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Persistent Organochlorine Pesticides and Oxidant/Antioxidant Status in the Placental Tissue of the Women with Full-term and Pre-term Deliveries

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Abstract: In India pre-term birth is the leading cause of death of infants and this number is continuously rising. Pre-term birth is an increasingly prevalent complex condition with multiple risk factors. Only handful of studies has focused on the effect of oxidative stress due to organochlorine pesticides in pre-term deliveries. The aim of this study was to analyze the organochlorine pesticide residues in placenta of the females with pre-term and full-term deliveries and assess the levels of glutathione and malondialdehyde as oxidative stress markers and to correlate them with organochlorine pesticide levels. Fifty cases of full-term deliveries and forty cases of pre-term deliveries were included in this case-control study. Residues of organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT) (p,p-DDE, p,p-DDT, o,p-DDD) and its metabolites and isomers of hexachlorocyclohexane (HCH) (α,β,γ and δ) were analyzed by gas chromatography equipped with electron capture detector. Oxidative stress was measured by the quantification of malondialdehyde (MDA) and reduced glutathione (GSH). In placental tissue from the pre-term delivery, significantly higher levels of α-HCH, total HCH, p,p-DDE and total DDT were recorded than in the placental tissue from the full-term delivery. The MDA level was significantly elevated while GSH level was significantly lower in placental tissue of women with pre-term delivery compared to women with the full-term delivery. There were significant positive correlations of placental pesticide (α-HCH, γ-HCH & total HCH) levels with MDA ($p<0.05$), suggest the pesticide-induced pre-term delivery. Conclusively, organochlorine pesticide residues are associated with oxidative stress and we cannot rule out the possibility of the role of organochlorine pesticides in pre-term deliveries.

Keyworks: Pre-term birth, placental tissue, Organochlorine pesticides, Oxidative stress

Introduction

An estimated 15 million babies are born as pre-term babies every year and around one million children die each year due to complications of pre-term birth[1]. According to WHO (2012) report[2], India reaches top of the countries, with maximum number of pre-term deliveries i.e., 3,519,100 almost 24% of the total number infant deaths. Risk factors coupled with pre-term birth and low birth weight include socioeconomic status, race/ethinicity, smoking and environmental contaminants[3-6]. Throughout the pregnancy period, women are exposed to a wide variety of foreign chemicals through lifestyle factors (smoking, drug abuse and alcohol consumption), maternal medication and also from occupational and environmental exposure[7,8]. In addition, there is no barrier to prevent inflowing of these chemicals and they are circulated between mother and foetus by simple diffusion. These foreign chemicals are capable to alter the usual functions of placenta [9,10] like production and release of hormones and enzymes, nutrients transport, waste products and maturation; consequently upsetting the foetal development and lastly, at the terminal phase of placental life, i.e., delivery[8.]

There are many likely cause of pre-term delivery, but it now appears that free radical generation in the trophoblastic placental tissue may play an important role [11,12]. Based on this information, present study, the first of its kind from Agra region because in this region the leading cause of infant deaths are prematurity and low birth weight (33.6%)[13] was designed to determine pesticide levels, lipid peroxidation and glutathione GSH in the trophoblastic placental tissue of women with the pre-term delivery and compared with those of women with the full term delivery.

The primary objective of the present study was to evaluate the association of pesticide exposure, with oxidative stress and pre-term deliveries of women residing in

Agra city area. We hypothesized that pesticide exposure would be positively associated with oxidative stress which may lead to premature birth.

The two organochlorines DDT and HCH persist in the environment, concentrate in the crops, continue to be detected in the food chain[14], accumulate in human tissues and fluid due to their lipophilic nature, and are excreted in breast milk[15-17]. Due to the human and environmental risks associated with the use of such pesticides, they have been banned in several countries but are still used in India [18]. While the use of DDT is restricted to vector control programs only and the production of technical HCH is being phased out, the annual production of γ-HCH (lindane) increased from 40 metric tons (mt) in 1996–1997 to 250 mt in 1997–1998[19]. Some of these compounds are suspected to disrupt the endocrine system by mimicking estrogenic activity and thereby increase the risk of hormone dependent disorders [20-23].

Presence of different organochlorines residues in placenta and their transplacentally transfer from mother to foetus have been already reported in several studies[24]. Such compounds are reported to be endocrine disrupters and capable of altering the hormonal balance by their antiandrogenic activities[25]. Studies have shown that organochorines have been associated with an increased risk of abortion, intrauterine growth retardation (IUGR), minor malformations, pre-term, small-for-gestational-age babies; with endometriosis, cryptochildism and hypospadias in the newborn[26,27]. In addition to estrogenic activity, organochlorine pesticides are also capable to induce lipid peroxidation, moderate oxidative stress and brings about changes in lipid composition and membrane activity[28,29]. Organochlorines are also known to influence oxygen free radicals; OFRs[30]; it has been reported that free radical generation in the trophoblastic placental tissue may have serious toxic effects and environment pollutants like organochlorines pesticides might be associated with it. Keeping in view

the above, present study is proposed to investigate if there is any potential interaction of accumulating organochlorine insecticide residues in placental tissues of pregnant women with biomarkers of oxidative stress and antioxidant defence system.

Materials and methods

To obtain the status of the organochlorine pesticides levels and antioxidant enzyme activities in pre-term and full-term deliveries and thus provide a comprehensive picture as to whether there exist any association between them, we measured the levels of some organochlorine pesticides, (Hexachlorocyclohexane) HCH and its isomers like α-HCH, β-HCH, γ-HCH, and δ-HCH, metabolites of (Dichlorodiphenyltrichloroethane) DDT such as p,p-DDE, p,p-DDT, p,p-DDD and antioxidant enzymes reduced glutathione (GSH). Lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), was assayed.

Patient's selection:

Ninety pregnant women from Agra (India), after 28 weeks of gestational age attended antenatal clinic of local hospital were included for this study. All placenta samples were collected from healthy pregnant women. A birth was classified as pre-term if the gestational age of the newborns were assessed as 37 weeks or less and premature rupture if the amniotic membranes ruptured spontaneously 24 hr or more the onset of labor.

A combination of interview and questionnaires was used to collected information on participants' age, height, weight, area of residence (rural/urban) smoking, drinking and tobacco habits, dietary habits, reproductive histories and gestational age based on the date of the last menstrual period. We calculated BMI (kg/m^2) from subject's height and weight. Also took permission for access their medical records. The questionnaire addressed the occurrence of several occupational hazards, including

the use of pesticides. The study population was rather homogeneous in terms of the socioeconomic status, age and nutrition habits. Women confirmed their participation by signing a consent form and this study was approved by the institutional human ethics committee.

Collection of placental samples and processing;

We selected mainly trophoblastic placental tissue without sign of calcification, avoiding the deciduas basalis and chorionic plate in accordance with other recent studies[31,32]. Placental tissues were collected during parturition. Approximately 20 gm of placental tissue were taken from each subject, collected in wide-mouthed, organochlorine pesticides free containers as coded samples and transported to the Department of chemistry, Dr. B R Ambedkar University, Agra immediately after collection in an ice container for biochemical assays and organochlorine pesticide analysis. An aliquot of homogenate was used for the determination of malondialdehyde (MDA) last product of lipid peroxidation[33] and determination of glutathione (GSH)[34].

All the reagents and solvents used in this study were procured from Merck chemicals. Standard of pesticides used in GC studies were procured from Dr. Ehrenstorfer Gmbh Chemicals, Germany. Extraction of pesticide residues was carried out as described by Saxena & Siddiqui[35] briefly described below. One gm of finely chopped placental tissue was homogenized with formic acid and n-hexane. The contents were quantitatively transferred to a 25-mL stoppered conical flask and shaken for 1 h at 37^0 C in a metabolic shaker, and n-hexane was collected (repeated thrice). For cleanup, pooled extracts treated with fuming sulphuric acid to remove the traces of tissue. Analysis was done in (AIRF-JNU, New Delhi.) on gas chromatograph equipped with ECD (electron capture detector) under the standardized conditions. Comparing peaks with those of standards enabled quantification and tentative

identification of pesticides. Further confirmation of pesticides was based on dual column gas chromatography/mass spectrometry. The main source of the detected pesticides is expected to be food chain contamination. However, exposures through air, water, and dust may also contribute.

Data analysis

Data were analyzed by using IBM-SPSS (version-22). Characteristics of the study subjects and levels of organochlorine pesticides were expressed as mean with standard deviation. Chi-square and students t-test were used to test the association between categorical and continuous variables, respectively. Linear regression analysis was performed to determine the strength of relationship between placental pesticide levels and oxidative stress parameters. A value of $p<0.05$ was considered statistically significant.

Results

This study included 90 females with full-term and pre-term deliveries with relatively homogenous group, having similarity in characteristics such as age, height, weight, BMI, drinking water supply and area of residence. The demographic and reproductive characteristics of the subjects are depicted in table 1 and table 2. Maternal age ranges between 17-33 years. The differences between Age, height, weight and number of children were not found statistically significant. Placental organochlorine pesticide levels in pre-term and full-term cases are shown in table 3. The levels of all the pesticides were found higher in pre-term cases but the difference were not found statistically significant, only α-HCH, total-HCH, p,p-DDE and total DDT were found significantly higher in pre-term cases. The level of GSH ($p<.05$) was found significantly increased in full-term group whereas, MDA ($p<.05$) was found significantly higher in pre-term group (fig.1 and fig.2).

Table 1: Demographic characteristics of the females from pre-term and full-term deliveries

Variables	Pre-term (n=40)	Full-term (n=50)
Age (Yrs)	24.23±3.20	25.54±3.91
Height (Cm)	154.85±4.24	155.04±5.47
Weight (Kg)	58.95±8.16	58.78±7.21
BMI (Kg/m^2)	24.57±2.93	24.50±2.74
Number of children	1.78±0.89	1.44±0.76
Duration of Lactation	15.10±8.52	11.50±8.6

Values represent mean±SD

Table 2: Demographic characteristics of the females from pre-term and full-term deliveries

Variables		Pre-term (n=40)	Full-term (n=50)	df	x^2
Abode	**Rural**	26 (65%)	19 (38%)	1	.00
	Urban	14 (35%)	31 (62%)		
Addiction	**Yes**	20 (50%)	24 (48%)	1	.04
	No	20 (50%)	26 (52%)		
Pesticide exposure	**Definite**	0 (0%)	0 (0%)	1	71.1*
	Possible	0 (0%)	5 (10%)		
	No	40 (100%)	45 (90%)		
Source of drinking water	**Pvt.**	25 (62.5%)	28 (56%)	1	2.84
	Govt.	15 (37.5%)	22 (44%)		
Dietary habit	**Vegetarian**	29 (72.5%)	27 (54%)	1	5.37
	Non- Vegetarian	11 (27.5%)	23 (46%)		

No. of subjects (%), df=degree of freedom, *x^2=Chi-square,*p<0.05*

Table 3. Organochlorine pesticides in the placenta of the females from pre-term and full- term deliveries

Name of pesticides	Pre-term (n=40)	Full-term (n=50)
α-HCH*	39.75±39.73	7.48±8.15
β-HCH	37.73±107.76	15.64±19.21
γ-HCH	28.70±11.38	28.52±18.71
δ-HCH	4.65±6.38	4.42±4.74
Total HCH*	110.85±120.57	39.0±28.90
p,p-DDE*	13.95±12.14	8.98±10.68
p,p-DDT	4.23±11.54	1.18±4.92
o,p-DDD	1.48±4.01	0.98±2.0
Total DDT*	19.68±17.55	11.14±12.92

Results are in ppb
*p<.05

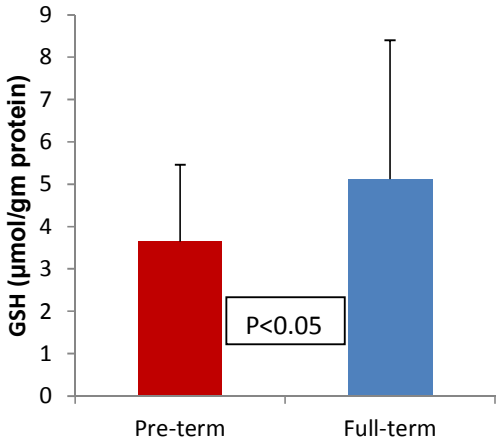

Fig 1. Comparison of levels of GSH in pre-term and Full-term delivery cases (Values are in mean±SD)

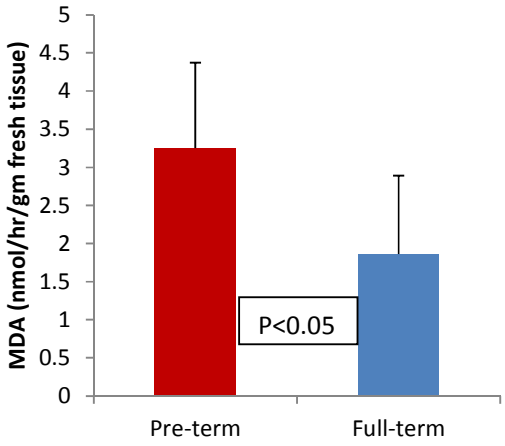

Fig 2. Comparison of levels of MDA in pre-term and Full-term delivery cases (Values are in mean±SD)

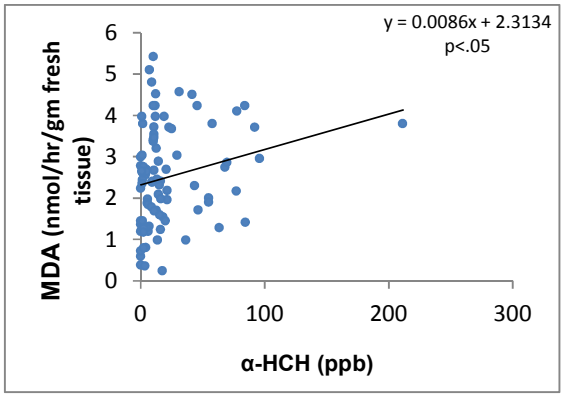

Fig 3. Placental MDA level plotted against placental α-HCH

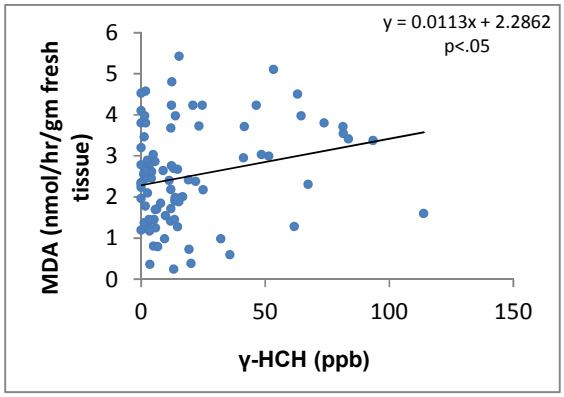

Fig 4. Placental MDA level plotted against placental γ-HCH

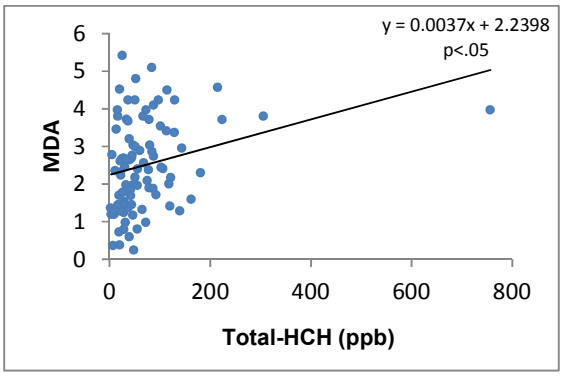

Fig 5. Placental MDA level plotted against placental total-HCH

Figure 3, 4 and 5 represents the strength of relationship between MDA and placental α-HCH (R^2=0.05, p<0.05), γ-HCH (R^2=0.05, p<0.05) and total HCH (R^2=0.07, p<0.05) respectively. The significant correlations of placental pesticide levels with MDA suggest the possibility of pesticide-induced pre-term delivery among women through disrupting the oxidant/antioxidant balance in the trophoblostic placental tissue.

Discussion

The problem of exposure to multiple chemical present in the environment is not confined to India. Population throughout the world are exposed to similar or even worse conditions of environmental contamination. Organochlorine pesticides are chemically stable, lipophilic in nature, and persist in the environment due to its long half life and are very slowly degradable. Organochlorine pesticides are ubiquitous in

the environment i.e., in air, water, soil, food items etc. and are biomagnified through food chain[36]. Increasing OCPs residues have been found in different human samples such as placental tissues, blood, amniotic fluid, semen, breast milk, adipose tissue, tumor tissue etc[37-40,20,23] and have wide range of acute and chronic health effects. Considering the higher percentage of body fat in women, the storage of these toxins is of great in them[41]. Owing to their xenoestrogenic nature, Organochlorine pesticides disturb the normal estrogen–progesterone balance, which is important in the maintaining the pregnancy[42,43,39,44]. Gamma HCH (lindane), is produced and used as pesticide on fruit, vegetables, animals and animal premises. Also available as a prescription medicine in the form of cream, lotion, shampoo to treat scabies (mites) and head louse in humans. Gamma-HCH alters the level of thyroid, pituitary and sex hormones in females and impaired level of sex hormone may interfere with the normal pregnancy. Concentration of α-HCH found five times elevated in pre-term group when compared to full-term group (39.75±39.73 vs 7.48±8.15) and maximum among the seven pesticides. Beta-HCH is the most persistent isomer of HCH having negligible insecticidal activity but measurable estrogenic effect[45]. Beta-HCH possesses the property to increases uterine contraction frequency in a concentration-dependent manner in rats[46]. These observations lend support to the role of β-HCH in reproductive toxicity and a possible association with pre-term labor due to its estrogenicity. A possible association between higher levels of β-HCH and preterm is in agreement with previous reports[43,24,47]. Dichlorodiphenyltrichloroethane (DDT) is a pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus. Most of the DDT present in the environment is a result of previous use [48,49]. *p'p'*-DDE tends to persist for much longer in comparison to the parent compound and is considered a marker of past exposure to DDT. Earlier studies have reported that p,p´-DDT and/or p,p´-DDE in

maternal, umbilical cord blood and serum are associated with pre-term deliveries[42,43,50,24]. In view of the reproductive toxicity of organochlorine pesticides, their presence in pregnant women and subsequent transfer to the developing fetuses has the potential to hamper growth and development of the baby in the womb[42]. We found higher levels of p,p-DDE, o,p-DDD and p,p-DDT in pre-term delivery cases than full-term delivery cases but only p,p-DDE (potent xenoestrogen) was reported significant.

Our results show high levels of MDA in pre-term cases than full-term (Fig 1). Malondialdehyde is a common oxidative stress marker due to lipid peroxidation[51.] Decreased levels of GSH in pre-term than full-term cases (Fig 2) indicate diminished ability to resist oxidative damage.

Our results are consistent with previous studies suggesting oxidative stress due to pesticide exposure and lead to premature birth. There are many likely cause of adverse pregnancy outcome including pre-term delivery but it now appears that oxidative stress in trophoblastic placental tissue play an important role. The generation of reactive oxygen species (ROS) in the human placenta can occur, as in other tissue[11]. The biochemical mechanism of pesticide-induced pre-term delivery through the modulation of oxidant/antioxidant status of the trophoblastic placental tissue has not yet been clearly understood.

The only published Indian study on organochlorine pesticides and oxidative stress among pre-term (n=30) and full-term (n=30) cases, originating from Delhi, indicated that organochlorine pesticides (β-HCH and α-endosulphan) were significantly higher in the maternal and cord blood of the pre-term cases[47]. That study did not include placental tissue that might have reflected the cumulative internal exposure at the target site.

In the present study, MDA level was significantly higher ($p<0.05$) while GSH level was significantly lower ($p<0.05$) in placental tissue of women with the pre-term delivery as compared to women with the full term delivery. This is in accordance with previous published studies[52,53] proposed that pesticide could enhance lipid peroxidation in the placental tissue, through the promotion of membrane physical changes, which would propagate oxidative stress.

Our observations of reduced levels of GSH are in agreement with previous studies[54,55]. The significant positive correlations of α-HCH, γ-HCH, and total HCH with MDA suggest that isomers of HCH may be associated with increased formation of ROS and thus produce oxidative stress in pregnant women (fig 3, 4 and 5). In a recent study, increased levels of MDA in scabies patients after treatment with γ-HCH suggested that the disruption of the organization of plasma membrane is the primary action of biologically active organochlorine pesticide isomers that may affect cell survivability and increased lipid peroxidation[56].

In conclusion, sociodemographic, obstetrical, and reproductive history as confounders of the pre-term delivery were closely matched between the two groups of women. However, some of the pesticides was significantly higher and significantly correlated with MDA suggests the pesticide-induced pre-term delivery in women of the present study through the modulating the oxidant/antioxidant status in placental tissue. Further studies are still needed to confirm the free radical mechanism of pesticide-induced pre-term delivery as observed in the present investigation.

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