Pesticides Level Mediates Oxidative Stress In Recurrent Pregnancy Loss Of Northern Indian Women.

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Introduction: Recurrent pregnancy loss (RPL) is defined as 3 or more consecutive pregnancy losses prior to 20th week of gestation. The prevalence of recurrent pregnancy loss is 1% - 3% of pregnancies. It becomes a challenging medical problem in case of unknown etiology. Environmental contaminant could be considered for adverse effect on the human reproductive health includes heavy metals, pesticides and other agents. Exaggerated maternal immune response and oxidative stress status may be responsible for RPL with unknown etiology.

Objective: The aim of the study is to evaluate the role of oxidative stress-related biomarkers and to find out the possible association of pesticides in the pathogenesis in RPL.

Methods: Oxidative stress markers and pesticides level were analysed in both cases and controls.

Results: The mean±SD values of blood level of antioxidant enzymes SOD, CAT, GR and GPx were observed to be significantly (p=0.0001) decreased in cases as compared to controls. While significantly (p<0.01) increased mean±SD blood level of lipid peroxides, protein carbonyls and conjugated dienes in cases and significantly decreased mean±SD values of blood level of LPO, PC and CD in control group was observed. Estimation of pesticide showed higher levels of α-HCH, β-HCH, γ-HCH, p,p'-DDT and p,p'-DDE in RPL as compared to control.

Conclusion: This study suggests that high blood levels of γ-HCH may be associated with risk of RPL. Pesticide exposure tends to increase oxidative stress which may result in various complications including peroxidation of vital body molecules resulting in increased risk for recurrent pregnancy loss.

Keywords: Recurrent pregnancy loss, oxidative stress, pesticides, antioxidant enzymes, Environmental contaminant.

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INTRODUCTION:
Recurrent pregnancy loss (RPL) is a devastating multifactorial problem that impacts a substantial number of couples. It has been defined by the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) by two or more failed pregnancies [1,2]. Although, different studies have been conducted to understand the etiology of this phenomenon, only limited factors have been suggested to be associated with this reproductive disorder. The recurrence rate of a spontaneous pregnancy loss is influenced by maternal age, parental karyotypes and abnormal laboratory findings [3]. It occurs in 2-4% of clinically diagnosed pregnancies [2] and constitutes a major emotional distress for couples. According to a recent study, the incidence of RPL is ~0.65%, although again this register-based study only included hospital-treated miscarriages [5]. The stability of reproductive cells and tissues is dependent on balanced concentrations of antioxidants and oxidants. Varied levels of Reactive Oxygen Species (ROS) can have both positive and negative impacts on female reproduction. Environmental pollutants including pesticides have been implicated in the pathogenesis of reproductive disorders [6]. Lifestyle factors such as maternal smoking, alcohol consumption, and recreational drug use stimulate production of unfavorable amounts of ROS leading to oxidative stress (OS), which renders physiological processes of female reproduction and the fetus vulnerable to oxidant-induced damage [7]. Exposure to environmental pollution can also give rise to excessive OS during pregnancy, and has increasingly raised concern about the impact of pollutant exposure on maternal and fetal health. Studies focusing on RPL have examined the anatomic, antiphospholipid syndrome, genetics, age, thrombophilias, autoimmunity, infection, sperm quality, and lifestyle issues factors [8]. However, oxidative stress (OS) factors are recently considered as the other potential causes of idiopathic RPL [9]. OS is one of the substantial factors affecting the pathophysiology of pregnancies.

A positive interconnection between smoking and occupational exposure to pesticides with elevated probability of RPL-abortion has been documented in recent years [10]. Rigorous diagnostics can recognize the specific cause only in few cases of RPL [13]. Hence, further environmental factors must be recognized that may affect pregnancy and play an etiological role in the pathogenesis of RPL. Current studies recommend the role of constant environmental pollutants such as organochlorine pesticides (OCPs) in the etiology of unfavorable reproductive effects [14]. OCPs have a tendency to cumulate in adipose tissues because of their lipophilic nature and half-life of months to years and also biomagnify through the food chain [16]. Because of this, OCPs can even be present in minute quantity in ecosystem, generating a constant exposure risk to human. Considerable amount of various OCPs have been present in human body tissues like blood, placenta, amniotic fluid, and in secretions like semen, breast milk, etc. [17].
OCPs can stimulate endocrine dysfunctions, immunological changes; oxidative stress and DNA damage [19]. Preceding studies have concluded elevated levels of DDT isomers and its metabolites in women with spontaneous abortion [13-14]. However, studies concerning the role of other OCPs in RPL are insufficient. Pesticide chemicals may induce oxidative stress leading to production of free radicals and alteration in antioxidants mechanism. Oxidative stress plays an important role in the toxicity of various xenobiotics, including organophosphates (OPs), synthetic pyrethroid, organochlorine (OC) and carbamate pesticides [22].
This study aimed to evaluate the oxidative stress-related biomarkers in RPL patients and to investigate the possible association of OCPs in the pathogenesis of RPL.

MATERIALS AND METHODS:
Study design and subject
This was a case control study conducted at Queen Mary’s Hospital, King George’s Medical University, Lucknow, UP, India from 2016 to 2018. 100 women (cases) with a history of at least three recurrent
pregnancy losses before the 20th week of gestation were included in this study. An equal number of women (100) undergoing normal vaginal labor at term with live healthy birth were recruited in the control group. Women with hormonal disorders (hyperprolactinemia, hyperandrogenemia, luteal insufficiency), uterine abnormalities (uterus fibroids, uterus bicornis, uterus subseptus), chromosomal translocation, antiphospholipid antibody syndrome, immunological causes of miscarriages, anemia, hypertension, bacterial vaginosis, TORCH infections, pre eclampsia, renal disease, heart disease, diabetes, urinary tract infections, metabolic disorders, tuberculosis, smoking, alcohol consumption or chronic drug intake and having complications during pregnancy and/or delivery were excluded from both the groups. We also excluded women with potentially confounding factors such as women of farming communities, occupational exposure to pesticides and industrial chemicals from this study. The spouses of these women were also non-diabetic with normal karyotype, normal sperm count and normal sperm morphology. Women confirmed their participation by signing a consent form and this study was approved by the institutional ethical clearance committee for human research. (ECR/262/Inst/UP/2013).

Sample collection:
Venous blood (3ml) was taken from each subject at the time of recruitment. Whole blood was transferred into heparin containing tube and then centrifuged; plasma was separated and used for the estimation of lipid peroxide levels (LPO), protein carbonyl contents (PC) and conjugated dienes (CD). The RBCs were lysed by mixing chilled water and RBC lysate was used for the estimation of antioxidant enzymes namely catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR).

Biochemical estimation:
Catalase (CAT) activity was determined spectrophotometrically by the method of Aebi [23]. Superoxide dismutase (SOD) activity was determined spectrophotometrically according to the method of McCord and Fridovich [24]. Glutathione peroxidase (GPx) and Glutathione reductase (GR) were assayed by the method of Pagila and Valentine [25]. Total protein content of RBC sample was determined by the method of Lowry et al [27]. Lipid peroxide (LPO) was estimated according to the method of Ohkawa et al [28]. Conjugated dienes (CD) were measured by the method of Racknagel and Ghosal [29]. The protein oxidation was measured by estimating the protein carbonyl (PC) levels by the method of Liu et al [30].

Pesticides (OCPs and OPPs) extraction and quantification:
The estimation of pesticides residue from blood was done after Bush et al. (1984) [31] with minor modification. In brief, 1 ml of blood was taken in separating funnel (100 ml) extracted with 10 ml hexane by shaking it at room temperature (30 °C) for 10 minutes. The organic hexane layer was collected and this process was repeated thrice; pooled hexane extract was collected. Organic layer (30 ml) was pooled together, collected and dried by using a vacuum evaporator (IKA RV 10 digital). Concentrated sample was transferred in the auto injector vial by passing through anhydrous sodium sulphate and dried with the flow of nitrogen. Sample was reconstituted to 1ml prior to injection, sealed and loaded on to auto injector for the analysis at condition. The trace level pesticide (ppb) in the blood samples was analyzed by using a Agilent technologies 7890A Gas Chromatograph, equipped with a micro Electron Capture Detector (ECD), capillary column DB-5MS (Perkin Elmer, CA, USA) and an auto injector 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 per min with hold time of 1.5, 0.5, 0.5, 0.5, 2 and 2.5 min, respectively and electron capture detector temperature was at 300°C.

RESULTS:
The comparison of oxidative stress parameters of both cases and control groups is summarized in Table I. It was observed that the mean±SD values of blood level of antioxidant enzymes, SOD, CAT, GR and GPx were observed to be significantly (p=0.0001)
decreased among the cases while above mentioned antioxidant enzyme mean±SD values of blood level found to be increased significantly in control group. However, the results shows significantly (p<0.01) increased mean±SD blood level of lipid peroxides, protein carbonyls and conjugated dienes in cases and significantly decreased mean±SD values of blood level of LPO, PC and CD in control group. A sum of total 14 pesticides (12 organochlorine and 2 organophosphate) selected on the basis of their persistent nature and consumption rate. These pesticides were known to be endocrine disruptors and having carcinogenic potential. Mean OCP levels (ng mL⁻¹) in cases and controls are listed in Table II. The results clearly showed that blood samples of women with RPL had higher levels of α-HCH, β-HCH, γ-HCH, p,p'-DDT and p,p'-DDE in comparison to control subjects. However, only significant association (p=0.01) was found between γ-HCH levels and RPL in comparison to controls in this study. Total HCH, Endo α, Endo β also show nearly significant association; if we increase the sample size, these too may fall in significant association category.

Table I: Comparison of oxidative stress parameters in cases and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases (n=100)</th>
<th>Controls (n=100)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>8.60±2.74</td>
<td>10.92±2.62</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Catalase (U/mg protein)</td>
<td>66.86±17.57</td>
<td>106.6±21.01</td>
<td>0.0001*</td>
</tr>
<tr>
<td>LPO (nmole MDA/mg protein)</td>
<td>4.22±3.17</td>
<td>3.02±1.82</td>
<td>0.007*</td>
</tr>
<tr>
<td>GPx (U/mg protein)</td>
<td>0.23±0.04</td>
<td>1.69±0.79</td>
<td>0.0001*</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td>0.67±0.32</td>
<td>1.04±0.47</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Protein carbonyls (nmole/mg protein)</td>
<td>0.09±0.05</td>
<td>0.01±0.001</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Conjugated Dienes (µM)</td>
<td>187.6±61.9</td>
<td>138.02±23.02</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>
**Table II: Distribution and comparison of OCP levels (ng/mL) in women with RPL (case) and control women.**

<table>
<thead>
<tr>
<th>OCP name</th>
<th>Cases (n=100)</th>
<th>Control (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>25% 50% 75%</td>
</tr>
<tr>
<td>α-HCH</td>
<td>5.28 ± 2.02</td>
<td>3.25 4.66 6.55</td>
</tr>
<tr>
<td>β HCH</td>
<td>5.64 ± 3.06</td>
<td>3.77 5.11 8.11</td>
</tr>
<tr>
<td>γ HCH</td>
<td>7.37 ± 6.97</td>
<td>2.78 5.29 9.06</td>
</tr>
<tr>
<td>Total HCH</td>
<td>18.3 ± 8.53</td>
<td>13.43 16.23 20.82</td>
</tr>
<tr>
<td>Endo β</td>
<td>1.55 ± 1.35</td>
<td>0.87 1.51 2.12</td>
</tr>
<tr>
<td>pp'-DDE</td>
<td>4.16 ± 2.67</td>
<td>2.24 3.92 5.51</td>
</tr>
<tr>
<td>pp'-DDT</td>
<td>1.45 ± 2.50</td>
<td>0.00 0.00 1.99</td>
</tr>
</tbody>
</table>

* Significantly different from control (p<0.05).

**DISCUSSION:**
Organochlorine pesticides exhibit hormonal activity in various tissues with mechanisms involving the steroidogenic pathway, receptor mediated changes in protein synthesis or antiandrogenic and estrogenic actions. Most of their endocrine effects result from the ability to mimic 17-β-estradiol [32] and may lead to miscarriage. It was supposed that the lipophilic nature of organochlorine pesticides disturbs the normal estrogen-progesterone balance which is particularly important in the maintenance of pregnancy [34]. Pathak et al. [35] reported that high β-HCH levels in cord blood were associated with pre-term labour and high γ-HCH levels were associated with a higher risk of recurrent miscarriage. Significant variation between women with RPL and control subjects regarding the levels of γ-HCH in blood was established. Human epidemiological data in relation to OCPs with miscarriages are uncertain and results vary [14,38]. Saxena et al. [38] revealed elevated amount of γ-HCH and other OCPs in women who had birth of babies before time (n=12) and/or one time voluntary miscarriage (n=8) compared with women who had faced full-term pregnancy. However, the importance in their study was specified to premature delivery only and nearly eight times increased level of γ-HCH was found in cases (56.09± 30.86 ng/mL) in comparison to subjects analyzed in this present study (7.37 ± 6.97ng/mL). Furthermore, our inclusion/exclusion criteria is clinically different in terms of define recurrent miscarriages i.e. women with minimum three recurrent miscarriages compared to study of Saxena et al. [38] where they took samples of women with single abortion. Pesticide poisoning may induce oxidative stress leading to generation of free radicals. More recently, environmental pollutants including pesticides have been implicated in the pathogenesis of reproductive disorders. During pregnancy, development of OS could affect placental expansion and/or increase pregnancy loss. Oxidative components are oxygen derived atomic particles and peroxides that are generally produced in small amount. Basically, produced during diverse pathways such as oxidative phosphorylation in mitochondria, and when the tissue is open to the elements of ischemia/reperfusion damage, they are formed in larger amounts. Pertaining to their enormously reactive features, they give rise to functional and structural damage to proteins, cellular DNA and cell membranes [39]. During pregnancy, the placenta is in a position to continuously generate OS. Placental tissues encompass a moderate activity of antioxidants such as SOD, CAT, GPx, because of low concentration of syncytiotrophoblasts, sensitizing the tissue against OS [40].
OS also give rise to certain issues such as preeclampsia, recurrent abortions and congenital disorders in diabetes [40]. Irregular placentation at the beginning of pregnancy pertains to OS and subsequent damage of endothelium giving rise to abortion. Safronova et al. [41] concluded that the creation of active oxygen species in the granulocytes of RPL patients is higher compared to normal reproductive function. Deficiency of antioxidant defense has been established to be linked with RPL [40]. It has also been documented that the concentration of lipid peroxides rises in the decidua of women experiencing primary pregnancy loss [41].

Antioxidants are classified as enzymatic and nonenzymatic. Common enzymatic systems include glutathione reductase, GPX, SOD, and CAT. Nonenzymatic agents are α-tocopherol (vitamin E), ascorbic acid (vitamin C), ceruloplasmin, ferritin, and transferrin. Low amount of GPX, vitamin A, vitamin E, β-carotene, SOD and CAT, and raised amount of reduced glutathione (GSH) are produced to balance the increased ROS levels as affirmed in patients with RPL [41]. It causes abnormal placentation during the beginning of pregnancy that result in damage of syncytiotrophoblast, accordingly leading to RPL.

**CONCLUSION:**
In conclusion, our study shows a connection between high blood levels of γ-HCH and women with RPL. A considerable decrease in antioxidant enzymes and substantial increase in LPO, conjugated dienes in cases was found out. Present study concluded that pesticide exposed patients suffering from recurrent pregnancy loss were significantly affected by oxidative stress. These parameters may be helpful in the prognosis or risk evaluation of RPL, however, detailed and extensive research is required. This study may draw attention to the consequences of environmental chemicals or xenobiotics on the “course of pregnancy” and women with a history of RPL may be benefited by knowing about their OCP burden in specialized clinics after consultation with physician/gynecologist. However, our study has several dependable limitations such as a small sample size. Moreover, we are also unable to determine if the association which we observed between γ-HCH and repeated miscarriages was due to exposure of the mother during pregnancy or early childhood of an individual that affected their subsequent reproductive development. Moreover, we must highlight that toxicity is linked to genetic predisposition, dietary habits and contamination due to other pollutants. OS and ROS-induced injury may be the missing fragments of the puzzle of miscarriage and RPL of unexplained etiology. A healthy pregnancy involves a lucid communication between maternal immune system and fetal cells. Since increased oxidative damage to the placenta occurs in patients with recurrent pregnancy loss, supplementary antioxidant therapy may be of benefit to these patients during preconception and early stages of conception. Further research is required to explore the mechanisms responsible for and preventing the oxidative damage due to pesticide exposure including antioxidant therapy in recurrent pregnancy loss.

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**CONFLICT OF INTEREST**
All the Authors declare that they have no conflict of interest.

**REFERENCES**
4. Rasmark R. E, Matthiesen L, Rylance R, Christiansen O.B. Is the incidence of recurrent pregnancy loss increasing? A retrospective


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