Original Article

Pesticide Risk and Recurrent Pregnancy Loss in Females of Subhumid Region of India

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Abstract

Objective: The objective of this study is to determine the level of pesticides and their role in cases of recurrent pregnancy loss (RPL). **Materials and Methods:** This was designed as a case–control study. Gas chromatography was used to characterize the pesticide level in 70 cases and 70 controls. Case refers to women with RPL, whereas controls refer to women with full-term delivery. **Results:** A higher level of pesticide, namely beta-hexachlorocyclohexane, malathion, chlorpyrifos, and fenvalerate was found in the case group as compared to control group ($P < 0.05$). **Conclusions:** The present study suggests that high exposure of pesticide (organochlorine and organophosphates) may increase the risk of RPL in females of the subhumid region of India.

Keywords: Organochlorine, organophosphate, recurrent pregnancy loss

Introduction

Based on the incidence of sporadic pregnancy loss, the incidence of recurrent pregnancy loss (RPL) should be approximately 1 in 300 pregnancies. Although the specific cause of RPL is not yet known, considerable evidence suggests that both genetics and the environment play an important role in the origin and evolution of this disease. Pesticides are the class of man-made environmental chemicals that can affect the body's development, growth, and hormone balance.[1] Pesticides are usually designed to target a particular pest, but due to its broad range of toxicity, it can also be directed to other nontarget species. In the majority of cases, however, human exposure is unintentional.

Previous studies also examined the role of other factors such as infections, hormonal aberrations, menstrual irregularities, malnutrition, psychological conditions, stressful events, high alcohol, nicotine, and caffeine intake, but the results are inconsistent.[2,3] In recent years, the high risk of miscarriages has been reported in smoking women.[4] Settimi *et al*. also reported the positive association between pesticides exposure and increased risk of pregnancy loss.[5] Pesticides exposure may also cause reproductive and developmental disorders.[6] A definite cause of RPL can be identified only through intensive diagnostics.

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Environmental pollutants or xenobiotics have been suspected for their strong role in causing RPL and other reproductive aberrations, but pesticides such as organochlorine (OCP) and organophosphate (OPP) are suggested as the main culprits. Pesticides such as dichlorodiphenyltrichloroethane and hexachlorocyclohexane are officially banned in India. However, they are still in use to control the disease-carrying vectors.[7]

OCP and OPP both have a long half-life. These pesticides accumulate in the adipose tissue and thus enter into the food chain and circulate.[8] On exposure through different means such as air, water, food, and soil, these may enter in blood circulation and can induce endocrinological disorders, immunological aberrations, oxidative damage, and eventually molecular damage.[9-11] The roles of di chloro di phenyl tri chloro ethane (DDT) exposure in spontaneous pregnancy loss have

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previously been reported.[3,12,13] However, more studies with diverse patient groups are required to establish the association of pesticides in the etiology of RPL. The present study was conducted to further investigate this claim. The levels of OCP and OPP were quantified and compared between the cases with repeated pregnancy loss and women having successful full-term delivery among the population of subhumid region of India.

Materials and Methods

After the agreement of the ethical committee, the womens' consent was obtained before they were included in the study. A total of 140 women were recruited between January 2012 and January 2015 from the Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, Utter Pradesh, India. The two different groups were evaluated.

The first group included 70 patients (case group) having two or more spontaneous pregnancy loss before the 20th week of gestation.

The second group included 70 apparently healthy females (control group) having had one or more successful deliveries during the period of investigation.

The participants with diabetes mellitus, hypertension, tuberculosis, immunocompromised symptoms, any endocrine disorder and genital, colon or breast cancer, or any other malignancies were excluded from the study. All were interviewed to collect the information, including age, alcohol consumption, smoking/tobacco chewing, and family history.

Sample collection

Five milliliter of venous blood was collected from every women included in the study on the 21th day after the commencement of the menstrual cycle for the control group and before the 20th week of gestation for the case group. Serum was separated from all the samples by centrifugation at 1000 × *g* for 10 min and was stored at −20°C till further analysis.

Pesticides extraction and quantification

The estimation of pesticides, OPPs and OCPs, residue from blood was done according to the method of Bush *et al*. with minor modification.[14] A total number of 14 pesticides (12°CP and 2 OPP) were selected for the study on the basis of their persistent nature and consumption rate. In brief, 1 ml of blood was taken in the separating funnel (100 ml) extracted with 10 ml hexane by shaken it at room temperature (30°C) for 10 min. The organic hexane layer was collected, and this process was repeated thrice; pooled hexane extract was collected. The organic layer (30 ml) was pooled together, collected, and dried by using a vacuum evaporator (IKA RV 10 digital). The concentrated sample was transferred into an autoinjector vial by passing through anhydrous sodium sulfate and dried with the flow of nitrogen. The sample was reconstituted to 1 ml before injection, sealed and loaded on to autoinjector for the analysis. The trace level pesticide (ppb) in the blood samples was analyzed using an Agilent technologies 7890A Gas Chromatograph, equipped with a micro-electron capture detector (ECD), capillary column DB-5MS (Perkin Elmer, CA, USA), and in an autoinjector Agilent technologies 7683B series split-less mode injector with an insert liner. The gas chromatograph temperature was programmed as follows: injector temperature: 250°C, oven temperature: initially ramped from 165°C to 180°C at a rate of 3°C, 200°C at a rate of 1.5°C, 230°C at a rate of 2°C, 260°C at a rate of 3.5°C, and finally to 280°C at a rate of 6°C/min withhold time of 1.5, 0.5, 0.5, 0.5, 2, and 2.5 min, respectively and ECD temperature was at 300°C.

Statistical analysis

The numerical tool for the demographic variables applied was the Chi-square test. The age, odd ratio, and confidence interval (95%) were substantiated by Vassar stats online calculator. The significance level of pesticides was evaluated by the nonparametric Mann–Whitney test. The univariate and multivariate binary logistic regression was carried out to find the strength of the association of the study parameters. The Hardy–Weinberg equilibrium was tested at each locus using the online available calculator. Statistical calculations were carried out using the advanced Statistical Package for the Social Sciences software version 16.0 (SPSS-16.0, IBM, Chicago, USA) Statistical Package for the Social Sciences provided by Institute of Business Management, India.

Results

We observed an elevated level of pesticide quartile in case with respect to the control group. Table 1 shows OCPs and OPPs levels in case and control groups in terms of descriptive statistics. The Mann–Whitney U-test was used to calculate the significance difference between the groups. The result revealed that levels of pesticides, beta-hexachlorocyclohexane (β-HCH), γ-HCH, δ-HCH, chlorpyrifos, pp-DDD, and fenvalerate were statistically significantly higher in the patients of the case group as compared with the control group (all $P < 0.05$) [Table 1].

The results for all the 14 pesticides (12°CP and 2 OPP) were analyzed through the Mann–Whitney test. We observed statistically significantly higher levels of β-HCH (*P*: 0.04), γ-HCH (*P*: 0.001), δ-HCH (*P*: 0.002), chlorpyrifos (*P*: 0.001), pp-DDD (*P*: 0.001), and fenvalerate (*P*: 0.001) in the patients of the case group in comparison to the patients of the control group. These pesticides were known to be endocrine disrupting and carcinogenic. The distribution prototypes of different pesticides in case and control groups are shown in Figures 1-4. Figure 1 describes the level of OPP pesticides in both groups studied. Figure 2 indicates the higher level of γ-HCH and δ-HCH in the case group as compared to the control group. Similarly, Figures 3 and 4 show the higher level of β-HCH, DDT, and pp-DDD in the case group as compared to the control group, respectively.

Discussion

The RPL and their causative factors are well demonstrated in this study and are compared with earlier studies.[15,16] The impact of environmental exposure and oxidative stress in the etiology

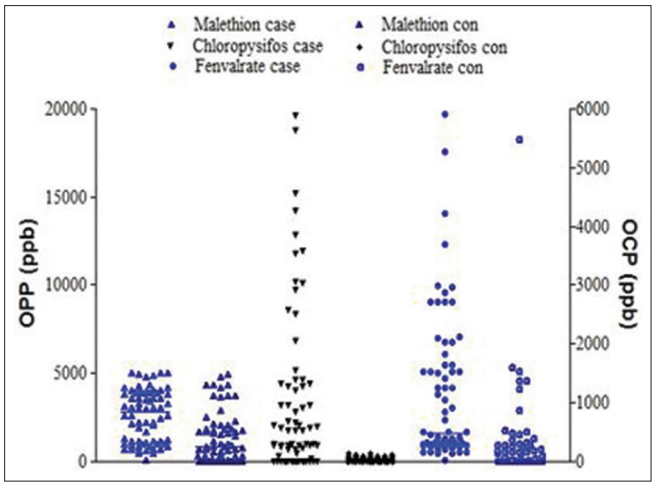

Figure 1: Level of organophosphate pesticides in control and recurrent miscarriage females

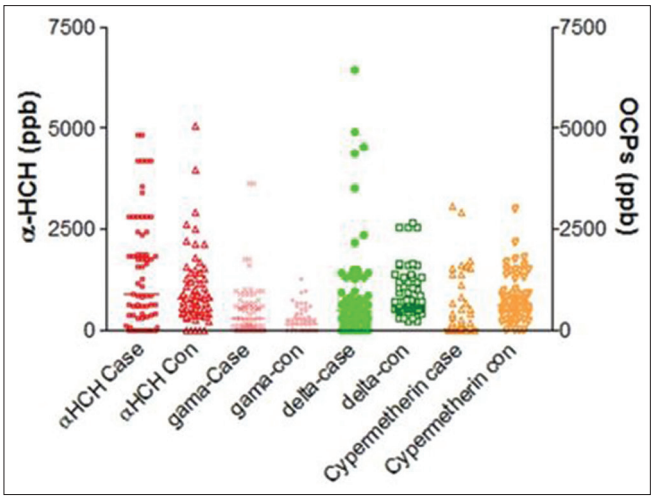

Figure 2: Level of organochlorine pesticides in control and recurrent miscarriage for females

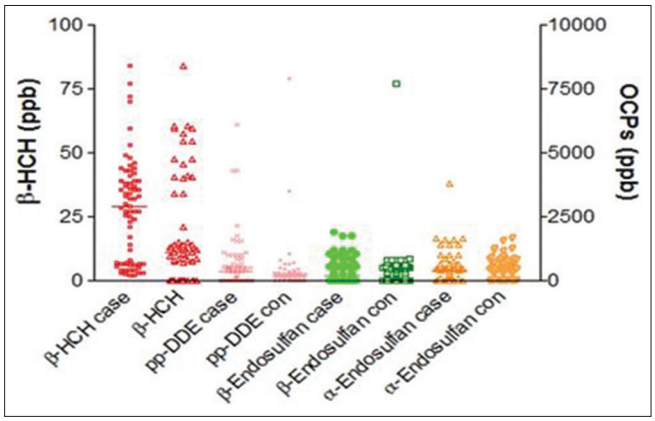

Figure 3: The distribution prototype of various pesticides

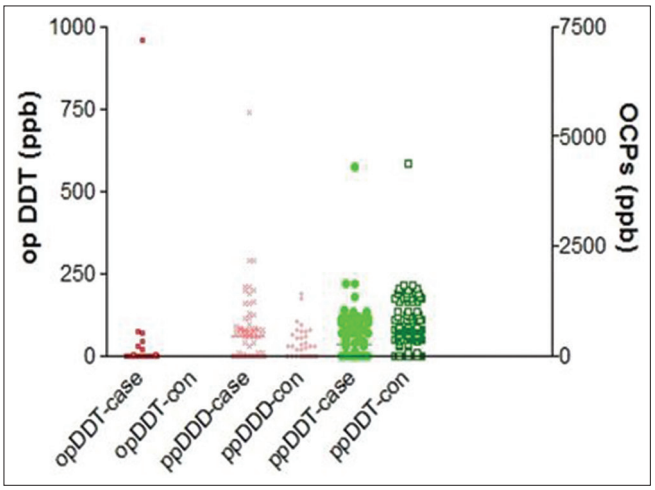

Figure 4: Patterns of pesticides distribution in case and control groups

of RPL was evaluated, and the findings were corroborated with Pathak *et al*.[17] The identification of environmental factors that enhance the threat of PCa must therefore be a goal for disease prevention. As previously outlined, pesticides and its derivatives, especially OCP (DDT, dioxins, and polychlorinated biphenyls) and OPP (malathion and chlorpysifos) possess weak estrogenic and androgenic effects. Their availability in the body may interfere at several control points in the hormone-signaling pathways. As a result, the response cascade of natural hormones can either be inhibited or excessively enhanced, at the wrong time, in the wrong tissue.

In this study, a statistically significant higher level of pesticides, namely β-HCH, γ-HCH, δ-HCH, chlorpysifos, pp-DDD, and fenvalerate ($P < 0.005$) is found in the case group compared to its control group. OCP pesticides exhibit hormonal activity in various tissues with mechanisms involving the steroidogenic pathway, receptor-mediated changes in protein synthesis, or anti-androgenic and estrogenic actions. Most of their endocrine effects are as a result of their ability to mimic 17-β-estradiol and may lead to miscarriage, but the evidence is inconclusive.[18,19] It was established that the lipophilic nature of OCP pesticides disturbs the normal estrogen-progesterone balance, which is particularly important in the maintenance of pregnancy.[20] Pathak *et al*.[21] reported that high β-HCH levels in cord blood were associated with preterm labor and high γ-HCH levels were associated with a higher risk of recurrent miscarriage.[17] In the earlier literatures, it was well established that cyfluthrin, a synthetic pyrethroid is a most frequently detected pesticide in the contaminants of breast milk in India.[22,23] Thus, these synthetic chemicals play a key role as a health risk to nursing infants. The concentration of a potent pesticide, chlorpyrifos in umbilical cord blood was negatively associated with birth weight, size, and neurodevelopment among infants born to low-income minority mothers.[24,25] This significance showed that the levels of pesticides are associated with an increased risk or excess incidence of pregnancy loss in the female population.

Conclusions

The current study demonstrates the importance of environmental factors in the context of RPL. Significantly higher levels of pesticides with endocrine-disrupting potential in cases suggest

Table 1: Different pesticide levels

Pesticides	Case (*n**; mean±SD)	25%	50%	75%	Controls (*n*; mean±SD)	25%	50%	75%	*P*
α-HCH	70; 1392±1340	316.4	876.6	1982.6	70; 1247.3±2533	431	787	1259	0.623
β-HCH	70; 25.4±17.9	6.38	27	38.2	66; 20.9±30.5	0	10.8	35.4	0.044**
γ-HCH	70; 494±686	60	288.2	673.7	67; 217.3±276	0	146.4	306	0.001**
δ-HCH	70;867±554	524.2	659	1100	67; 901.3±124	173.2	485	1256	0.002**
Malathion	70; 2556±1027	1027	2767	3868	70; 1253.7±1421	0	789	1790	0.22
Chloropysifos	70; 1036±1413	33.7	516.1	1327.3	69; 106.3±129	0	0	213	0.001**
α-Endosulfan	70; 454.3±624	0	393.6	566.4	70; 414.9±412	0	425.1	659	0.151
pp-DDE	70; 587.7±1065	0	353.2	624.9	70; 338.0±1020	0	145.9	269.6	0.099
β-ensosulfan	70; 397.2±490	0	159.3	638.2	70; 349.5±929	0	63.4	507.2	0.78
pp-DDD	70; 531.7±810	0	444.6	642.1	69; 145±291	0	0	209	0.001**
OP-DDT	68; 17.6±116	0	0	0	2; BDL	0	0	0	0.52
PP-DDT	70; 455.7±636	0	237	745.2	69; 634.2±638	239.6	579	803	0.714
Cypermethrin	70; 836.5±662	0	15.2	342.4	68; 338±847	3890	626	944	0.59
Fenvalerate	70; 3832.8±4193	969.2	1558.8	5451	63; 1034±256	0	142	907	0.001**

*Number of observations are varying as per the variable due to missing data; **Mann-Whiney U-test used to calculate the significance level.
SD – Standard deviation; HCH – Hexachlocyclohexane; α-HCH – Alpha-HCH; β-HCH – Beta-HCH; γ-HCH – Gamma-HCH; δ-HCH – Delta-HCH; PP-DDE – Para para dichloro diphenyl dichloro ethelen; PP-DDD – Para para dichloro di phenyl di chloro ethane; OP-DDT – Ortho para di chloro di phenyl tri chloro ethane; PP-DDT – Para para di chloro di phenyl tri chloro ethane; BDL – Below detection level

the possible role of these compounds as one of the causes of RPL. Increased pesticide level appears to indicate the increased levels of oxidative damage that has been associated with possible cause of RPL, and it may reflect indirect evidence of toxicity rather than a direct cause. However, our study has several limitations such as a small sample size. Moreover, we are also unable to determine if the observed association between γ-HCH and repeated miscarriages was due to the exposure of the mother during pregnancy or early childhood of an individual that affected their subsequent reproductive development. Furthermore, we must emphasize that toxicity depends on numerous additional factors such as genetic predisposition, dietary habits, and contamination with other pollutants. Hence, there is a need for further epidemiological studies with a larger cross-sectional population to be carried out to clearly determine the relationships between OCPs exposures and recurrent miscarriages along with the assessment of endocrine disruption, genetic polymorphism, and genetic environmental interaction.

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Conflicts of interest
There are no conflicts of interest.

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