious to desirable non-target organisms. In fact, however, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species including man, and other desirable forms of life that co-inhibit the environment. Therefore, lacking highly selective pesticidal action, the application of pesticides must often be predicted on selecting quantities and manners of usage that will minimize the possibility of exposure of non-target organisms to injurious quantities of these useful chemical. Challenge for producing more food for the ever-increasing population of the globe by proper management of crop pests and control of vector borne diseases necessitated the application of more pesticides on the earth. 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 population 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 and mortalitiess. 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 theblood of pregnant women, cord bloodand breast milk using gas liquid chromatography. 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. A statistically significant relationship exists among the residue levels ofOCPs between the mother's blood and cord blood and the maternal milk as indicated by the calculated correlation coefficient.A highly significant correlation was observed between human milk and maternal blood and cord blood for OCPs.As the concentration of OCPs increases in the mother's blood and cord blood, its accumulating concentrations in the milk also increase correspondingly.

KEY WORDS:
Mother's blood, Organochlorine pesticides, Contamination, Residues, Gas Chromatograph, Placenta, Cord blood

INTRODUCTION

Contamination offood chain with persistent chlorinated pesticide residues is now a well-accepted fact leading to their absorption from the lumen ofthe gut in human beings and then they appear in the blood. Human milk contains 3.5% fat on an average and these lipophilic pesticides do appear in milk in high concentrations Factors involved in the transfer of these pesticides from plasma to milk include those of solubility, ionization, concentration and special transport mechanism [1].

Since, organochlorine pesticides are lipophilic in nature; they will accumulate in fat and fat rich tissues ofhumans and animals in high concentrations. Therefore, weight might playimportant role in accumulation oforganochlorinepesticides. 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 [2].

These findings have given rise to considerable concern among paediatrician, 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 ofthe milk. Ifthe 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 ifthese substances have a high degree ofenvironmental and metabolic persistence, which together with a high fat solubility, means an ability to be bioaccumulated in organisms and biomagnified through natural food chains.

As human beings are placed at the top of most food chains [3] it is, therefore, not surprising that human adipose fat and milk fat usually have more than ten times higher levels ofpersistent chlorinated pesticides and PCBs as compared to milk fat from cows [4-7]. The low level of contamination of cow's milk may be further explained by the cow's daily and continuing mobilization offat and the contaminants therein, followed by excretion with the milk [4]. In fact, milk secretion is the most important route of excretion of those types of persistent compounds in human beings and other mammals [8-11].

The idea behind most investigations ofchemical contamination ofhuman milk has been to elucidate the infant burden ofthose chemicals from nursing. Such studies, however, may also be used as a general biological monitoring tool. As a result, it is possible to encircle pollution sources and prevent further exposures and, hopefully, any adverse health effects.

The first recognition that human breast milk may be contaminated by environmental chemicals came with the findings ofLaug and coworkers (1951), during that the milk from normal and healthy black American women contained considerable amounts of the organochlorine insecticide, DDT [12]. Since then, many investigations on contamination of human milk have been made in countries all over the world, andDDT, together with some other organochlorine pesticides, has been detected and/or determined in most of these investigations. Until now the contaminants found most frequently in human milk have been DDT, its main metabolite DDE, hexachlorocylohexanes, dieldrin and heptachlor epoxide. Among the more locally found contaminants are the organohalogens, such as Aldrin, heptachlor, mirex, oxychlordane, t-nonachlor, pentachlorobenzene, hexachlorophene, perchloroethylene and halothane. Most human milk monitoring studies have concentrated on analyzing a few compounds in the milk, mainly the organochlorines DDT, DDE, HCHs, dieldrin and heptachlor epoxide.

Since, the organochlorine compounds are lipid soluble and tend to accumulate in the food chain and store in high concentrations in tissues and lipid rich organs such as adipose tissues and liver. Human milk is the most important and indispensable food for the newborn; during lactation, fat mobilization could take place from adipose tissue and therefore, organochlorine compounds are mobilized and excreted together with the milk. Thus, milk secretion is the most important route Human milk is the primary and the potent source ofinfant nutrition. It is rich in fat and stands at the end of the food chain. In view of their lipophilic nature, pesticides could accumulate in milk in alarming concentrations. Therefore, human milk can be used as one ofthe evaluation indexes ofenvironmental contamination by these 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.

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 [13]. Earlier reports have drawn attention to the pesticide burden in the human milk from India and from all over the world [14]. 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 [15], [16] mutagenic [16],[17] teratogenic [17],[18] immunosuppressive [19],[20] create endocrine dysfunction such as hypothyroidism or high estrogenic activity [21],[22] disturb reproductive processes [23],[24] growth depressants [25],[26] induces several psychogenic and neurogenic abnormalities in adult stages [27],[28], and are associated with abortions, premature deliveries, still births and infants with low birth weights [29]-[32]. 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 [33]. 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 [34-39].

The exposure of the general population to pesticide may occur through several environmental media, including food, water and air and to a lesser extent soil. There is evidence that greatest exposure to pesticides for the general population takes place as a result of ingesting food and water containing small residues of pesticides. This type of incidental exposure of human beings may result in the accumulation of OCPs in their blood in quite high concentrations. Presence of pesticides in the blood is the indication of environmental pollution by these toxins or the body burden of these pesticides which in turn is a risk to the human health.

Taking above points into consideration, a continued surveillance on the levels of pesticide pollutants in human population 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 and mortalitiess. 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 theblood of pregnant women, cord bloodand breast milk using gas liquid chromatography. 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. A statistically significant relationship exists among the residue levels ofOCPs between the mother's blood and cord blood and the maternal milk as indicated by the calculated correlation coefficient.A highly significant correlation was observed between human milk and maternal blood and cord blood for OCPs.As the concentration of OCPs increases in the mother's blood and cord blood, its accumulating concentrations in the milk also increase correspondingly.

MATERIALS AND METHODS

101 pregnant women admitted to Zanana Hospital and Mahila Chikitsalya: attached to the Deptt. of obstetrics & Gynecology. S.M.S. Medical College, Jaipur (India) and two private hospitals "Sanjeevani Hospital" and " Meera Hospital", Bani Park, Jaipur are Included in the present study. 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 [40] by interviewing the subjects at the time of collection of samples.

Sample Collection

Maternal blood,Cord blood and Mothers milk:

Five ml of maternal blood from each case was collected by venipuncture in pre-heparinzed vials 4-8 hours before parturition and stored at -10°C in a deep freeze until analysed. Umbilical cord blood was collected by squeezing the cord into pre-heparinzed vials All the stored samples were analysed within 48 hours of their storage. The breast milk, approximately 5 ml, from all the subjects were collected at the third dayof parturition directly by the manual expression of the breast into cleaned, pesticide free,50 ml wide mouthed glass tube with a cap lined with aluminium foil. No breast pumps orother devices were allowed to be used for the collection of milk samples Immediatelyafter the milk samples were collected the containers were capped and kept in deep freeze, till analysed, generally within 48 hours.

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 blood, 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 [41]. Specimens of milk were extracted by the methodology given by Takie and his coworkersin 1983 with little modifications according to the prevailing laboratory conditions [42].

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, Cord blood and Breast Milk of 101 pregnant women (ppb).

S. No.	Organochlorine pesticides	Breast Milk	Maternal Blood	Cord Blood
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
1.	Σ HCH	0.2033 ±0.0264	142.8 ±20.45	142.1 ±28.13
2.	Σ Heptachlor	4.3932±2.9294	1566.9±317.38	214.8±148.95
3.	Σ DDT	0.2999±0.0724	107.8± 18.66	75.4± 12.82

Table 2: Correlation Coefficients between average levels of total HCH, total Heptachlor, total DDT equivalent in breast milk (BM), maternal blood (MB) and cord blood (CB).

Sr. No.	Pesticide	Samples	BM	MB	CB
1.	Total HCH	Breast Milk	1	0.1275	0.1990*
		Maternal Blood	0.4336#*	1	0.2947#*
		Cord Blood	0.2967#*	0.2957#*	1
2.	Total DDT	Breast Milk	1	0.0254	0.2546#*
		Maternal Blood	0.5632*	0.3426*	1
		Cord Blood	0.3904*	0.3426*	1
3.	Total Heptachlor	Breast Milk	1	0.0557	0.2627#*
		Maternal Blood	0.3659#*	1	0.2960#*
		Cord Blood	0.2692#*	0.3110#*	1

* Statistically Significant (P<.01)
\# Statistically Significant (P<.05)
Σ HCH-total HCH
Σ Heptachlor-Total Heptachlor
Σ DDT-Total DDT

Correlation among milk, maternal blood and Cord blood.

1. Table:1 is showing the Concentration of organochlorine pesticides in Maternal blood, Cord blood and Breast Milk of 101 pregnant women (ppb).
2. Table:2 is showing Correlation Coefficients between average levels of total HCH, total Heptachlor, total DDT equivalent in breast milk (BM), maternal blood (MB) and cord blood (CB).
3. 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.
4. In the Table 2 total DDT in the maternal blood has been plotted as a function of total DDT in milk. The values in the table 2 indicates that total DDT in human milk increases with its

increase in the maternal blood, hence increase of total DDT concentration in the human milk is a function of its increase in blood of the mother and values clearly shows a significant relationship as evident from calculated correlation coefficient.

5. Table 2 illustrates the significant relationship between the level of total DDT in the maternal milk and the cord blood. The values clearly indicates that with the increase in the cord blood level of total DDT, there is an increase in the residue levels of milk as well. Hence statistically significant relationship exists among the residue levels of total DDT between cord blood and the maternal milk, it is also indicated by correlation coefficient.
6. A highly significant correlation was observed between human milk and maternal blood with regard to total HCH levels (Table 2). It is described by the values in the Table 2 that human milk HCH concentration is a function of HCH concentration in the circulating blood of the mother. As the concentration of HCH increases in the blood, its accumulating concentrations in the milk also increase correspondingly.
7. Table 2 depicts a statistically significant relationship between the level of total HCH in human milk and cord blood as shown by the calculated correlation coefficient of 0.26967. It is clear that the increase in cord blood HCH also results in an increase in human milk HCH concentration. But the significant observation noted from the Table 2 is that accumulating rate (increase in concentration) of HCH in milk is much higher than that of same in cord blood, however, both are slightly interrelated.
8. In the Table 2, total heptachlor in the maternal blood has been plotted as a function of total heptachlor in milk. The values indicates that total heptachlor in the human milk increases with its increase in the maternal blood. Hence, a statistically significant relationship exists among the residue levels of total heptachlor among the cord blood and the maternal milk as indicated by the calculated correlation coefficient (Table 2).
9. Table 2 indicates a statistically significant relationship between the levels of total heptachlor in the human milk and cord blood as shown by the calculated correlation coefficient of 0.2692 (Table 2). It is obvious from the values, that as the concentration of total heptachlor increases in the cord blood, its accumulatory concentration in the milk also increases correspondingly. Table 2 also depicts that the accumulatory rate of total heptachlor is much higher than that of same in cord blood, may be because the fat content of both the tissues varies from each other.

DISCUSSION

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.

The newborns are not simply small adults is worth reiterating, albeit trite. They differ morphologically, physiologically and biochemically from adults. For example, the neonate's central nervous system is only partially developed and is a site of rapidly differentiating and highly mobile cells. Similarly, the physiology of the hypothalamic/ hypophyseal axis is undergoing rapid and permanent change. It is exquisitely sensitive to the negative feedback effects ofgonadal steroids, but quickly loses this responsiveness. Other physiological control systems are undergoing similar change. Even the metabolic fuel differs; newborns operate on fat, adults on carbohydrate. Of great toxicological importance is the immaturity ofthe neonate's hepatic drug - metabolizing enzyme system. The activity of this system is very low in newborns and, in the rat the various enzymes do not reach adult levels until 30-50 days after birth, i.e. at or after puberty.
Because of such differences, the neonate and the adult frequently respond differently to pesticides and other agents. This difference is not necessarily predictable. It may be quantitative, either greater or lesser in the neonate, or qualitative, and must be determined empirically. The response will depend upon a host of pharmacodynamic factors, which can be conveniently considered as the innate responsiveness of the target tissue(s) and upon pharmacokinetic factors which determine how much of the agent reaches the responsive tissue and for how long it remains.

Water comprises the major part of milk, typically from 87 to 95% [43]. The protein content of human milk is relatively constant at about 0.9% (W/W), and the essential amino acid requirement are met. [44]. The content ofcarbohydrates, mainly lactose, is 6.5 to 7.4%with littlevariation [45]. The fat content probably is the most variable nutrient. This variability is very important in relation to our subject, because most organic pollutants detected in human milk are fat soluble and occur mainly in the fatty phase of the milk. On reviewing the literature, the results in general are given on a milk fat basis. This is useful for comparing monitoring results, bearing in mind the general correlation between milk fat and residue contents. But since, we are more concerned with the infant intake and burden of pesticides, we have detected pesticides on the whole milk basis.

A highly significant correlation was observed between human milk and maternal blood and cord blood for OCPs.As the concentration of OCPs increases in the mother's blood and cord blood, its accumulating concentrations in the milk also increase correspondingly.

CONCLUSION

It is quite clear from the foregoing discussion, that in all over the world Indian mothers have got the significant and may be highest body burden of OCPs. This is because in the third world countries such as India, because of the cost - benefit ratio OCPs are still the major pesticides used in agriculture and public health sector. This is in accordance with the findings of Dale and his coworkers (1965) that the Indians have got the highest body burden of OCPs [45]. The present study directly reflects the national scene of magnitude of pesticide pollution which signifies the distribution and accumulation of non-biodegradable lipophilic pesticides in pregnant women on one side and subsequently the vulnerability of the successive generation from its very inception in the womb of the mother on other side and then the exposure of the neonates through mothers' milk which further makes the scenario petrifying.

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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